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Preface

This Special Issue of *Applied Sciences*, titled "Advances in Endodontics and Periodontics", aims to improve the state of the art in relation to the most relevant advances in Endodontics and Periodontics. Topics addressed in this Special Issue include alternative treatment strategies such as "green" antimicrobials, relationship with systemic diseases, age-related dentistry, as well as updating evidence-based knowledge pertaining to endodontics and periodontics. Recent advances in endodontics have been stressed, incorporating new devices and materials which have enabled a reduction in therapy failures, though these have not been quite correlated with the expected positive outcomes in populations. On the other hand, due to increased life expectancy, geriatric dentistry has highlighted the need to be prepared to manage oral age-related diseases, such as apical periodontitis and tooth mobility, caries, and periodontal disease. Of equal importance is the implementation of preventive and conservative measures, including pulp vital and regenerative therapies, in view of the maximum possible preservation of a functional natural dentition. Dentists should be aware of the medical complexities, poor cognitive function, and lack of autonomy of some elderly patients, which can be challenging for the entire team. Thus, there is huge pressure on dental professionals worldwide, leading to prioritizing the need to update contemporary knowledge involving medical conditions, drug prescription, or concomitant oral illnesses involving periodontics and endodontics. Reinforcing its interdependency with differential diagnosis, prevention, and treatment of dental and periradicular pathologies in an integrated systemic health of the patient is, thus, crucial. We hope that this Special Issue will provide an opportunity to discuss the state of the art of endodontics and periodontics, and we look forward to addressing the best protocols to raise the quality of treatments.

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Review

The Novel Role of Solvents in Non-Surgical Endodontic Retreatment

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Abstract: Non-surgical endodontic retreatment is a reliable conservative option for managing post-treatment apical periodontitis. However, effective microbial control, based on the maximization of filling removal and disinfection protocols, is not yet predictable. Traditional gutta-percha solvents, which are indistinctively used for both the core and sealer filling materials, became obsolete due to unprecedented advances in endodontic technology. Nonetheless, microtomography, scanning electronic microscopy findings, and histobacteriological analysis tend to confirm the persistence of filling materials and the lack of association between root canal enlargement and superior disinfection. There is a controversy regarding the most suitable clinical protocols surrounding the shaping procedures and the supplementary disinfection steps. Based on the literature and the previous work of the team, the authors aimed to summarize the current knowledge regarding specific solvent formulations that target filling materials. Additionally, the advantage of an additional irrigation step to optimize disinfection was highlighted. This adjunctive procedure serves a dual role in the dissolution of filling materials, and in conferring an antibiofilm effect. Further research is needed to understand the novel contribution of these strategies upon clinical practice outcomes.

Keywords: endodontics; filling materials; gutta-percha; irrigating solutions; non-surgical endodontic retreatment; sealer; solvents

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1. Introduction

Non-surgical endodontic retreatment (NSER) is a conservative option for managing persistent apical periodontitis (AP) associated with root-filled teeth, or where a new disease has emerged after root canal filling. Its main objective is to reduce the interradicular bacterial load to levels that are compatible with periapical healing, relying on maximum filling removal, repreparation through the most complete and canal-centered shaping techniques, and disinfection protocols [1]. However, the current therapy still focuses on the main root canal.

Reducing old filling remnants is crucial, as they may harbor intraradicular biofilms, the main cause of post-treatment AP [2]. The relative difficulty of NSER is related to variables such as the design of the retreatment/instrumentation systems, the age and type of the root canal filling, and previous preparation errors, besides the complex root canal anatomy [3]. After regaining access to the apical foramen, chemo-mechanical preparation (repreparation) aims to further remove filling residues and disrupt persisting adhered biofilms. Current retreatment techniques include rotary files, ultrasonic instruments, heat, laser, hand files, and solvent solutions [3]. Although their combination is generally required, removing the bulk of the obturations has greatly improved with the development of nickel–titanium (NiTi) rotary systems. This improvement has led to clinicians rarely using solvents.

Two main strategies have been proposed to optimize disinfection before the new filling: (i) a further apical enlargement [4], with the risk of weakening the root structure; or

(ii) using adjunctive procedures, such as sonic/ultrasonic processes or recently developed finishing instruments, to activate the standard sodium hypochlorite (NaOCl) irrigating solution [5,6]. Laser and photoactivated therapies have also been mentioned, despite their inherent high costs [7,8]. The subsequent sealing and the remaining cervical and radicular dentin structure have also been considered to be factors of favorable outcomes [9,10]. However, the current state-of-art, which involves combining adequate mechanical shaping and activated antimicrobial irrigating solutions, namely NaOCl, is still not able to provide a predictable outcome for NSER. Furthermore, there is no evidence for an improvement in the periapical status of populations that are concomitant with the extensive advance in endodontic knowledge and research [10].

Following the evolution path of NiTi instruments, new engine-driven NiTi instruments, for purposes other than shaping, such as glide path preparation, retreatment, or irrigation enhancement, have emerged. The ProTaper retreatment (Dentsply Maillefer), the self-adjusting file, or the XP-endo Finisher (FKG Dentaire) are some elucidative examples [11]. On the other hand, recent investigations using proposals that are safer and as effective as chloroform, such as methyl ethyl ketone (MEK), ethyl acetate, and novel solvent mixtures (MEK/tetrachloroethylene (TCE) and MEK/orange oil (OOil)), have highlighted an additional role for endodontic solvents. There is no intention of promoting the use of solvents per se, but essentially the purpose is to uncover different paths for optimizing disinfection in retreatment procedures without neglecting all of the available options. Apart from filling dissolution, its antibiofilm efficacy, enhanced by agitation, opens new perspectives in the current retreatment disinfecting protocol [12–14]. Built on the literature search and the previous work of the team, one of the major goals was to identify and summarize new solvent proposals concerning their specificity, their moment of use, their enhancement through agitation and biocompatibility, their effects on dentin structure, and their antimicrobial/antibiofilm activity in NSER.

2. Evolution of Endodontic Solvent Compounds

Traditional gutta-percha solvents are chemical substances, usually organic, whose primary objective is the dissolution or softening of filling materials (particularly gutta-percha). Studies on the advantages of their use are not consensual [15]. Some authors have stated that solvents should only be used when the working length is hard to reach [16]. Eventual disadvantages have also been reported, such as slowing of the retreatment process due to a higher accumulation of filling material remnants [16,17]. On the other hand, the isolated use of mechanical instruments has been associated with several problems, including perforating roots and straightening canals, preventing their original shape from being preserved [3].

Chloroform is one of the most popular gold-standard gutta-percha solvents, with a long history in endodontics. Although it is recognized as being one of the most effective for both gutta-percha and sealers [18,19], its use has been questioned due to its cytotoxicity and carcinogenic potential [20–22]. Although, in general, the cytotoxicity of gutta-percha solvents depends on their exposure time and dose, chloroform also has a considerable storage risk, as it is highly flammable. In turn, halothane, also associated with a high level of toxicity, has been discontinued [23]. Other solvents such as xylene and eucalyptol, which have been proposed as being alternatives to chloroform, although quite effective [24,25], have been shown to address similar concerns, namely regarding biocompatibility [20,26,27]. TCE was reported as having a strong dissolution effect, particularly over gutta-percha [28]. Although it has been pointed out as also promoting the dissolution of endodontic sealers, it was clearly less effective than chloroform [12,29]. Essential oils, such as OOil, which have recently been stressed as “green compounds”, were considered as being quite safer but less effective, particularly for sealer dissolution [24,27,28].

3. Solvent Specificity

Targeting the chemistry of a resin epoxy-based sealer, MEK (also known as 2-butanone or methyl ethyl ketone) and ethyl acetate (also known as 1-acetoxyethane or acetic ester) have raised attention as being novel endodontic solvents [12]. MEK is an organic, colorless, water-soluble solvent with a sweet odor that is reminiscent of acetone, and is categorized in group D (not carcinogenic to humans) [30]. It has been especially highlighted for the dissolution of one of the most commonly used endodontic sealers: AH-Plus [12]. Based on the same principle of a targeted approach to a sealer′s chemistry, 10% formic acid and 17% ethylenediaminetetraacetic acid (EDTA) have recently been suggested for hydraulic sealer dissolution in the clinical retreatment protocol [31].

Although traditional solvents have been indistinctively used for both filling materials—gutta-percha and sealers (such as resin and zinc-eugenol-based)—there has always been a special focus on their gutta-percha dissolution profile. However, different compounds have emerged as quite specific sealer solvents, such as EndoSolv E (Septodont) (a tetrachloroethy lene-based compound) and EndoSolv R (Septodont) (a formamide and phenyl ethylic alcohol-based compound) for zinc oxide–eugenol-based and resin-based sealers. Recently, they have been replaced by EndoSolv (Septodont), the main constituents of which are ethyl acetate (50–100%) and pentyl acetate (2.5–10%) [32]. Even though its manufacturer claims that it can be used for different types of sealers, there are no sound reports regarding its efficacy.

The development of new rotary retreatment files may have contributed to a lesser focus on investigating solvents for NSER. However, microtomography, scanning electronic microscopy findings, and histobacteriological analysis have shown that, independently of the instrumentation system or the supplementary irrigating approach, filling residues and resistant biofilms still persist in root canals or dentinal tubules after NSER conventional procedures [2,15,16].

The use of traditional gutta-percha solvents is mostly isolated. However, a few studies have assessed some associations for better performance. Faria-Junior et al. [33] revealed that TCE potentiated the effect of OOil and eucalyptol in different types of gutta-percha and Resilon. The association between Citrol+TCE obtained the best results on Resilon′s dissolution, while OOil (citrol) alone obtained the worst; however, they were still quite milder. In the same sense, Citrol+TCE and Eucalyptol+TCE were the most successful among associated and isolated compounds against EndoREZ cones. The lack of a deeper explanation and concerns regarding their biocompatibility pointed out the need for further research. Recently, with the same methodology of weight loss percentage, Ferreira I et al. [12] presented MEK as having a higher efficacy for resin-based-sealer dissolution. The values obtained reached those of chloroform; thus, they were quite different to the traditional gutta-percha solvents studied. Additionally, the authors confirmed the efficacies of two binary mixtures with MEK as a common compound and organic/essential oil as a co-solvent: MEK/TCE and MEK/OOil. A synergistic effect explained their increased efficacy for gutta-percha and resin sealer dissolution. The mixtures' performances reached the gold standard of chloroform and, importantly, with a safer profile [13].

4. Moment of Use

Traditionally, solvents were applied at the initial stages of the NSER, when fillings are more compact, through the deposition of a few drops into the space created by the coronal filling removal [3]. The main objective was to soften gutta-percha, enabling the initial penetration of the file into the remaining obturation [5]. Some authors reported a negative impact of the solvents' deposition (chloroform and eucalyptol) in the medial and apical parts of the retreated ex-vivo canals, with reduced the filling remnants in the root canal surfaces of the nonsolvent groups [16]. Different methodologies, such as the type and moment of solvent deposition (before/after repreparation), may have influenced the results.

Flooding the canal with solvent after removing the bulk of the remaining gutta-percha, and further enlargement, have also been investigated. One of the studies assessed the effect of xylene (1 min) on cleaning the root canal with paper points; the outcome was comparable to 2.5% ultrasonically activated NaOCl [34]. In turn, Fruchi et al. [35] emphasized the cleaning performance of the reciprocating instruments with xylene (1 min) and concluded that, even with passive ultrasonic agitation (PUI), the solvent did not improve filling removal. Similarly, Barreto et al. [36] also showed no improvement with PUI with OOil or NaOCl. Contrarily, Ferreira I et al. [37,38] showed promising results, advising specific solvents (MEK/TCE and MEK/OOil) as an additional step after the conventional repreparation and NaOCl/EDTA treatment. Due to their high dissolution rate in short periods, the same solvent mixtures might also be considered, to assist with the initial penetration of well-compacted obturations.

5. Solvent Agitation

The goal of combining solvents with ultrasonic agitation (UA) was for endodontic instruments to reach difficult-to-access areas, enhancing their effectiveness, as with the current irrigating protocol [39]. Moreover, the apical root canal, which is considered a "critical zone" due to its strategic position for microorganisms, remains a challenge for several instrumentation techniques or irrigating/dressing proposals [40].

SEM assessments found no improvement in root canal walls cleanliness using PUI with EndoSolv R as a final step after further enlargement (repreparation), independent of the root canal thirds; thus, its efficacy remains unclear [41]. Additionally, with contradictory outcomes, a few ex-vivo studies with microtomography quantified the volume of the remnants of filling materials after retreatment protocols with solvent agitation. Barreto et al. [36] found no significant differences between static NaOCl, PUI/NaOCl, and PUI/OOil, but stressed that all groups showed a significant reduction in filling residuals (gutta-percha and epoxy resin-based sealer). The lack of superiority of the solvent group was justified with the formation of a paste that penetrated the dentinal tubules and canal irregularities, making its removal harder. Fruchi Lde et al. [35] concluded that solvent agitation (PUI for 1 min, with xylene) slightly increased filling material removal, but without statically significant results.

On the other hand, in vitro studies assessing the dissolution rate using a sample weight comparison concluded that UA increased the efficacy of solvents such as eucalyptol and OOil. However, independent of the solvent, the greatest dissolution was obtained with the ZOE sealer [42]. Another study [43] with chloroform and eucalyptol corroborated an increased efficiency of solvents in the dissolution of sealers with UA, although with a significant decrease concerning the mineral trioxide aggregate sealer (MTA Fillapex). Ferreira I et al. [12] also reported a positive impact of UA on solvent efficacy, which was first evidenced with MEK over an epoxy resin-based sealer (AH-Plus). Similarly, traditionally milder solvents, such as OOil, were clearly improved via UA with regard to gutta-percha dissolution [28].

Because MEK had little effect on gutta-percha dissolution, studies with the MEK/TCE and MEK/OOil associations have confirmed previous findings and a clear benefit of UA in filling dissolution [13]. The suggested protocol assessed in ex-vivo studies with microtomography, including MEK/TCE, and claimed to target the most common filling materials: gutta-percha and epoxy resin-based sealer (AH-Plus). These performances was reported as being similar to a further enlargement to the next file size, thus preventing an excessive reduction in the thickness of the root canals [38]. The authors also found that the benefit of solvent agitation was independent of the device, whether ultrasonic or XP-endo Finisher R [37]. The specificity and synergism of the solvents in the mixture, their moment of use, and the exposure time, as well as sonic/ultrasonic agitation, were given as explanations for the performance obtained.

6. Biocompatibility

NSER procedures are inevitably associated with more post-operative complications, due to a higher risk of extrusion. In addition to necrotic infected pulp residues and debris that can be pushed out of root canals, there is a risk of extrusion of filling materials and/or irrigating solutions and dressings. The biocompatibility of any compound used is, thus, a safety requirement. An ideal root canal irrigating solution should be biocompatible because of its close contact with the periodontal tissues, and should respect the biological and mechanical integrity of the tooth [44].

Although solvents have almost fallen into disuse with the advent of new retreatment instruments, a recent review emphasizes the heterogeneity of the studies published and encourages a pursuit of the comparison of compounds in different scenarios [45]. Despite the disparity of methodologies, most of the reported findings are based on the performances of traditional solvents, namely chloroform, eucalyptol, EndoSolv R, and xylol; with new and less cytotoxic proposals, such as orange essential oils, having insufficient dissolution properties to justify their use. Although the most effective solvents are generally recognized as being highly cytotoxic, using small amounts inside treated root canals may prevent concerns regarding the risk of extrusion [20,26,27,46,47]. Nevertheless, inadvertent contact with the periapical tissues could pose a risk to the patient.

The new strategy of combining solvents with agitation in the empty root canal after filling removal might raise additional concerns. One example is the suggested protocol with MEK/TCE or MEK/OOil, even though in-vitro studies have reported a lower cytotoxicity from these novel proposals compared to the isolated compounds or the gold standard, chloroform [13]. Moreover, the use of solvents as an adjunct to mechanical instrumentation has not been associated with higher extrusion [48,49] or poor post-operative conditions [50]. Nonetheless, prospective randomized studies are always needed to assess the clinical performances of new strategies.

7. Effects on Dentin Structure

During NSER, solvents are inevitably in contact with dentin for some time. For a long time, investigations have highlighted a decrease in enamel and dentin hardness, due to the significant softening effects of chloroform, xylene, and halothane, with a time-dependent effect [51]; however, others do not confirm these findings [52,53]. Recent protocols suggest longer periods of dentin exposure to solvents after removing the bulk of the obturations. Some apprehension has, thus, arisen as to whether solvents can alter the dentin surface's chemical composition, with potential changes in its microhardness, and consequences on the bond strength of the sealers [53,54]. A recent systematic review [55], including push-out assessments, has stressed that the heterogeneity of the studies prevented a reliable conclusion from being reached. However, chloroform and xylene seemed to raise further concerns.

Despite reducing dentin's hardness, the novel solvent proposals of MEK and ethyl acetate are reported as being preferred over chloroform, which caused the most significant decrease [56]. A different experimental design associating MEK with the specific co-solvents TCE and OOil significantly increased dentin hardness after NaOCl and EDTA treatment [57]. Regarding direct dentin exposure, the MEK/TCE group showed no significant differences from the control (saline). MEK/OOil produced a significant hardness increase, independently of being used directly or after the NaOCl/EDTA standard final irrigating protocol.

The effect of solvent agitation on dentinal structure, per se, has been scarcely studied, and with ambiguous results. UA was reported to elicit a decline in dentin hardness when using MEK, ethyl acetate, and chloroform [56]. On the other hand, a study with the solvent mixtures MEK/TCE and MEK/OOil found no evidence of UA causing an additional decrease in dentin's hardness [57]. Findings from endodontic irrigating solutions such as NaOCl, chlorhexidine, or EDTA also tend to diverge. Investigations on EDTA's effect on dentin microhardness found that diode laser agitation caused higher hardness reduction than EDTA alone. However, there were no significant differences with UA or photon-induced photoacoustic streaming [58,59]. The different methodologies and chemistries regarding the compounds might explain the contradictory outcomes.

8. Antimicrobial/Antibiofilm Activity

AP is currently recognized as a biofilm-induced disease [60]. This causal link explains the increased resistance of endodontic intra-radicular infections to conventional disinfection procedures associated with the number of unprepared areas where root canal microorganisms, in planktonic and especially biofilm form, may persist [11,60]. These are considered to be the main causes of treatment failure. Moreover, the awareness that bacterial biofilms occur with particular relevance in the apical portion is crucial for the treatment, indicating the importance of primary and post-treatment AP therapeutics [61].

Research focusing on the antimicrobial properties of conventional gutta-percha solvents, such as halothane, eucalyptol, and OOil, has not been deep. The reported assays are almost exclusively against planktonic bacteria, such as *Enterococcus faecalis* (*E. faecalis*) and *Staphylococcus aureus* (*S. aureus*). In general, findings agree upon a stronger degree of antibacterial activity that is associated with the most cytotoxic solvents; OOil, for example, shows no antibacterial activity against the species mentioned [62]. Ex vivo studies emphasize that chloroform reduces intracanal levels of cultivable *E. faecalis* during endodontic retreatment [63]. By also stressing the role of *E. faecalis* as being the prime etiological agent of post-treatment infection, Subbiya A et al. [64] highlighted that RC Solve, a derivative of OOil, had superior antibacterial activity compared to xylene and EndoSolv E, which has tetrachloroethylene as its major compound. That study considered the minimal inhibitory concentration against *E. faecalis* ATCC and a clinical isolate from a failed root canal.

Maximum antimicrobial activity against *E. faecalis* biofilm has been reported with the association of a surfactant, such as cetrimide, and with chloroform, eucalyptol, or OOil. Although the combinations with cetrimide achieved a 100% kill rate, cytotoxicity assessments or the dissolution efficacy of the suggested associations were missing [65]. Biofilm removal strategies include its disruption via chemo-mechanical preparation with specific shaping techniques, and antimicrobial irrigating solutions/dressings. Increased concern over its resistance to conventional antimicrobial drugs should be considered [66,67].

Supplementary procedures for activating the final irrigating protocol with NaOCl with recent devices, such as ultrasonics or XP-endo Finisher R, have been suggested [5,6]. However, microorganisms are reported to regrow after NaOCl treatment. Although the final exposure with the chelating EDTA had an additional antimicrobial effect, authors claim there is a flaw in its ability to completely eliminate resistant biofilms, such as *C. albicans*, the most prevalent fungi isolated from persistent endodontic infections [14,68]. A recent study highlighted that the association of MEK/OOil could eradicate *C. albicans* biofilm cells remaining after the conventional NaOCl and EDTA final irrigating protocol [14]. To our knowledge, this is one of the first reports regarding the antibiofilm activity of solvents over endodontic microorganisms, refractory to the NaOCl and EDTA protocol, while exhibiting excellent dissolution ability over the most common filling materials.

9. Future Directions

The causative agents of post-treatment disease have been exhaustively investigated, confirming a less diverse set of microbiota and lower cell counts in well-treated teeth. *Streptococcus* species and the usually reported *E. faecalis* are among the most common bacteria that are isolated in secondary infections [69]. *E. faecalis* has been especially implicated, probably due to its ability to survive in mono-infections under adverse conditions [69]. Nevertheless, nearly 55% of the microbial community belongs to uncultivated or uncharacterized phylotypes, which may be dominant in some cases, and the common association of *E. faecalis* with secondary infections is not definitively supported [70]. Moreover, *Fusobacterium* spp. and *Pseudomonas* spp. with *Streptococcus* and *Actinobacteria* spp. have recently been reported as the most dominant taxa. Regarding fungi, *C. albicans* is recognized as the most frequently identified [61].

In addition to the wider microbial knowledge regarding endodontic microbial diversity, new concepts are developing, such as the awareness of proteins, which are often associated with virulence and with resistance to antibiotics, and the dependency on the host's individual profile [61]. Rapid and accurate chair-side tests for quick microbial detection, together with the knowledge of antibiotic resistance genes, could eventually address a rapid microbiological diagnosis, enabling the best therapy. Meanwhile, advances in antibiofilm-effective adjunctive protocols might be important for reducing the bacterial load, improving the success rate of endodontic treatments.

10. Concluding Remarks and Limitations

One of the limitations of the present paper is the risk of bias in the strategy of the literature search. However, a systematic review was not the objective here, but rather, the identification of relevant directions for endodontic investigation, from the authors' point of view.

Research findings on new endodontic solvent proposals changed the paradigm by considering the use of solvents, not only for initial filling softening, but also in the final process as an adjunctive in removing filling residues and disrupting refractory biofilms. There is some evidence for the advantage of an additional step with specific and safe solvent proposals, such as the dual role of promoting the dissolution of filling materials, and an antibiofilm effect. These nonantibiotic agents may be a strategy for optimizing retreatment procedures in order for a better outcome of post-treatment disease.

Basic science is important for investigating singular hypotheses that can contribute to a deeper understanding of complex processes. However, prospective studies clarifying the roles of novel protocols in the outcome of the clinical (re)treatment of endodontic infections are missing. The development of novel strategies that understand and that reach endodontic microbial communities is crucial for achieving the necessary level of infection control that results in an improved long-term treatment outcome.

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Review

Removal of the Previous Root Canal Filling Material for Retreatment: Implications and Techniques

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Abstract: Adequate removal of the previous filling material may be pivotal to a favorable outcome of root canal retreatment of teeth with post-treatment periodontitis in order to permit the access of irrigants and medicaments to persistent bacteria. However, even with recent technological advances, including the introduction of specially designed instruments, no technique has been shown to predictably promote complete filling removal. Supplementary approaches used after chemomechanical preparation, including the use of finishing instruments, ultrasonics and laser, have shown promising results in enhancing root canal cleaning and disinfection. This narrative review addresses the importance and implications of maximal filling removal during retreatment and discusses the effectiveness of different techniques and supplementary approaches used for this purpose.

Keywords: filling removal; gutta-percha; non-surgical endodontic retreatment; root canal sealers

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1. Introduction

The ultimate goal of root canal treatment is to prevent or treat apical periodontitis (AP) [1]. Consequently, the development or persistence of clinical signs and symptoms of AP following treatment indicates an unsuccessful outcome. The disease associated with root canal-treated teeth can be termed post-treatment AP and is managed by nonsurgical root canal retreatment, periradicular surgery or extraction. Because both primary and post-treatment AP share the same etiology, i.e., intraradicular infection, the same basic antimicrobial treatment principles and practice can be applied to approach both conditions.

Numerous epidemiological studies revealed that one of the most important risk factors for post-treatment AP is the technical quality of the previous treatment [2–7]. Teeth with low-quality treatment exhibit normal periradicular tissues in less than 40–50% of the cases, while a successful outcome is observed in about 85–95% of the adequately treated teeth [8,9].

It is apparent that even some well-treated teeth can fail. Teeth with persistent lesions after root canal treatment are eligible for retreatment or surgery [8]. The primary cause of post-treatment disease in well-treated teeth is usually bacteria located in areas of difficult access to intracanal antimicrobial procedures [10].

The success rate of retreatment in teeth with post-treatment AP, in turn, ranges from about 60% to 85% [11–15]. The lower rate of healing when compared to the initial treatment is likely related to the following: limited access to residual bacteria, which can be more complicated because of the need to completely remove the previous filling material; difficult to access to residual bacteria located in areas distant from the main canal lumen; and resistance of residual bacteria [8].

Because of the intraradicular location of most persistent/secondary bacterial infections [16,17], intracanal management by retreatment has great potential to promote healing. For this, disinfection of the entire canal system should be one of the most important goals of retreatment. A favorable outcome of non-surgical endodontic retreatment in teeth with post-treatment disease will depend on proper infection control [18], including not only the main root canal but also difficult-to-reach areas, including lateral and apical ramifications, isthmuses, recesses and dentinal tubules. In this context, adequate removal of the previous filling material is of fundamental importance for disinfection during retreatment, as it permits the access of irrigants and medicaments to residual bacteria [19].

Removal of the previous filling material is the first intracanal phase during retreatment, and should ideally be complete to expose areas of the root canal system where infection foci may exist. However, even with recent technological advances, which included the development of different instruments specially designed for retreatment, no technique has been shown to predictably promote thorough filling removal. This problem is even more critical in the apical canal region, especially in the presence of curvatures and anatomic irregularities [20,21].

Understanding the importance of attaining complete filling removal during retreatment as well as the variables and technical issues that may influence it is of paramount relevance for clinicians to improve the outcome. The purpose of this narrative review is to address diverse aspects of root canal filling material removal during retreatment.

1.1. The Importance of Complete Filling Removal

The actual impact of the amount of residual filling material on the outcome of root canal retreatment is unknown and it is very difficult to evaluate in the clinical setting, as the potential methods for evaluation, such as periapical radiographs and cone-beam computed tomography (CBCT) may not have sufficient resolution to exhibit small filling remnants on the canal walls. These evaluations have usually been performed in ex vivo studies using either direct observation under the microscope [22–24] or micro-computed tomography [21,25–29], which has a much higher resolution than radiographs and CBCT [30]. However, it is possible to infer the consequences of leaving residual material during retreatment. Histobacteriological studies have clearly demonstrated the intracanal location of bacteria in teeth with post-treatment AP, including irregularities and recesses in the main canal lumen, dentinal tubules, isthmuses and ramifications [17,31,32]. If the residual filling material covers some of these areas, bacteria may be physically protected and will remain unaffected by the antimicrobial substances used during retreatment [10]. If the unaffected bacteria are those that are actually causing periradicular inflammation, then the retreatment outcome will be compromised [18].

In teeth with an inadequate or absent coronal restoration, saliva may seep into the canal and bacteria may colonize the interface between the filling material and the dentinal wall [33–37]. If the previous material is not completely removed for retreatment, these areas will remain contaminated and may serve as a reservoir of bacteria to induce or maintain AP [38].

One of the many challenges for the clinician during retreatment is to completely remove the previous filling material, especially in curved and well-filled canals [39]. The resistance imposed by the material makes its removal difficult, increasing the risk of procedural accidents such as ledges, perforation and instrument fracture. Furthermore, unlike gutta-percha, which is usually confined to the main canal, the endodontic sealer may penetrate into the dentinal tubules. Although this is required for a fluid-tight seal, the depth of sealer penetration and the blockage of the dentinal tubules can add an extra challenge to canal retreatment. Once the sealer penetrates into dentinal tubules, its total removal during retreatment is physically impossible [40].

Non-surgical endodontic retreatment can be divided into two phases: filling removal and re-instrumentation. Considering that canal enlargement during re-instrumentation also helps remove the previous filling material, evaluations of canal cleaning after retreatment should always take into account both phases, that is, the whole procedure. Different retreatment techniques have been proposed, with the most recent ones based on engine-driven nickel-titanium instruments. Some systems have been specially designed for use in retreatment.

1.2. Effectiveness of Different Protocols

Numerous nickel-titanium (NiTi) instrument systems for root canal preparation have been introduced into the market over the years. Unfortunately, most of them are released without sufficient information regarding their mechanical and geometric properties and, even worse, without any demonstration of their effectiveness. As for retreatment, rotary NiTi instruments have been used for both removal of the previous filling material and re-instrumentation [41]. Depending on the system, it is even possible to perform both steps simultaneously [42].

Chemical solvents (chloroform, xylol or eucalyptol) have traditionally been used in association with manual or rotary instruments to facilitate instrument penetration into the filling mass. In addition, the solvent may contribute some antimicrobial effects [43], though the magnitude and relevance of this property remain questionable, given the small amount of substance used and the fact that more potent antimicrobial agents, such as sodium hypochlorite, will be used next in the procedure and in much higher volumes. A study evaluated the influence of a solvent (eucalyptol) in enhancing the removal of filling material for retreatment of Vertucci's type II mandibular molars [21]. The Mtwo instrument system (VDW) was used for filling removal, with or without a solvent, and then the residual filling volume was assessed by micro-computed tomography. Findings revealed that the amount of material removed from the canal, including the isthmus area, was 83.2% without solvent and 83.8% with solvent, with no significant difference [21]. Other studies have confirmed these findings, not showing superior removal of filling material when using solvents [44,45]. Besides, the formation of a thin layer of chemically softened gutta-percha on the root canal wall may impair further cleaning and disinfection. This, along with solvent toxicity and unproved effectiveness, may discourage the use of these substances during retreatment [46,47].

Regardless of the retreatment technique, studies have shown that the complete removal of filling material is not commonly achieved [28,48,49], especially in the apical portion of root canals (Figures 1 and 2) [19,42,50]. The mean percentage of residual filling material has been demonstrated to range from 0.02% [51] to 43.9% [52] of the initial filling volume, with the great majority of studies reporting values below 10%. For this reason, supplementary approaches have been recommended to enhance filling material removal [42,50] and improve root canal disinfection [53–57] (see below).

Figure 1. Microcomputed tomographic images of the apical segment of a mesial inferior molar root (class II of Vertucci, with isthmus) subjected to retreatment. **Left**, initial microtomographic scan performed after root canal obturation. **Middle**, post-preparation image obtained after retreatment with the Mtwo system (final instrument 35/0.04) in both canals. **Right**, final image after using the XP-Endo Finisher R.

Figure 2. Image obtained in a stereomicroscope (16×) after root canal retreatment in a lower incisor. The system used was the Universal ProTaper (final instrument F4).

1.3. Main Variables Influencing Filling Material Removal

1. Anatomy. The outcome of non-surgical endodontic retreatment can be affected by the tooth type; studies revealed that multi-rooted teeth have a significantly lower percentage of success than premolars and anterior teeth [58,59]. Certainly, the complex internal anatomy, including isthmuses and ramifications, as well as the occurrence of curvatures can make the retreatment of infected teeth more difficult. Root canals that are oval or flattened in cross-section also pose additional difficulties to proper cleaning and disinfection [60]. Rotary or reciprocating instrumentation often produces a circular and centered preparation, with limited lateral action on the recess areas of oval /flattened canals [61,62]. Although brushing or circumferential filling might help overcome this problem, a high percentage of the canal surface still remains unprepared in these teeth [63,64].

2. Coronal access. Adequate coronal access is expected to influence the filling material removal. Studies reported that minimally invasive access cavities resulted in a greater amount of filling remnants compared to conventional cavities [65,66].

3. Instrument. A systematic review concluded that NiTi instruments specially designed for retreatment do not have advantages over conventional techniques with regard to filling material removal [49]. Although these instruments may more easily penetrate into the filling mass and reduce the procedure time, they have been shown not to be essential for better material removal [41,49]. On the other hand, the use of hybrid techniques and larger preparation diameters are associated with greater cleaning [49]. Studies have shown that differences in taper, tip and cross-sectional shape, as well as the operation mode and the number of instruments used, usually failed to promote significant differences in filling removal [28,41,48,50,67].

4. Quality of filling. A prospective study with long-term follow-up showed that the outcome of endodontic retreatment was significantly better in teeth, with previous obturation ranked as inadequate [15]. Conceivably, thorough removal of the previous filling material in these cases is more predictable. This increases the possibility of further antimicrobial procedures to reach the residual infection.

5. Canal enlargement. Larger preparation sizes are associated with greater filling material removal [42]. The final size of the preparation in retreatment should incorporate the diameter of the previous preparation along the entire canal length. This is justified not only for maximum filling material removal, but also to promote proper cleaning and disinfection [68,69]. In addition, a prospective cohort study [70] reported a high success rate (89%) for the retreatment of lower molars using contemporary techniques and preparation sizes of 0.35–0.40 mm, with tapers varying from 0.04–0.06 mm/mm. Nevertheless, it is important to emphasize that excessive

enlargement should be avoided, especially in the pericervical area, in order not to weaken the root, which could predispose the tooth to fracture.

6. Patency. Foraminal patency and the extent of root canal cleaning as close as possible to the apical terminus were identified as factors that positively influence the root canal treatment/retreatment outcome [71–73]. This is because, in some infected teeth, the most advanced frontline of infection may be located close to or at the apical foramen. In addition, increasing the apical level of the root canal filling during retreatment also positively affected the outcome when compared to teeth in which such an increase was not observed [18]. This can be explained by the fact that the apical advance of root canal procedures during the retreatment of infected teeth can lead to better disinfection of the apical part of the canal, where the bacteria commonly associated with the post-treatment disease are usually located.

7. Irrigant agitation. It has been demonstrated that the removal of the filling material in the canal can be enhanced by supplementary approaches for agitation of sodium hypochlorite after canal preparation (see below).

8. Solvent. Its benefit is observed only in the initial stage of the retreatment as it may facilitate penetration of the instrument into the filling mass. As discussed above, solvents may impair proper cleaning because of the formation of a thin layer of soft gutta-percha, which can cover the canal wall [47,74]. With regard to filling removal, some studies have not observed greater benefits when a solvent was used [44,75,76].

9. Magnification. The use of magnification under abundant illumination is of utmost importance in many phases of endodontic treatment/retreatment and surgery. The impact of magnification on filling material removal is more evident in straight canals [23,77,78]. Furthermore, an improved retreatment outcome was observed when the operating microscope was used [15].

10. Type of filling material. The different types of filling materials may cause diverse levels of difficulty for removal during treatment. Of the most currently used materials, the greatest challenge has been reported for bioceramic sealers [67,79–82].

11. Age of filling. Although the effects of the root canal filling age and its impact on gutta-percha removal are not well known, a recent study using a tricalcium silicate sealer showed that filling removal was less effective in freshly filled canals than aged filled canals [83].

1.4. Retreatment with Hand or Engine-Driven Instruments

The results of different studies evaluating the removal of the filling material by hand and engine-driven instruments are conflicting. While some studies showed a better performance for hand instruments [51,84], others have not reported significant differences [28,85], and even others observed better results for engine-driven instruments [60,86]. A systematic review concluded that, unlike engine-driven instruments, retreatment with hand instruments was not associated with iatrogenic errors [49]. Hand instrumentation usually spends more time for retreatment when compared to rotary or reciprocating systems [28,84,87]. However, speed should not be the main factor when selecting one system over another. The difference in time is usually limited to only a few minutes and a longer action time also results in a longer retention time for antimicrobial irrigants, which can improve disinfection [88].

1.5. Retreatment with Rotary or Reciprocating Instruments

In general, studies comparing rotary and reciprocating systems for filing material removal during retreatment have shown a similar performance [28,48,67,86,89–94]. Because these systems are often compared in teeth with similar anatomy, under standardized irrigation conditions, working length and apical preparation sizes, the isolated effects of the operation mode on filling removal may not be of great significance. These findings are in line with many other studies that showed similar shaping [95–97] and disinfection [98–100] effects when using NiTi instruments in continuous rotation or reciprocation.

1.6. Canal Transportation during Retreatment

The safety of retreatment procedures is a matter of concern because of the risk of procedural accidents, including instrument fracture, zips, ledges and perforation. The occurrence of root canal transportation during filling removal and re-instrumentation has been evaluated, especially in curved canals and in the apical third [14,101]. Transportation can complicate proper cleaning, disinfection, and filling of the canal and, if unnoticed or not corrected, may evolve to more drastic accidents, including perforation and zips [102]. Furthermore, excessive dentin removal, especially in the most coronal parts of the root canal, may weaken the root structure and put the root at risk of fracture [103].

Studies have shown a high frequency of canal transportation during retreatment [45,101,104,105]. For instance, a recent study revealed the occurrence of apical transportation in all mesial canals from mandibular molars retreated with either rotary multifile or single-file NiTi systems [105]. In general, the studies show a similar performance of the different NiTi systems in terms of centralization, including when compared with manual instruments [45,101,104,105]. However, any conclusion about a specific retreatment system/technique is premature because of the restricted number of studies on the subject.

Many variables can interfere with canal transportation during retreatment, including canal anatomy, type of filling material, dentin hardness, preparation technique, operator experience, curvature angle, radius and position, and instrument geometry, motion, and alloy.

1.7. Supplementary Approaches

These procedures are recommended for use after instrumentation with the main purpose of maximizing cleaning and disinfection before placing an intracanal medication or the final obturation. Supplementary approaches include mechanical, sonic, ultrasonic and laser means [56].

Mechanical effects are usually represented by the agitation of the irrigant in the canal by manually pumping instruments or gutta-percha points or by engine-driven instruments operated at low speed (e.g., lentulo spirals, plastic instruments, or NiTi finishing instruments). Finishing instruments have received a great deal of attention over the last years and studies have demonstrated that they can significantly improve disinfection after instrumentation [53,106]. One of the most commonly used is the XP-endo Finisher R instrument (FKG Dentaire, LaChaux-deFonds, Switzerland), which was specially designed for retreatment. This finishing instrument is a variant of the XP-endo Finisher (FKG Dentaire), with a larger core diameter (ISO 30). It exhibits a zero taper and, at the body temperature, undergoes morphological changes to acquire a spoon-like shape that expands its reach in the canal system. Both the XP-endo Finisher and the XP-endo Finisher R have been demonstrated to be highly effective in removing the residual filling material during retreatment [21,25,26,107–109]. The additional reduction in residual material provided by these finishing instruments may reach a mean of 40% to 69%, including the isthmus area [42,75]. A study showed that XP-endo Finisher R was more effective than sonic and ultrasonic devices in removing the previous filling material in oval canals [110]. A systematic review concluded that the supplementary approach with XP-Endo Finisher or XP-Endo Finisher R is effective in enhancing the removal of root canal filling materials during root canal retreatment [111].

The Self Adjusting File (SAF) (ReDent-Nova, Ra'anana, Israel) is a non-conventional hollow and flexible instrument for root canal preparation, which was devised to adapt itself to the canal morphology in cross-section. Because the instrument surface is lightly abrasive and can scrape dentin, there is a good potential for cleaning the canals free of residual filling material during or after preparation [112,113]. When used for canal preparation during retreatment, the amount of residual material after using SAF was not significantly different from conventional instruments [25,114]. Nonetheless, favorable results have been reported for the SAF instrument when used as an adjunctive step after the preparation of oval [113] or curved canals [115].

A commonly used approach to supplement disinfection after preparation is the agitation of sodium hypochlorite by sonic or ultrasonic devices. Sonic frequencies range from 20 Hz to 20 kHz. The EndoActivator is a sonic handpiece that operates at 167 Hz or 10,000 cpm and uses non-cutting polymer tips to agitate the irrigant in the canal [116]. Studies have evaluated its effects on filling material removal. Some showed that EndoActivator might increase the removal of filling material [117], even though it was significantly less effective than ultrasonics [117] and XP-endo Finisher [110,118]. However, another study observed no significant improvement in filling material removal [94].

Ultrasonic frequencies are greater than 20 kHz. Ultrasonic units for endodontic use usually oscillate at frequencies ranging from 25 to 30 kHz [119,120]. Ultrasonic activation of irrigant solutions is another widely used supplementary approach. The main effects of ultrasonics result from the phenomena of cavitation and acoustic streaming and possibly warming of the irrigant [119,121–123]. Ultrasonic effects can affect and disorganize bacterial biofilms and wash away detached bacterial cells from the canals [120,124,125]. Moreover, cavitation may weaken the bacterial membranes and potentiate the antimicrobial effectiveness of NaOCl [120,126]. The phenomena of acoustic streaming and cavitation are dependent on the free displacement of the ultrasonically activated instrument [122,123,127]. Many studies have addressed the effects of ultrasonics in improving disinfection by NaOCl after preparation, and the results are conflicting [128–131]. Ultrasonics have also been used to enhance the removal of the filling material during retreatment and studies have shown significant improvement [50,132,133]. For instance, a study revealed that passive ultrasonic irrigation reduced, on average, 43% of the volume of the remaining filling material [50]. However, other studies showed no significant improvement [89,94,134,135].

Laser has also been proposed for a supplementary step of irrigant activation during canal treatment and retreatment, to improve disinfection and also to enhance filling material removal. A study used the Er:YAG laser, Er:YAG laser-based photon-induced photoacoustic streaming (PIPS), and Nd:YAG laser and reported a significant improvement in material removal; a comparison between them showed better results for the Er:YAG laser [136]. PIPS was significantly better than sonic and ultrasonic approaches in removing filling remnants from oval canals [137]. The additional cleaning effects of PIPS seem not to be significantly affected by the type of filling material [138]. However, a study reported that the PIPS method did not have a significant effect on the removal of filling remnants in comparison with conventional needle irrigation [139]. When the Er:YAG laser-initiated shockwave-enhanced emission of photoacoustic streaming (SWEEPS) was used in curved canals, it improved the removal of filling remnants and performed better than PUI when no solvent was used [140]. The superior cleaning effects of SWEEPS over PUI were confirmed by another study [141]. A study compared PUI and super short pulse (SSP) and SWEEPS modes of Er:YAG laser-activated irrigation (LAI) with two different laser tips for removing filling materials in curved canals and concluded that all of them were significantly effective; the LAI/SSP showed better results than the others [142].

2. Conclusions

It is salient to point out that, regardless of the method used for the removal of filling remnants, using or not a supplementary step, virtually all studies demonstrated that the canals are rarely, if ever, completely cleaned after these procedures. In the large majority of studies, the previous obturation was adequate in terms of apical length and homogeneity, which arguably increases the difficulties for full removal. This is not the rule in the clinical setting, as most cases presenting with post-treatment AP show inadequate root canal fillings [4,7,143]. Filling removal in these cases may not be so challenging and this helps explain the better outcomes for teeth with post-treatment disease that have inadequate fillings when compared to the ones with previous adequate fillings [15].

Virtually all studies on the subject of filling removal had an ex vivo nature (extracted teeth). Most studies were based on recently placed fillings; therefore, their results cannot be extrapolated to aged fillings, which are the most common in the clinical scenario. Despite the limitations of ex vivo studies, there is currently no effective and reliable means to evaluate filling material removal in the in vivo situation.

The impact of filling material removal on the success of endodontic retreatment still needs to be confirmed by long-term outcome studies. However, given the difficulties in performing such evaluation in the clinical setting, this question may long remain to be answered. Logical thinking indicates that complete removal may improve disinfection of the canal system by permitting the antimicrobial irrigants and medications to reach residual bacteria that are the cause of post-treatment disease. Taking into account the performance of the current retreatment techniques, in which no one predictably promotes complete filling removal, supplementary procedures should be considered indispensable in non-surgical retreatment. Further research is also encouraged to develop and evaluate techniques and strategies that can successfully clean root canals during retreatment.

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Systematic Review

Prevalence of Root Canal Treatments among Diabetic Patients: Systematic Review and Meta-Analysis

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Abstract: (1) Apical periodontitis (AP) is the inflammatory response of the periapical tissue to bacterial antigens and toxins arriving from inside the root canal after pulp necrosis. To control AP, it is necessary to interrupt the passage of antigens from the root canal to the periapex, which is achieved via a root canal treatment (RCT), which is the indicated endodontic therapy in cases of AP. The prevalence of root-filled teeth (RFT) is an indicator of the frequency of endodontic infections and the degree of dental care. Diabetes is associated with AP and has been identified as the main prognostic factor in RCT. The aim of this study was to carry out a systematic review with meta-analysis answering the following question: What is the prevalence of RFT among diabetic patients? (2) This study was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines 2020. A literature search was undertaken without limits on time or language until 12 January 2023 in PubMed-MEDLINE, Embase and Scielo. All studies reporting the prevalence of RFT among diabetic patients via radiographic examination; both panoramic and periapical radiographs were included. Meta-analyses were calculated with Open Meta Analyst software. The main outcome variable was the prevalence of RFT, calculated as the total number of RFT divided by the total number of teeth, which is expressed as a percentage. As a secondary outcome variable, the prevalence of diabetic patients with at least one RFT, expressed as a percentage, was also calculated. The quality of evidence of the included studies was analyzed according to the guidelines provided by the Centre for Evidence-Based Medicine in Oxford. The risk of bias was assessed using the Newcastle–Ottawa Scale, which was adapted for cross-sectional studies. To estimate the variance and heterogeneity amongst the trials, the Higgings I2 test was employed. (3) Eight studies fulfilled the inclusion criteria. Four studies were classified as having a high risk of bias, and four were classified as having a moderate risk of bias. The prevalence of RFT was estimated for 37,922 teeth and 1532 diabetic patients. The overall calculated prevalence of RFT among diabetic patients was 5.5% (95% CI = 4.1–6.9%; $p < 0.001$). The percentage of diabetics who had at least one RFT was 42.7% (95% CI = 23.9–61.4%; $p < 0.001$). (4) This systematic review and meta-analysis concluded that the prevalence of RFT among diabetic patients is 5.5%. More than 40% of diabetics have at least one RFT. In daily clinics, dentists should suspect that patients are undiagnosed diabetics when multiple RCT failures are observed in the same patient.

Keywords: diabetes; endodontics; epidemiology; root canal treatment; root-filled teeth; prevalence; survey; population-based study

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1. Introduction

When bacteria, their toxins or their antigens reach the dental pulp, the pulpal inflammatory reaction, pulpitis, ends up inducing pulp necrosis [1]. If an adequate treatment is not instituted, toxins and antigens will pass through the apical foramen into the periapical tissues, inducing the immune and inflammatory reaction characteristic of apical periodontitis (AP) [2]. To control AP, it is necessary to interrupt the passage of antigens from the root canal to the periapex, which is achieved via a root canal treatment (RCT) [3]. RCTs are focused on eliminating bacteria that cause an infection in the root canal system using chemical and mechanical methods [4]. Both AP and RCT are highly prevalent, with at least one tooth affected by AP in 52% of people [5], and 8% of the teeth of the world population being root-filled teeth (RFT) [6].

On the other hand, several epidemiological studies on endodontic medicine have reported the high prevalence of AP among patients affected by some systemic diseases [7–10]. In the specific case of diabetes, there are many cross-sectional and case–control epidemiological studies that have found a higher prevalence of AP among diabetic patients [11–16]. Diabetes mellitus is characterized by an inadequate carbohydrate, lipidic and protein metabolism; its primary aspect is hyperglycemia [17,18]. Hyperglycemia acts as the main cause of the incidence and progression of microvascular complications associated with the disease (retinopathy, nephropathy and neuropathy). Two main types of diabetes have been established: type 1 diabetes (insulin-dependent diabetes) is characterized by deficient production of insulin by the pancreas and requires external administration of this hormone; type 2 diabetes (non-insulin dependent diabetes) is characterized by the ineffective use of insulin by the cells of the body, representing 95% of all diabetics [19,20].

Given the high prevalence of AP among diabetic patients [11,13,15,16,21,22], it can be expected that the prevalence of RFT is also high among those patients. However, several systematic reviews have concluded that diabetes is a major preoperative prognostic factor in root canal therapy [23–25], negatively influencing the treatment outcome and RFT survival rate [26,27]. Therefore, diabetics could have a lower prevalence of RFT than the general population does. On the other hand, diabetic patients have a high prevalence of periodontal disease [28,29], which also leads them to lose a greater number of teeth than the healthy non-diabetic population does [30].

The aim of this study was to conduct a systematic review and meta-analysis investigating the prevalence of RFT among diabetic patients.

2. Materials and Methods

This study was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines 2021 [31] and the methodological guidance for systematic reviews of observational epidemiological studies reporting prevalence and cumulative incidence data [32]. The CoCoPop mnemonic has been followed to formulate the review question [32], as follows: What is the prevalence of RFT (Co, condition) among diabetic patients (Pop, population) around the world (Co, context)?

The main outcome was the percentage of RFT. As a secondary outcome, we took into account the percentage of people with at least one RFT.

2.1. Literature Search Strategy

A literature search was undertaken without limits on time or language until 12 January 2023 in PubMed-MEDLINE, Embase and Scielo. The most frequently cited descriptors in the previous publication on this theme were used in the electronic search strategy using combining Medical Subject Heading (MeSH) terms and text words (tw). The search strategy is shown in Box 1.

Box 1. Key words and search strategy.

(Diabetes OR Diabetes Mellitus OR Hyperglycemia OR Diabetic) AND (nonvital tooth OR nonvital tooth OR tooth nonvital OR tooth nonvital OR nonvital teeth OR teeth nonvital OR devitalized tooth OR tooth devitalized OR devitalized teeth OR teeth devitalized OR pulpless tooth OR tooth pulpless OR pulpless teeth OR teeth pulpless OR endodontically-treated teeth OR teeth endodontically-treated OR endodontically-treated tooth OR tooth endodontically-treated) AND (cross-sectional studies OR cross-sectional OR cross-sectional design OR cross-sectional research OR prevalence studies OR prevalence study OR survey OR prevalence OR epidemiologic studies OR epidemiologic study OR cohort studies OR cohort study OR concurrent studies OR concurrent study OR incidence studies OR incidence study OR case-control studies OR case-control study)

Complementary screening was performed to look for any additional studies among the references of the included studies.

2.2. Eligibility Criteria

The studies that have been included are all those that provided information on the frequency of endodontic teeth among diabetic patients, as determined by radiographic examination.

The following exclusion criteria were applied: studies that evaluated the prevalence of RFT only among non-diabetic patients, those that did not report data on the prevalence of RFT, those that did not provide full mouth information, those that included patients with mixed dentition, and those that did not contrast their findings with radiographic examination, as well as reviews, letters, posters, conference proceedings or case series, and dissertations

2.3. Study Selection

The studies were selected by three of the authors (D.C.-B., M.L.-L., and J.J.S.-E.) by evaluating titles and abstracts. The full text was accessed when the title and abstract did not allow the authors to judge the study. Next, full texts were analyzed and the articles that met the eligibility criteria were selected. In case of disagreement, it was resolved by the three authors reaching a consensus.

2.4. Data Collection/Extraction Process

The same authors collected information about the selected studies. For each article, the following information was extracted: authors, year of publication, participants, radiographs used, the number of teeth, the number of RFT and the number of people with at least one RFT.

2.5. Quality Assessment and Risk of Bias of Individual Studies

The guidelines provided by the Centre for Evidence-Based Medicine in Oxford [33] were used to analyze the quality of evidence in the included studies. The risk of bias was assessed using the Newcastle–Ottawa Scale, which was adapted for cross-sectional studies [6,34].

Three authors (D.C.-B., M.L.-L., and J.M.-G.) independently assessed the risk of bias of each of the included studies. In case of disagreement, the authors discussed it until they reached an agreement.

Two domains were taken into account when analyzing the quality assessment and risk of bias of the individual studies: sample selection and outcome. The domain sample selection included the following items: the representativeness of the sample, sample size and non-respondents. The domain outcome included the following items: the assessment of the outcome, the inclusion of third molar in the outcome, the inclusion of edentulous in total sample and the number of observers. The evaluation of each item was conducted according to the criteria previously described [6]. The maximum possible score was 12 points. A high risk of bias was defined as from 0 to 4 points, a moderate risk of bias was considered for the studies scoring from 5 to 8 points, and finally, a low risk of bias was assigned to studies scoring between 9 and 12 points.

In studies whose sample included edentulous patients, only dentate patients were considered for statistical analysis.

2.6. Outcome of Interest

The main outcome variable was the prevalence of RFT, which was expressed as a percentage. As a secondary outcome variable, the prevalence of diabetic patients with at least one RFT, expressed as a percentage, was also calculated.

2.7. Data Synthesis and Statistical Analysis

The prevalence of RFT among diabetic patients was calculated by carrying out a meta-analysis using OpenMeta Analyst version 10.10 software [35] using the binary random effects model. Another meta-analysis was performed also using subgroup based on the number of total diabetic population with at least one RFT. Higgings I^2 test was employed to estimate the variance and heterogeneity amongst the trials. A slight degree of heterogeneity was considered when I^2 was 25–50%, a moderate degree was considered when it was between 50 and 75%, and a high degree was considered when it was >75% [36].

3. Results

3.1. Selection of the Studies

Figure 1 shows the flow diagram of the search strategy, according to PRISMA 2020 instructions. After the initial search, 26 published studies were selected. There were no duplicate studies. After examining the titles and abstracts, 15 of the 26 eligible papers, those that did not investigate RFT, were excluded. Before, the full text of the remaining 11 studies were comprehensively read. Three studies were excluded: one was included because it only included RFT [37], and two others were included because they did not provide data on RFT [38,39]. Finally, eight studies were selected for the systematic review and meta-analysis [11,13–16,21,40,41].

3.2. Characteristics of the Included Studies

Table 1 shows the main features of the included studies [11,13–16,21,40,41]: sample size, age and sex distribution, type of diabetes suffered by the patients, radiographs used and the prevalence of RFT. Seven of them also provided data on the percentage of diabetic people with at least one RFT [11,13–15,21,40,41].

3.3. Outcome of the Primary Meta-Analysis and Publication Bias

The eight studies added a total of 1532 people who had 37,922 teeth in total, of which 2156 were RFT [11,13–16,21,40,41]. Figure 2 shows the forest plot of the primary meta-analysis. The overall calculated prevalence of RFT among diabetic patients was 5.5% (95% CI = 4.1–6.9%; $p < 0.001$). The heterogeneity value was $I^2 = 96\%$.

Another analysis was carried out including the seven studies, providing information about patients with at least one RFT (Figure 3) [11,13–15,21,40,41]. This meta-analysis included a total of 1387 patients, of which 203 had at least one RFT (42.7%; 95% CI = 23.9–61.4%; $p < 0.001$). The heterogeneity value was $I^2 = 98\%$.

3.4. Quality Assessment and Risk of Bias

Quality assessment and risk of bias was evaluated for each study (Table 2). According to the guidelines provided by the Centre for Evidence-Based Medicine in Oxford [33], all the studies were classified as level 4. Four out of eight studies were classified as having a high risk of bias [11,15,21,41], and four of them were classified as having a moderate risk of bias [13,14,16,40]. None of the included studies were classified as having a low risk of bias.

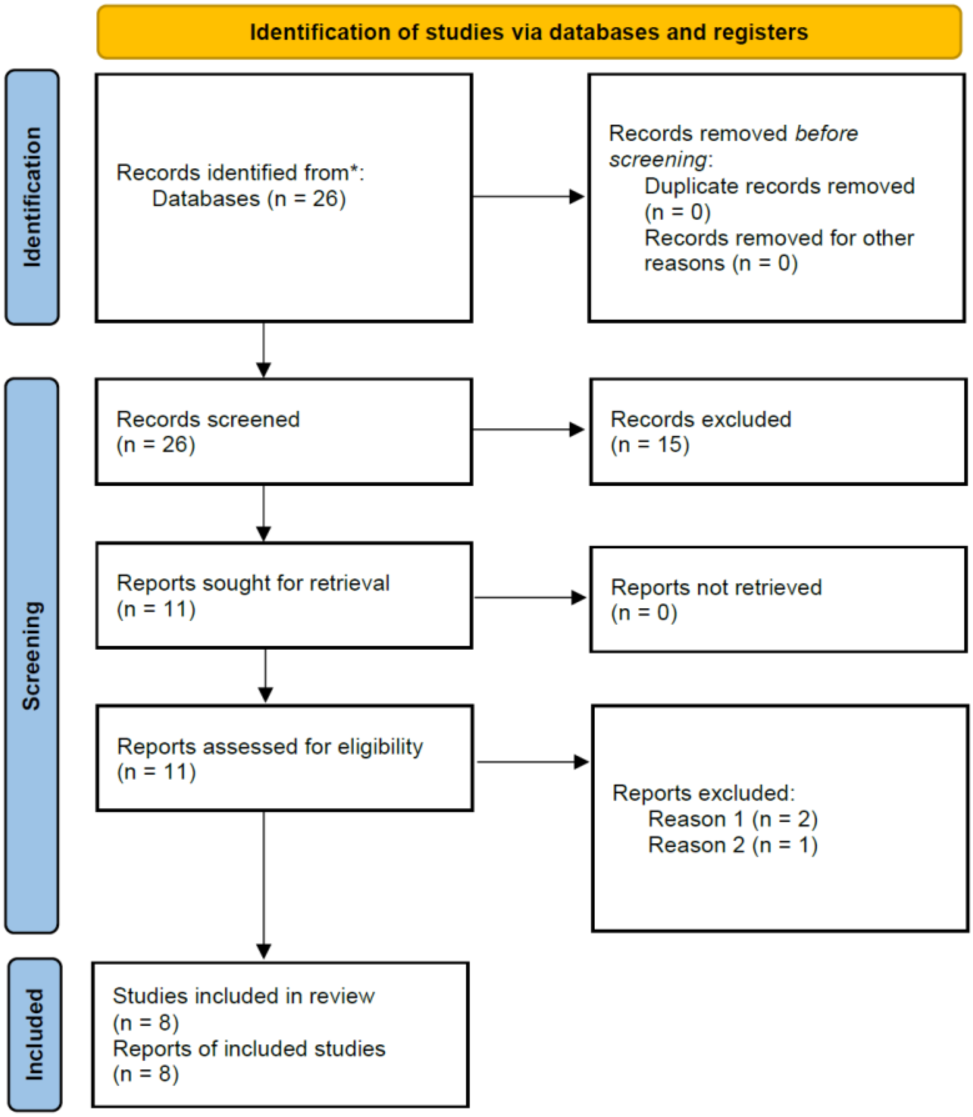

Figure 1. Flow diagram of the search strategy of the systematic review and meta-analysis following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines 2020 [31].

Table 1. Characteristics of the included studies, main outcomes and type of evidence.

Authors	Year	Study Design	Sample Size	No of Teeth	Gender (%)	Age	Radiographs	No. of RFT	Prevalence of RFT (%)	People with at Least One RFT (%)	Type of Evidence [33]
Segura-Egea et al. [21]	2005	Cross-sectional	32 type 2	692	12 men; 20 women	43–74	Periapical (1 observer)	12	2	31	4
López-López et al. [11]	2011	Cross-sectional	50 type 2	1095	20 men; 30 women	44–83	Panoramic	85	7.8	70	4
Marotta et al. [15]	2012	Cross-sectional	30 type 2	652	12 men; 18 women	40–69	Periapical + Panoramic (2 observers)	85	13	76.7	4
Sánchez-Domínguez et al. [14]	2015	Cross-sectional	83 type 2	1751	49 men; 51 women	66.6 ± 10.6	Panoramic (3 observers)	58	3.3	32.5	4
Al-Nazhan et al. [40]	2017	Cross-sectional	926	25,028	540 men; 386 women	>18	Panoramic (2 observers)	1541	6.16	4.6	4
Smadi [16]	2017	Cross-sectional	145 type 2	3111	71 men; 74 women	Not provided	Panoramic (2 observers)	130	4.18	Not provided	4
Pérez-Losada et al. [13]	2020	Cross-sectional	216 type 2	4514	117 men; 99 women	Not provided	Panoramic (3 observers)	173	3.8	12.5	4
Limeira et al. [41]	2020	Cross-sectional	50 type 2	1079	23 men; 27 women	18–45	Panoramic (1 observer)	72	6.7	76	4

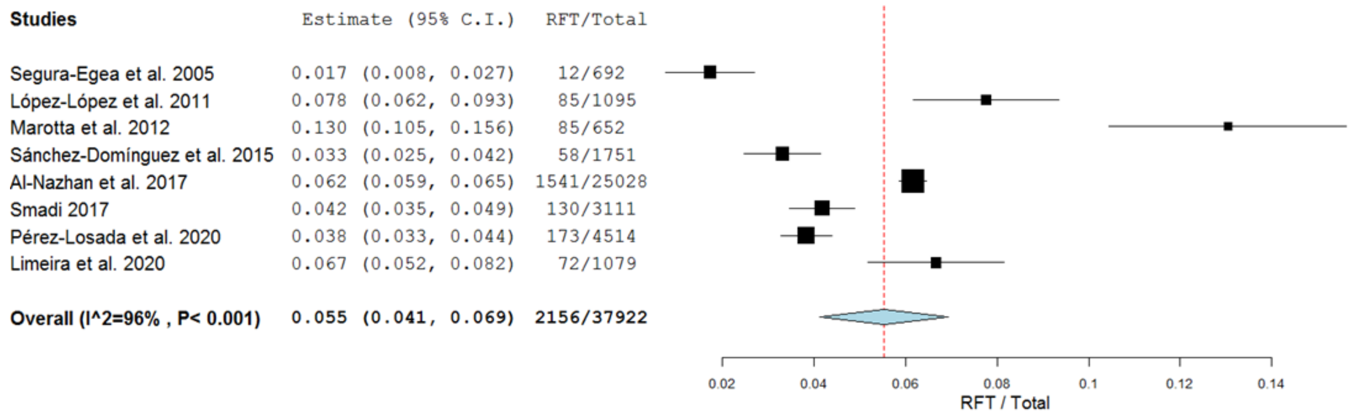

Figure 2. Forest plot of the primary meta-analysis showing the prevalence of RFT in the diabetic adult population [11,13–16,21,40,41].

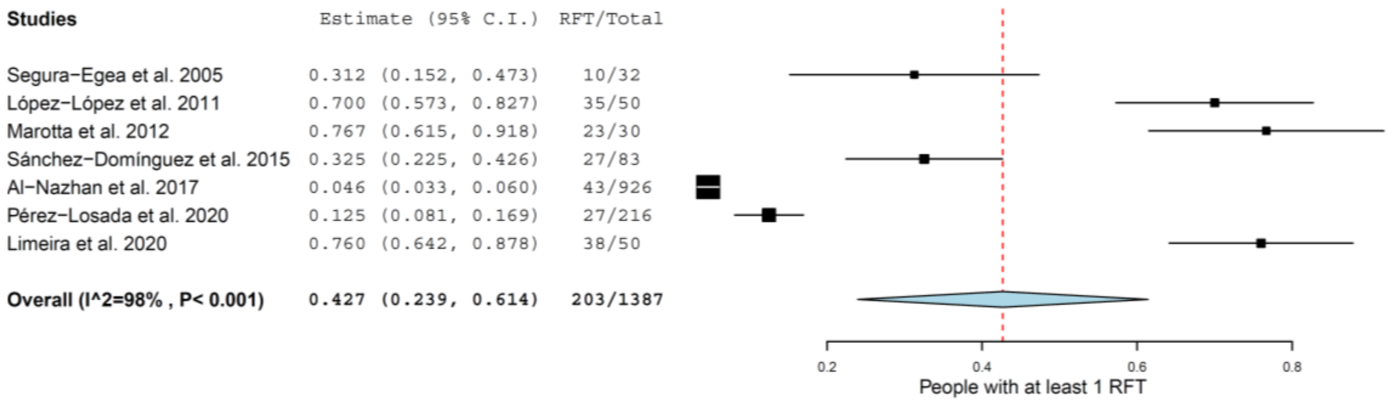

Figure 3. Forest plot of the studies that have calculated the percentage of people with at least one RFT in the total sample [11,13–15,21,40,41].

Table 2. Quality assessment and risk of bias of individual studies according to the criteria previously described [6]: High risk of bias was defined as from 0 to 4 points, a moderate risk of bias was considered for the studies scoring from 5 to 8 points, and finally, a low risk of bias was assigned to studies scoring between 9 and 12 points. Each * is one point.

Authors	Year	Study Design	Selection				Outcome			Risk of Bias
			Representativeness of the Sample	Sample Size Calculation	Non-Respondents	Asse-ssment	Inclusion of Third Molar	Inclusion of Eden-tulous in Sample	No. of Observers	
Segura-Egea et al. [21]	2005	Cross-sectional	*			**	*			High
López-López et al. [11]	2011	Cross-sectional	*			*	*		*	High
Marotta et al. [15]	2012	Cross-sectional	*			**			*	High
Sánchez-Domínguez et al. [14]	2015	Cross-sectional	*			**	*	**	*	Moderate
Al-Nazhan et al. [40]	2017	Cross-sectional	**			*	*	**	*	Moderate
Smadi [16]	2017	Cross-sectional	*		*	**			*	Moderate
Pérez-Losada et al. [13]	2020	Cross-sectional	*			**	*	**	*	Moderate
Limeira et al. [41]	2020	Cross-sectional	*			*	*		*	High

4. Discussion

The aim of this study was to conduct a systematic review to determine the prevalence of RFT among the diabetic adult population. According to the raw data from the primary study, it can be concluded that the prevalence of RFT among the diabetic adult population over 18 years is 5.5%, with 42.7% of diabetic people having one or more RFT. The systematic review and meta-analysis of prevalence and incidence are emergent methodologies in the field of evidence synthesis. The traditionally used PICO strategy does not agree with prevalence studies, so the CoCoPop rule was used [32].

Taking into account the worldwide prevalence of RFT (8.3% of teeth and 55.7% of people) [6], the results of this study show a strikingly lower prevalence of RFT among diabetics compared to that of the general population. Moreover, the prevalence of RFT is an indicator of the frequency of endodontic infections and, at least apparently, these results are not consistent with the higher prevalence of AP among diabetic patients that has been shown [13–15,21,22,38]. On the contrary, an increase in the prevalence of RCT could be expected among the adult diabetic population. However, the explanation for the lower prevalence of RFT among diabetics probably lies in the fact that diabetic patients suffer from post-treatment AP more frequently [24,42], which is possibly consecutive to a delay in the healing of periapical tissues [43,44]. The persistence of AP among diabetics after RCT leads, in some cases, to tooth extraction. In fact, type 2 diabetes is associated with greater loss of RFT [23], and most of the studies included in the present study [11,13–16,21] refer to type 2 diabetics. However, since it is not possible to learn about the quality of RCT or other possible prognostic factors, no definitive conclusions can be drawn in this regard.

Diabetes mellitus includes a group of disorders of the metabolism of carbohydrates, lipids and proteins, the main manifestation of which is hyperglycemia, as a result of a deficiency in insulin secretion, a lack of insulin action, or both [19]. Chronic hyperglycemia is associated with glucotoxicity and the damage and dysfunction of various organs, especially the eyes, kidneys, nerves, heart and blood vessels [19,20]. The results of several studies support a relationship between the prevalence of AP and diabetes [7,11,15,16,21,22,39]. Furthermore, several systematic reviews and meta-analyses have found a significant association between the endodontic treatment outcome and diabetes [23,24,26]. On the other hand, several studies have found a correlation between the higher prevalence of AP and poor glycemic control among diabetic patients [13,14,16]. In short, there seems to be a mutual influence between diabetes and AP [7,45].

The pro-inflammatory state and impaired immune response associated with diabetes may affect the reparative response of the periapical tissue, influencing the two main endodontic variables: the prevalence of AP and the frequency of RCT [46]. Innate immunity is the first line of defense against pathogens. Systemic conditions that alter the functions of innate immunity cells, such as diabetes, decrease neutrophil phagocytosis or macrophage chemotaxis, causing an inflammatory state that alters cell proliferation, delaying lesion healing. Especially in poorly controlled diabetic patients, a stronger systemic inflammatory reaction may be induced, with the activation of NF-κβ in macrophages and increased cellular oxidative stress, which may impair bone turnover and periapical wound healing [47]. These clinical situations are characterized by increased C-reactive protein levels in serum and the release of potentially tissue-destructive substances, such as reactive oxygen species, collagenase, serine proteases and the up-regulation of pro-inflammatory cytokines (IL-1b, IL-6, IL-8, IL-10 and TNF-α) [7]. All these biological changes result in further progression of the periapical inflammation and impaired periapical healing, ending with the loss of the RFT [45].

The outcome of RCT among diabetic subjects and controls has been prospectively investigated [48,49]. The rate of periapical healing was significantly lower in the diabetic group (43%) compared to that in the non-diabetic group (80%) ($p < 0.05$) after a 12-month follow-up [49]. In another study, the clinical and radiographic cure results of a RCT performed in a single visit on patients with type 2 diabetes mellitus with PA were evaluated, concluding that type 2 diabetics had larger chronic lesions compared to those of the control subjects, with there being slower and delayed clinical and radiographic healing among diabetic patients [48].

A recent umbrella review concluded that diabetes is a risk factor for the outcome of RCT [26]. Diabetes can be considered as a key preoperative prognostic factor in endodontic treatment [26,45]. In short, the greater rate of the loss of endodontically treated teeth could explain the low prevalence of RCT among diabetic patients compared with that of the general population [6].

On the other hand, a possible explanation for the lower prevalence of RFT among diabetics is also periodontal disease. Diabetic patients have a high prevalence of periodontal disease [28]. Diabetes and periodontal disease are closely linked and amplify one another, if they are not successfully controlled [50]. Considering that periodontal disease is also a leading cause of tooth loss [51], the low prevalence of RFT among diabetics could also be explained by the loss of endodontically treated teeth caused by periodontal disease. The combined effect of diabetes itself and periodontal disease can reasonably explain the low prevalence of RFT among diabetics.

Finally, another possible explanation that should also be taken into account is the increase in the number of dental implant treatments that has occurred in the last three decades [52]. Dentists and diabetic patients might prefer the extraction of teeth affected by endodontic infections and their replacement by dental implants, instead of performing RCT.

Regarding the articles included in the systematic review, the initial database search provided twenty-six articles. When the inclusion criteria were applied, it resulted in a systematic review of eight studies published in the first quarter of the 21st century. All the included studies were cross-sectional studies investigating both the prevalence of AP and RCT among diabetic patients. Most of the studies [11,13,14,16,40,41] used panoramic radiographs to detect RFT, another [21] used periapical radiographs and another one [15] used both periapical and panoramic radiographs. Although it might be thought that the detection of RFT can be performed with the same precision with panoramic and periapical radiographs, in previous studies, the prevalence of RFT has been found to be higher with the use of periapical radiographs [6].

The results of this study should be translated to the clinical practice [53]. As we have collected data on poor healing and the tendency to tooth extraction among diabetic patients, it is important to bear in mind that the prognosis of RCT can be poor among diabetic patients. However, this should not be an excuse for not focusing all the attention on making a good quality RCT. In addition, this can help dentists suspect undiagnosed diabetic patients when they recognize numerous failures of RCT. If in doubt, the patient should be referred for blood tests and additional tests to rule out diabetes.

Some limitations of this systematic review should be noted. One important limitation is the low numbers of included studies and patients. The reason lies in the fact that a few studies followed a strict protocol for the selection of the individuals included in the sample. This is also the reason why none of the studies included in this systematic review and meta-analysis had a low risk of bias. Thus, the results of this systematic review must be carefully assessed taking into account the quality of the included studies. Four of the included studies were classified as having a high risk of bias [11,15,21,41], while the other four studies were classified as having a moderate risk of bias [13,14,16,40]. None of the included studies calculated the sample size, which is necessary to ensure a correct sample size to justify the study results. Moreover, more than a half of the studies did not mention if edentulous patients were included in the sample, which alters the results of meta-analyses [11,15,16,21,41]. Only one [40] of the included studies had a reasonable representativeness of the sample, but none of them used the random sampling method. Given the very low proportion of RCT performed on third molars, whether or not the third molar was included in the study does not represent a major limitation. So, a low risk of bias was considered if the third molar was not included in the total patient sample. Similarly, if edentulous patients were not included in the total patient sample, a low risk of bias was also considered. Nevertheless, when the study did not specify whether it included edentulous patients in the total sample, it was considered to having a very high risk of bias. Lastly, the total number of diabetic patients included in this review, almost 1500, is too low to reach a strong conclusion. Moreover, the heterogeneity of the studies was greater than 95%, which indicates that the differences in the design, samples and characteristics of the population are high, and this can lead to very different results, compromising the results of the meta-analysis.

5. Conclusions

This systematic review and meta-analysis concluded that the prevalence of RFT among diabetic patients is 5.5%. More than 40% of diabetics have at least one RFT. In daily clinics, dentists should suspect that patients are an undiagnosed diabetics when multiple RCT failures are observed in the same patient. A blood test that assesses blood glucose can help to rule out the presence of diabetes.

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Article

Effect of Traditional and Conservative Endodontic Access Cavities on Instrumentation Efficacy of Two Different Ni–Ti Systems: A Micro-CT Study

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Abstract: This study aims to compare the shaping efficiency of the nickel–titanium instrument systems, One Reci and ProTaper Ultimate, using micro-CT (micro-computed tomography) in traditional and conservative endodontic access cavities. The experimental groups were formed according to the type of access cavity and Ni–Ti file system to be used. Sixty mandibular molar teeth were randomly divided into two main groups, the conservative access cavity (CAC) group and the traditional access cavity (TAC) group, and randomly divided into two subgroups according to the file system. The groups were compared with a two-way ANOVA analysis in terms of volume change, surface area, non-instrumented area transportation, and thickness of the dentin in the danger zone area after root canal preparation. The groups showed no statistically significant differences in terms of volume change, surface area, or the thickness of the dentin in the danger zone area after root canal preparation ($p > 0.05$). However, in the percentage of non-instrumented areas post-instrumentation between groups, the percentage of non-instrumented areas was statistically higher in specimens with CAC compared to TAC ($p < 0.05$). Canal transportation was higher in CAC at all distances from the apical region ($p < 0.05$). Within the limitations of this study, CAC can also be used with some precautions as an alternative to TAC.

Keywords: conservative access cavity; One Reci; ProTaper Ultimate; micro-CT; minimally invasive endodontics

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1. Introduction

Creating an access cavity to reach the complex root canal anatomy is the first important step in non-surgical endodontic procedures. Obtaining information about the complex composition of the root canal system is imperative to comply with the principles to be followed during traditional and conservative access cavity preparation and to find solutions to the problems encountered [1]. The main goal of endodontic procedures is to adequately shape, clean, and obturate the root canal system to prevent apical periodontitis [2].

Recently, different designs of minimally invasive root canal access cavities have been proposed to remove as little tissue as possible to achieve the goal of root canal treatment. The goal of this concept is to increase the resistance of the tooth to cracks and fractures after root canal treatment by maximally preserving the healthy structure of the tooth during the preparation of the access cavity [3]. However, the smaller the access cavity, the more difficult the visibility and disinfection of the root canal system become, and the more difficult it can be to find, shape, clean, and obturate the root canals. At the same time, poor visibility because of the small access cavity may increase the risk of iatrogenic complications, which may affect treatment outcomes [4].

Lately, technological developments have brought innovations in the field of endodontics, such as new-generation rotary systems, magnification and lighting systems, and cone beam computed tomography (CBCT), that provide clinicians convenience during treatment. Using these technologies allows endodontic procedures to preserve healthy dental tissues more and prevent the loss of teeth [5].

Traditional access cavity (TAC) preparation aims to provide direct access to the root canal system. Direct access helps to increase biomechanical efficiency and prohibits errors that may occur in various treatment stages [6]. In contrast, dental tissue removed during TAC preparation can reduce the resistance of teeth to fracture under occlusal forces [7,8]. Current developments recommend choosing the access cavity design that preserves more dentin and brings the endodontic-restorative relationship to an ideal state in terms of the preservation of tooth structure [9].

One of the most common causes of root canal treatment failure is reported to be the significant loss of dental tissue due to untreated caries and fractures of endodontically treated teeth [10]; this is also directly related to the amount of tissue lost and specific cavity configurations [11].

Conservative access cavity (CAC) preparation requires a dynamic approach that follows a conservative dental tissue removal procedure from the beginning of the procedure, which is expanded until adequate vision is achieved, rather than a predetermined access cavity form. This approach involves partial roof removal of the pulp chamber to protect the pulp horns, accompanied by cavity walls that are slightly concave and beveled towards the occlusal surface. This design aims to visualize the floor of the pulp chamber and all root canal openings from different angles [12], which means that clinicians can visualize the pulp chamber and floor by changing the angle of the mirror, though not at the same angle [13].

The minimally invasive endodontic concept, in addition to the differences in the access cavity, uses endodontic files with smaller tapers and innovative designs that aim to minimize the removal of tooth tissue [14].

Two different Ni–Ti instrument systems were used in this study. The first is One Reci (OR) (MicroMega, Besançon, France). This file is a recently released single-file system that works with a reciprocating motion. OR files undergo a heat treatment (C-wire) aimed at increasing the instrument's flexibility and ability of the file to stay at the center of the canal. Its variable off-center section, which begins as a triple helix and gradually turns into an S-shape towards the body, together with a deep groove, provides more space for debris removal in the coronal direction, giving it good cutting efficiency. This system completes the cutting action by rotating 170° counterclockwise and 60° clockwise [15]. The files of this system consist of 20.04, 25.04, 25.06, 35.04 and 45.04. The other file system used in this study is ProTaper Ultimate (PU) (Dentsply Sirona, 2021). The ProTaper rotary file system was introduced in 2001, evolved into ProTaper Universal (PTU) in 2006, and then progressed to ProTaper Gold (PTG) in 2014. The ProTaper system is known for its ability to precisely prepare both anatomically complex and simple canals while offering a simple workflow. PU by Dentsply Sirona is a more advanced version of PTU and PTG and has a similar working principle as previous ProTaper generations. The manufacturer claims that PU offers additional advantages by showing increased elasticity and greater resistance to cyclic fatigue. According to the manufacturer's instructions, the files of this system should be used at a constant speed of 400 rpm and with torque values of 4–5.2 Ncm. In this system, SX(020.003v), S1(016.002v), S2(020.004v), F1(020.007v), F2(025.008v), F3(030.009v), FX(035.012v), FXL(050.010v) files are available. SX, FX, and FXL files are auxiliary files in this system and were not used in this study [16].

This study aims to compare the shaping efficiency of the One Reci and ProTaper Ultimate Ni–Ti instrument systems using micro-CT in traditional and conservative endodontic access cavities approached in extracted mandibular molar teeth. In this respect, the study is novel due to the comparison of two different endodontic access cavities with two different Ni–Ti systems.

2. Materials and Methods

2.1. Sample Size Determination and Power Calculation

After ethics committee approval (protocol no. 18/02), the sample size was estimated based on studies that compared TACs and CACs [17,18]. Fixed effects one-way ANOVA was selected from the F tests family and ran in G*Power 3.1 software (Henrick Heine-Universität, Düsseldorf, Germany). Accordingly, for an analysis with α = 0.05 and 80% power, at least 10 teeth were allocated for each of the experimental groups as the ideal size required to observe significant differences. A total of 60 extracted mandibular molar teeth were selected for the experiments, and 15 samples/groups were used to compensate for possible sample loss.

2.2. Sample Selection

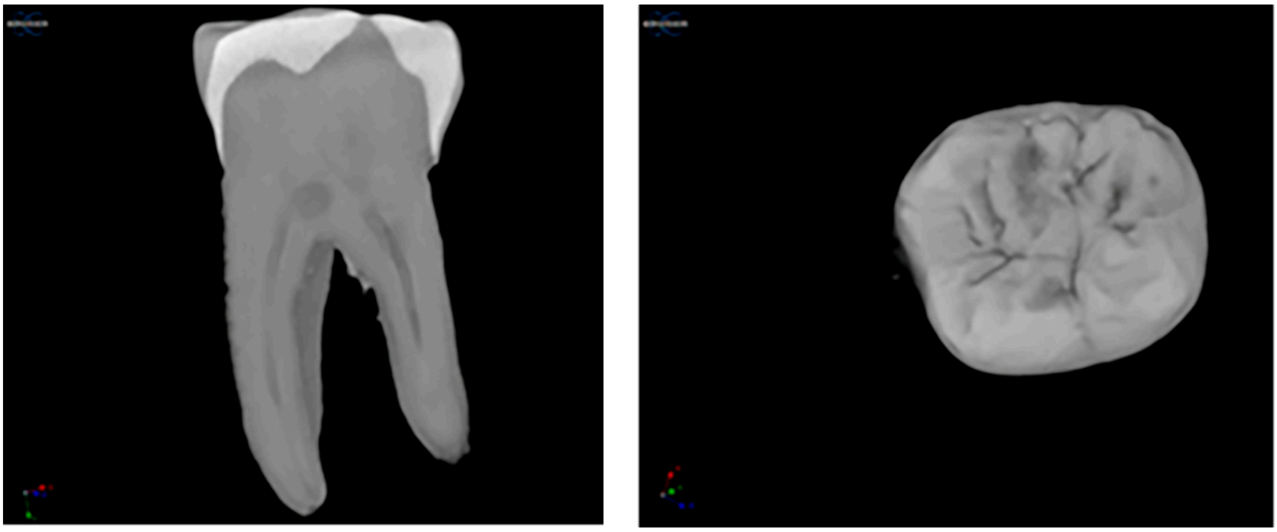
Sample selection was carried out with a dental operation microscope (Leica M320, Wetzlar, Germany) under 10× magnification. The first and second mandibular molar teeth were included in this study. The teeth were extracted due to periodontal diseases. Teeth with fractures, cracks, and caries on the root surface in the examination were excluded from the study. Additionally, teeth with root canal curvatures of more than 35° according to the Schilder technique were not included in the study. To ensure standardization in all samples, the root canal curvature was selected to be less than 35°. After the samples were kept in 2.5% NaOCl for 24 h, hard and soft tissue residues on the root surfaces were removed with the help of a periodontal curette. The teeth were numbered according to the study groups and stored at 4 °C and in 0.1% thymol solution until the experiment.

2.3. Micro-CT Scans

To conduct the first micro-CT scan before access cavity and root canal preparation, the samples were fixed on the turntable of the micro-CT device and scanned with the X-ray source of the device at 80 kVp, 125 mA, 26.7 µm pixel size, and 0.2 rotation. The images obtained after the scan were transferred to the NRecon software (ver. 1.6.10.4, Bruker X-ray, Kontich, Belgium). A total of 1800 two-dimensional sections were obtained from each sample. For the reconstruction, the ring artifact correction was fixed to 7, and the smoothing parameter was set to 3. The beam hardening artifact correction was set to 38%. Contrast settings were kept between 0 and 0.05 for all the samples. Examples of images obtained from micro-CT scanning before cavity and root canal preparation are given in Figure 1.

Figure 1 shows the results of the first micro-CT scan. In this scan, teeth were examined regarding fractures, cracks, caries, and radii of curvature to avoid missing possible errors in the dental operating microscope. Samples from micro-CT scanning were placed in silicone molds (Express XT, 3M ESPE, Neuss, Germany) with root tips visible to stabilize and isolate them from the environment during access cavity preparation. The experimental groups were formed according to the type of access cavity and the Ni–Ti file system to be used, as shown in Figure 2.

Figure 2 shows the four groups and the study design. Two different micro-CT scans were carried out for the control and analysis of all groups. The samples used in the study were randomly assigned to four main groups (n = 15). Of the randomly selected teeth, the groups were as follows: Group 1 included traditional access cavity and root canal preparation using One Reci(TAC-OR) (n = 15); Group 2 included traditional access cavity and root canal preparation using ProTaper Ultimate (TAC-PU) (n = 15); group 3 included conservative access cavity and root canal preparation using One Reci (CAC-OR) (n = 15); and Group 4 included conservative access cavity and root canal preparation using ProTaper Ultimate (CAC-PU) (n = 15). All specimens were removed from the silicone molds after cavity and root canal preparations and placed in the holder platform of the micro-CT. The second scans were repeated with the same parameters used in the first scans. Figure 3 shows examples of images obtained after the second scan.



(a) (b)

Figure 1. Micro-CT images of the lower molar tooth before cavity and canal preparation. (**a**) A sagittal section. (**b**) An axial section.

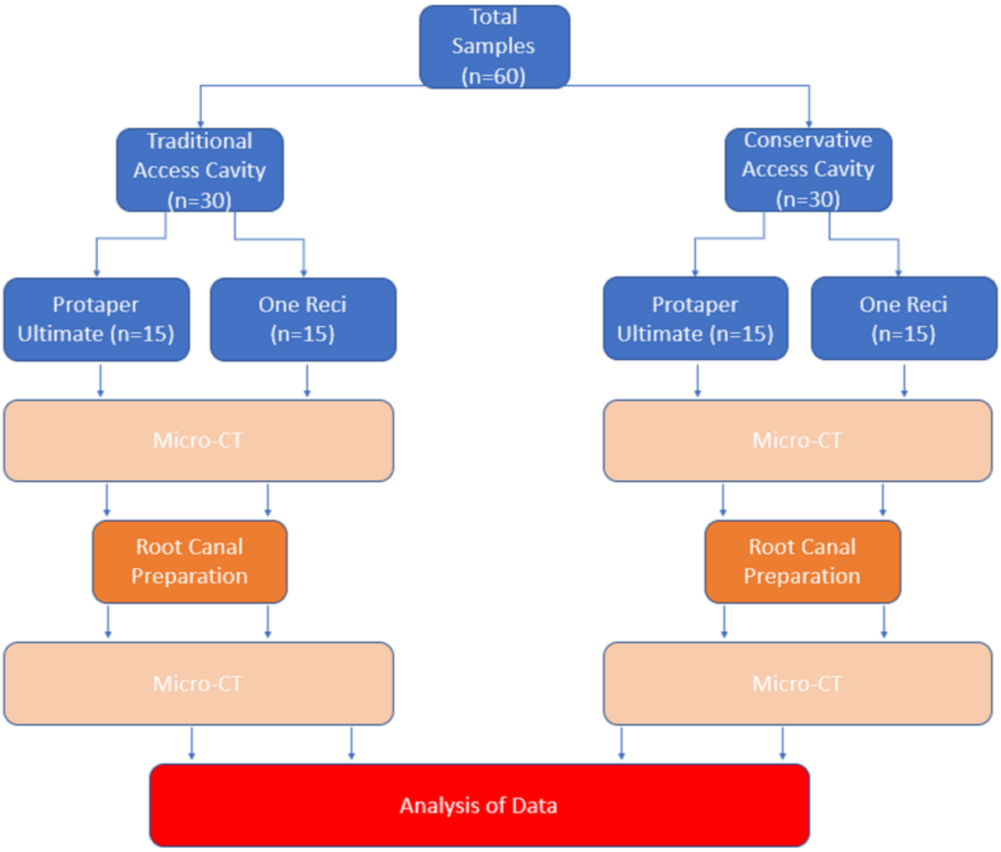


Figure 2. Flowchart of the study groups and procedures.

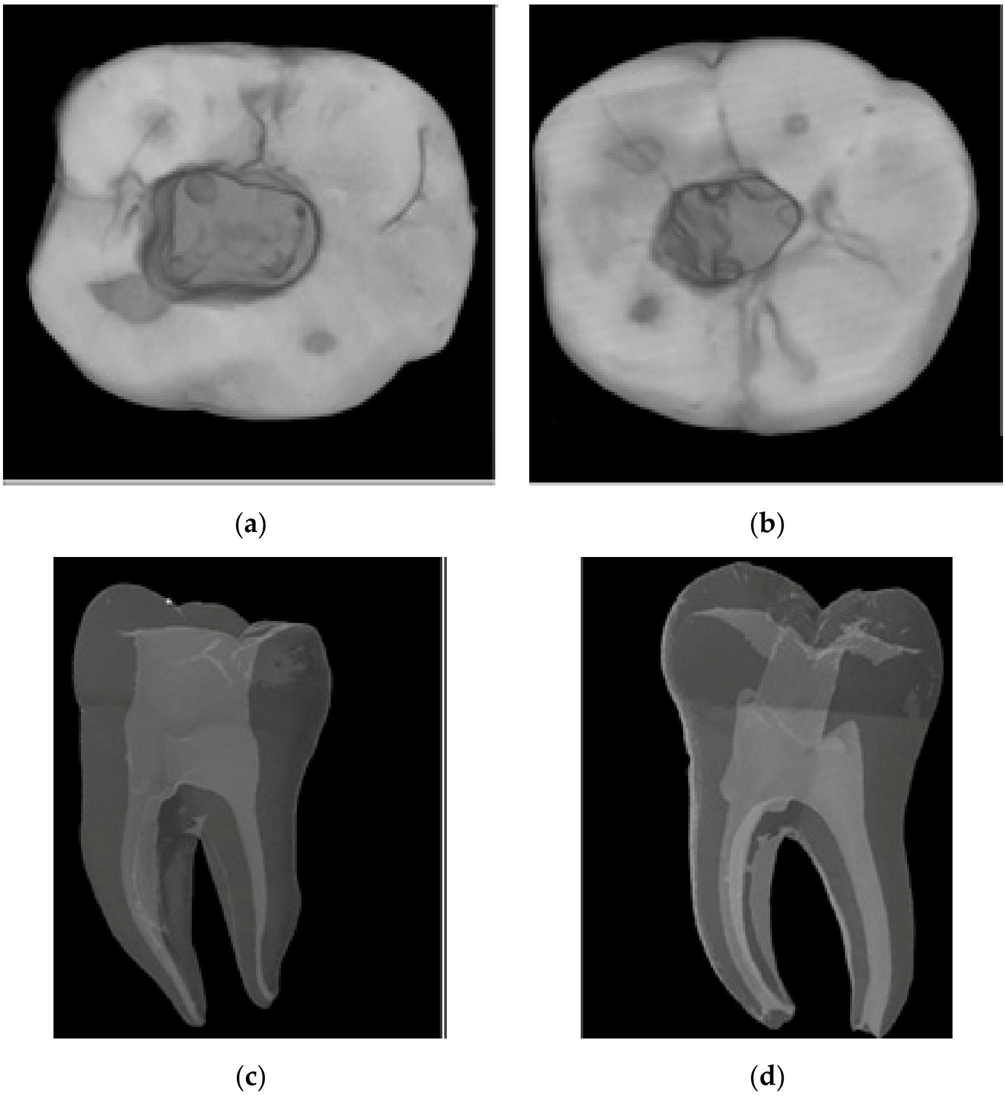


Figure 3. Micro-CT images illustrate the differences between traditional and conservative access cavities. (**a**) Axial section of a traditional access cavity. (**b**) Axial section of a conservative access cavity. (**c**) Sagittal section of the conservative approach. (**d**) Sagittal section of the traditional approach.

The images obtained after micro-CT scanning of traditional and conservative access cavities are shown in Figure 3. Figure 3 shows the differences between the access cavities of the two different methods.

2.4. Specimen Preparation

Access cavity preparations were made with a dental operating microscope (Leica M320, Wetzlar, Germany) under 6.4× magnification. Following the principles for traditional access cavities [19], the specimens in Group 1 and Group 2 were accessed from the central fossa with a number 14 diamond round bur. The cavity walls were then paralleled with a number 12 diamond fissure bar to allow direct access of files to the coronal third of the canal.

Conservative access cavity preparation was performed following the principles previously described in the studies of Clark and Khademi [13]. Cavity preparation of the specimens in Group 3 and Group 4 was started slightly mesial to the central fossa using the number 12 diamond round bur. The cavity walls were made slightly parallel with the diamond fissure burr numbered 11. Organic tissue residues and calcifications in the pulp chamber were removed with the help of ultrasonic tips.

After the cavity preparations of all specimens, to determine the working length in the mesio-buccal and mesio-lingual canals, a #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was inserted and advanced until it could be visible through the apical foramen. By measuring this length and subtracting 1 mm from that, the working length was determined for each canal and recorded. Then, the canal preparation process was carried out using an endodontic motor (Changzhou Eighteenth Medical Technology Co., Ltd., Changzhou, China). According to the determined working lengths of the samples belonging to Group 1 and Group 3, a glide path was created with a One G file (MicroMega, Besançon, France) using 300 rpm speed and 1.2 Ncm torque value. Each canal was then rinsed using 2 mL of 2.5% NaOCl. After this step, the preparation was made according to the manufacturer's instructions using the OR 25/06 file up to the working length with 170° counterclockwise and 60° clockwise motion.

In Group 2 and Group 4, the samples were prepared using PU according to the manufacturer's instructions with S1 and S2 files and F1 and F2 files, respectively. During the preparation procedures, the root canals were rinsed with 2 mL of 2.5% NaOCl at each file. Final irrigation was carried out with 5 mL of distilled water. All these procedures were performed by a single operator.

2.5. Image Processing

Images before and after instrumentation were repositioned in all three planes of space using the DataViewer software (ver. 1.5.6.2; Bruker X-ray, Kontich, Belgium). These images were imported into CTAn software (ver. 1.20.3.0; Bruker X-ray, Kontich, Belgium) and DataViewer to calculate canal volume, surface area, non-instrumented area, transportation, STL model preparation, and the thickness of the dentin in the danger zone.

Changes in volume and surface area were determined by subtracting the values before and after instrumentation using the CTAn software. The software was used to determine the region of interest (ROI). Furthermore, by adjusting the values of brightness and opacity, it is possible to remove "unwanted" voxels before calculating the final volume of the pulp and the canal cavity. Therefore, the software allows to obtain the desired volume, and the changes in volume and surface area can be compared between pre- and post-instrumentation [20]. CTVol software (ver. 2.3.2.0; Bruker X-ray, Kontich, Belgium) was used for the visualization of the STL models. The STL models were obtained with CTAn software by selecting the ROIs that include the root canals and adjusting the threshold to select the air in the canal for the preparation of the models.

The percentage of the non-instrumented area was calculated according to the study by Arias et al. [20] calculated using CTAn software as the percentage of the number of static voxel surfaces to the total number of surface voxels from the fitted 3D model before and after root canal preparation.

Canal transportation, the shortest distances from the orifice to the root tip before and after instrumentation, were measured in the mesial and distal directions using the DataViewer software. Then, using these data, the amount of transportation was obtained for each canal with the formula

$$\text{The amount of transportation} = (m1 - m2) - (d1 - d2), \qquad (1)$$

This formula was previously used in the study of Rover et al. [18]. In this formula, m1 represents the shortest distance from the mesial border of the root to the non-instrumented mesial margin, m2 represents the shortest distance from the mesial border of the root to the mesial margin of the instrumented canal, d1 represents the shortest distance from the distal border of the root to the non-instrumented distal margin, and d2 represents the shortest distance from the distal border of the root to the distal margin of the instrumented canal.

A value for canal transportation equal to 0 means no transportation. If the transportation value is negative, it indicates transportation in the distal direction, and if it is positive, it indicates mesial transportation. In Figure 4, images from the DataViewer software, where the measurement of the transportation amount is made, are displayed.

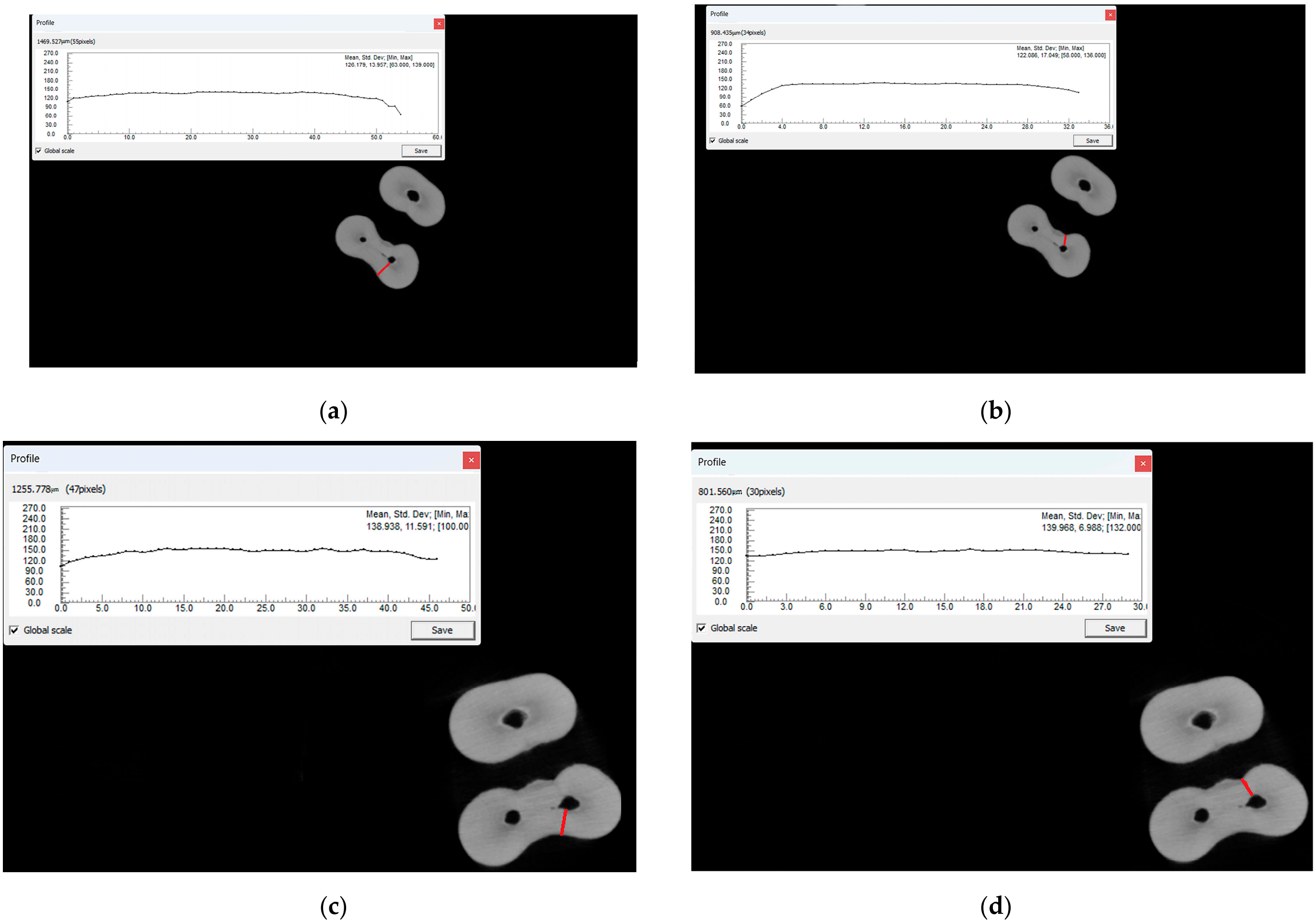


Figure 4. Determination of transportation with Equation (1) in DataViewer software. (**a**) m1, (**b**) d1, (**c**) m2, (**d**) d2. Insets show the distances in micrometers.

Figure 4 shows the measurement of the transportation. The transportation was measured using the Dataviewer software. In Figure 4, red lines show the shortest distances between borders and margins.

To measure the thickness of dentin in the danger zone area after canal preparation, sections were created from the images obtained from DataViewer software. In these slices, the thickness of the canal wall was measured in the axial plane. To assess changes in dentin thickness, the shortest distance from the instrumented and non-instrumented canal inner wall to the corresponding outer wall of the canal was measured. Radii of curvature were measured for all the samples, and the mean value for this measurement was 6.52 ± 1.49 mm using the method from Schafer et al.'s study [21]. All images were evaluated and measured by a single observer with 12 years of Micro-CT experience, and all measurements were made twice by the same observer.

2.6. Statistical Analysis

Statistical analyses were performed using SPSS software (ver. 20.0.1; SPSS Inc., Chicago, IL, USA). The normality of the data distribution was obtained by the Kolmogorov–Smirnov test, and its homogeneity was obtained by the Levene test. The data showed a normal distribution. Two-way analysis of ANOVA was used to statistically compare the means of the changes in the canal volume and surface area of the groups, as well as values for the danger zone, transportation, and non-instrumented area. Post hoc analyses were performed with Tukey and Bonferroni tests. In all analyses, $p \leq 0.05$ was taken to indicate statistical significance.

3. Results

There was no statistically significant difference between the groups in terms of volume change after root canal preparation in the mesio-buccal and mesio-lingual canals ($p > 0.05$). The use of OR or PU did not affect volume change ($p > 0.05$). Table 1 shows the values for volume change, surface area change, non-instrumented area change and danger zone change after canal preparation of the mesio-buccal and mesio-lingual canals.

Table 1. The mean ± standard deviation values of volume change, surface area, non-instrumented area, and the thickness of dentin in the danger zone area.

Parameters	Groups					
	TAC-OR (*n* = 15)	**CAC-OR (*n* = 15)**	***p* Values**	**TAC-PU (*n* = 15)**	**CAC-PU (*n* = 15)**	***p*-Values**
Δ Volume Change (mm^3)	MB 4.16 ± 0.28	MB 4.48 ± 0.27	$p = 0.38$	MB 4.06 ± 0.44	MB 4.28 ± 0.66	$p = 0.74$
	ML 4.41 ± 0.52	ML 4.33 ± 0.33	$p = 0.87$	ML 4.87 ± 0.48	ML 4.04 ± 0.42	$p = 0.06$
Δ Surface Area (mm^2)	MB 6.93 ± 0.31	MB 6.40 ± 0.4	$p = 0.21$	MB 7.03 ± 0.25	MB 6.80 ± 0.41	$p = 0.37$
	ML 5.79 ± 0.33	ML 5.89 ± 0.27	$p = 0.75$	ML 6.01 ± 0.31	ML 5.83 ± 0.25	$p = 0.59$
Non-Instrumented Area (%)	MB 11.82 ± 0.25	MB 16.08 ± 0.46	$p < 0.001$	MB 11.76 ± 0.17	MB 15.41 ± 0.31	$p < 0.001$
	ML 11.69 ± 0.15	ML 16.76 ± 0.14	$p < 0.001$	ML 11.44 ± 0.18	ML 15.55 ± 0.08	$p < 0.001$
Δ Danger zone (mm)	MB 0.30 ± 0.05	MB 0.29 ± 0.05	$p = 0.91$	MB 0.25 ± 0.07	MB 0.23 ± 0.06	$p = 0.67$
	ML 0.26 ± 0.04	ML 0.24 ± 0.08	$p = 0.83$	ML 0.28 ± 0.05	ML 0.22 ± 0.04	$p = 0.17$

TAC-OR: traditional access cavity—One Reci, TAC-PU: traditional access cavity—ProTaper Ultimate, CAC-OR: conservative access cavity—One-Reci, CAC-PU: conservative access cavity—ProTaper Ultimate, MB: mesio-buccal, ML: mesio-lingual.

There was no statistical difference between the groups in terms of changes in the surface area of the mesio-buccal and mesio-lingual canals after root canal preparation ($p > 0.05$). The use of OR or PU did not affect the change in surface area ($p > 0.05$). Table 1 shows the values of the surface area change after root canal preparation of the mesio-buccal and mesio-lingual canals.

The non-instrumented area in the TAC-OR group was 11.82 ± 0.25% in the mesio-buccal canal and 11.69 ± 0.15% in the mesio-lingual canal, respectively, and in the TAC-PU group, values of 11.76 ± 0.17% in the mesio-buccal canal and 11.44 ± 0.18% in the mesio-lingual canal were found. In the CAC-OR group, the non-instrumented area in the mesio-buccal canal was 16.08 ± 0.46%, and in the mesio-lingual canal, this value was 16.76 ± 0.14%; in the CAC-PU group, the non-instrumented area in the mesio-buccal canal was 15.41 ± 0.31%, and this value in the mesio-lingual canal was 15.55 ± 0.08%. The percentage of non-instrumented areas post-instrumentation in the mesio-buccal and mesio-lingual canals between the groups and the percentage of non-instrumented areas was statistically higher in specimens with a CAC compared to TAC groups ($p < 0.05$). The use of OR or PU did not affect the percentage of the non-instrumented area ($p > 0.05$). Table 1 shows the percentage values of the non-instrumented areas of the mesio-buccal and mesio-lingual canals post-instrumentation.

There was no statistical difference between the groups in terms of the thickness of the dentin in the danger zone after root canal preparation in the mesio-buccal and mesio-lingual canals ($p > 0.05$). The use of OR or PU did not affect the thickness of the dentin in the danger zone ($p > 0.05$). Table 1 shows the numerical values of the thickness of dentin in the danger zone after root canal preparation of the mesio-buccal and mesio-lingual canals.

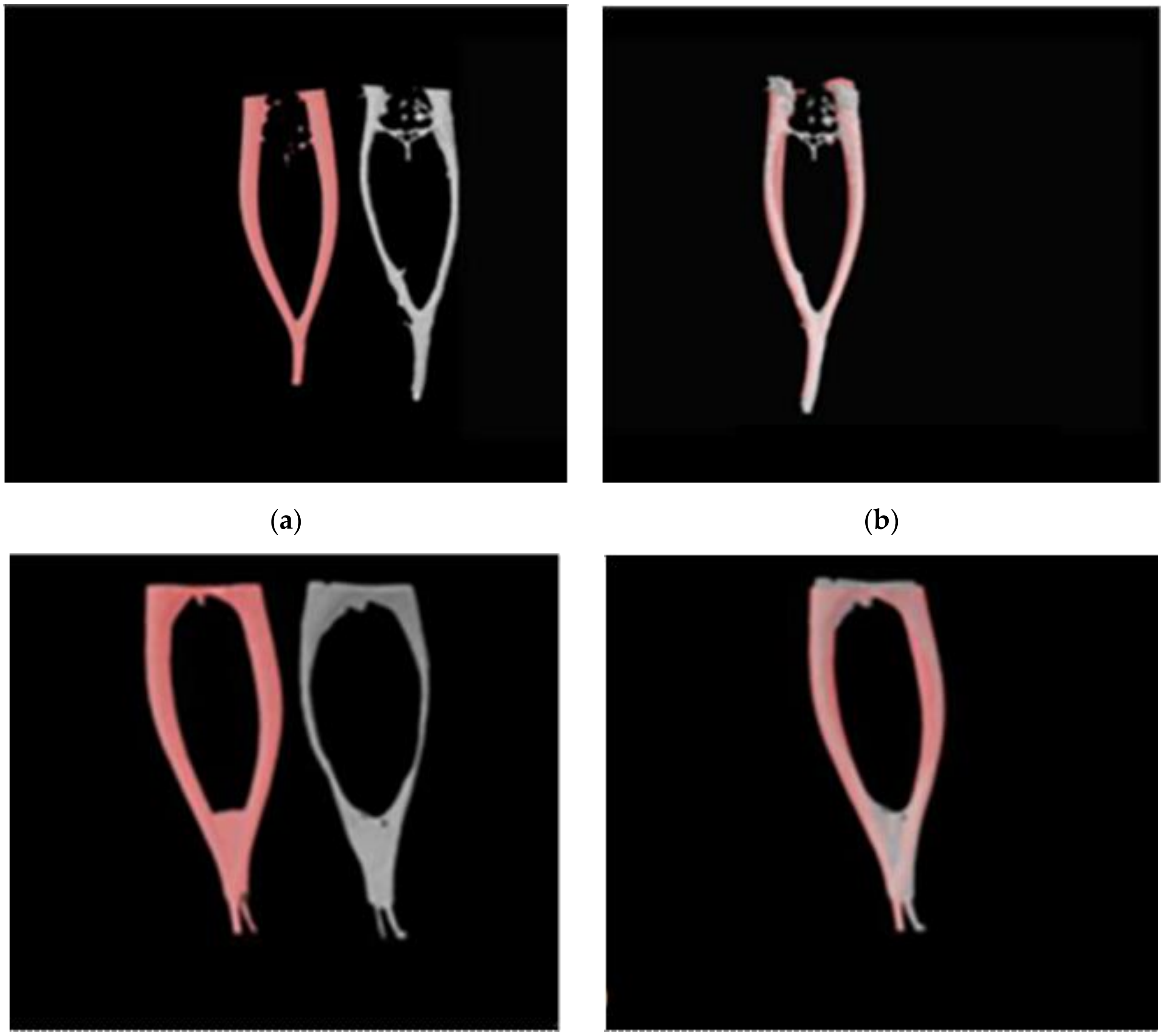
In Figure 5, the STL images obtained by micro-CT pre- and post-instrumentation were presented to all groups in the study, as well as the images obtained by superimposing these images.

Regardless of the file system used, more canal transportation was observed in the CAC groups at a distance of 2 mm, 5 mm, and 8 mm from the apical region ($p < 0.05$). The use of OR or PU did not affect canal transportation ($p > 0.05$). In Table 2, the mean transportation values of the mesio-buccal canals at a distance of 2 mm, 5 mm, and 8 mm from the apical region are given.

Table 2 shows statistically significant differences between the CAC and TAC groups without the effect of the PU and OR subgroups in terms of transportation in all measured distances from the apical region in the mesiobuccal canals.

In Table 3, the mean transportation values at distances of 2 mm, 5 mm, and 8 mm from the apical region of the mesio-lingual canals are given.

Table 3 shows statistically significant differences between the CAC and TAC groups without the effect of the PU and OR subgroups in terms of transportation in all measured distances from the apical region in the mesiolingual canals.



(**a**) (**b**)

(**c**) (**d**)

Figure 5. *Cont.*

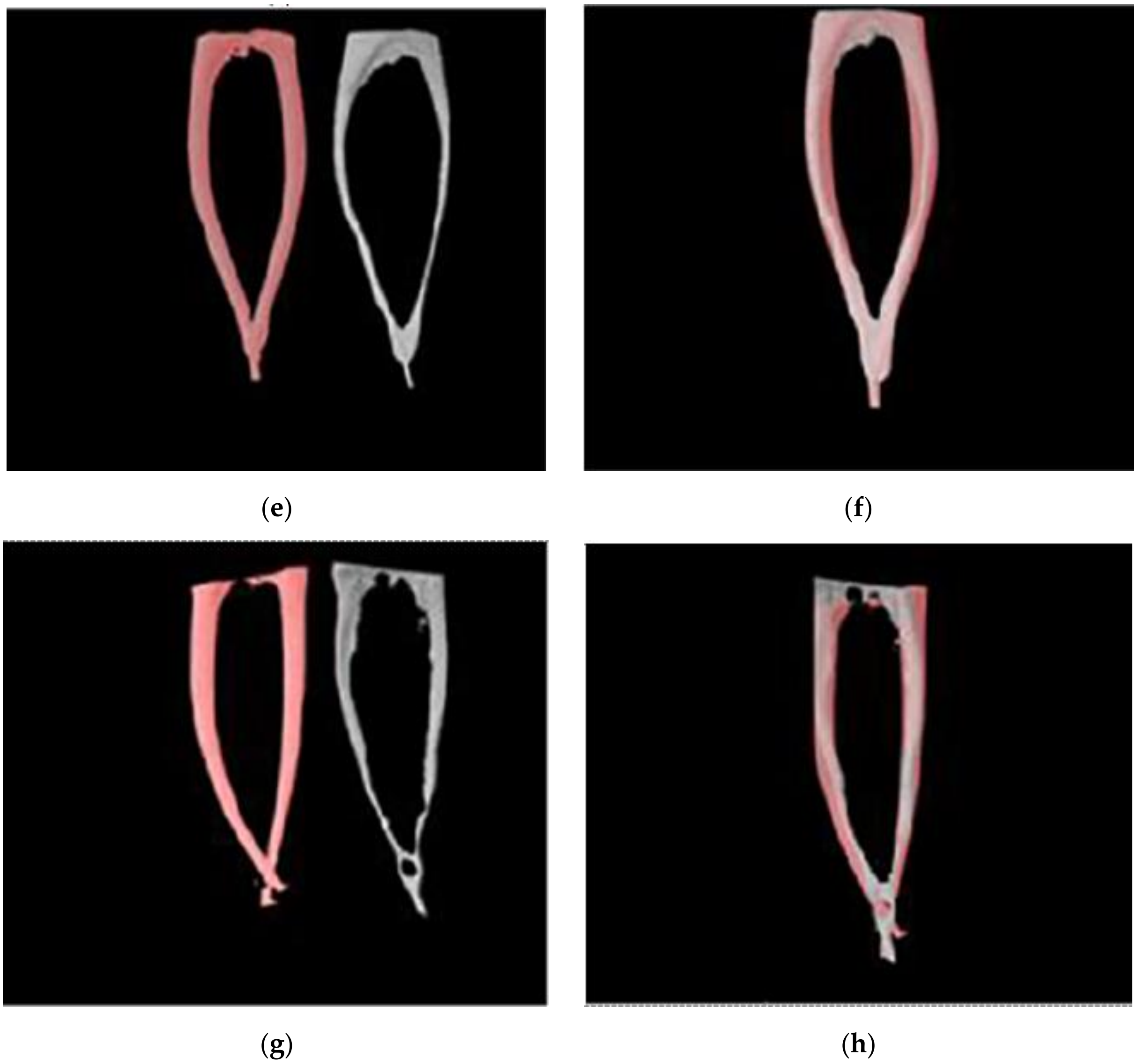


Figure 5. STL images of the micro-CT scans, before (gray) and after canal preparation (pink) and with superimposed STLs. (**a**) TAC-OR; (**b**) superimposed on TAC-OR; (**c**) TAC-PU; (**d**) superimposed on TAC-PU; (**e**) CAC-OR, (**f**) superimposed on CAC-OR, (**g**) CAC-PU, (**h**) superimposed on CAC-PU.

Table 2. The mean transportation values of the groups in the mesiobuccal canals are shown at a distance of 2 mm, 5 mm, and 8 mm from the apical area.

	2 mm	**5 mm**	**8 mm**
TAC-OR	0.100 ± 0.001 **	0.134 ± 0.002 *	0.156 ± 0.004 *
TAC-PU	0.099 ± 0.002 **	0.132 ± 0.002 *	0.154 ± 0.004 *
CAC-OR	0.121 ± 0.001 **	0.144 ± 0.003 *	0.172 ± 0.004 *
CAC-PU	0.123 ± 0.001 **	0.146 ± 0.003 *	0.175 ± 0.001 *

TAC-OR: traditional access cavity—One Reci; TAC-PU: traditional access cavity—ProTaper Ultimate; CAC-OR: conservative access cavity—One Reci; CAC-PU: conservative access cavity—ProTaper Ultimate. * Indicates a statistically significant difference ($p < 0.05$), ** Indicates a statistically significant difference ($p < 0.01$).

Table 3. The mean transportation values of the groups in the mesiolingual canals are shown at a distance of 2 mm, 5 mm, and 8 mm from the apical area.

	2 mm	5 mm	8 mm
TAC-OR	0.101 ± 0.002 **	0.128 ± 0.002 *	0.162 ± 0.004 *
TAC-PU	0.103 ± 0.001 **	0.131 ± 0.002 *	0.167 ± 0.004 *
CAC-OR	0.125 ± 0.001 **	0.142 ± 0.004 *	0.182 ± 0.003 *
CAC-PU	0.122 ± 0.001 **	0.143 ± 0.002 *	0.184 ± 0.004 *

TAC-OR: traditional access cavity—One Reci; TAC-PU: traditional access cavity—ProTaper Ultimate; CAC-OR: conservative access cavity—One-Reci; CAC-PU: conservative access cavity—ProTaper Ultimate. * Indicates a statistically significant difference ($p < 0.05$). ** Indicates a statistically significant difference ($p < 0.01$).

4. Discussion

In endodontic procedures, minimally invasive access cavity approaches, minimally invasive instrumentation and shaping, activation and agitation of irrigation solutions with different methods, and pressureless canal filling methods are all used for minimally invasive treatment purposes [22,23]. Many studies in the literature have emphasized the importance of preserving healthy tooth structure, especially pericervical dentin, using conservative endodontic cavities [13,17,24,25]. In agreement with the results of this study, Volster et al. revealed that CAC preserved more healthy dentin and pericervical dentin in comparison with TAC after root canal preparation using Wave One Gold and TruNatomy files [25]. Pericervical dentin is dentin located 4 mm coronal and 4 mm apical to the cementoenamel junction. Preservation of pericervical dentin, especially in the molar area, plays a critical role in the longstanding optimal functioning of the teeth [24].

Ni–Ti instrument systems, which increase shaping efficiency and shorten the working time, have created a revolutionary change by raising endodontic treatments to a new level. Two different Ni–Ti systems were used in this study. Due to the findings of Bergmans et al. [26], the files used in studies must have similar apical diameters and tapers to compare morpho-geometric changes after root canal preparation. Parallel to this, in the present study, root canal preparations with OR and PU were made by selecting files with 25 apical diameters and similar tapers. OR and PU are files that have been introduced to the market by manufacturers for minimally invasive root canal shaping purposes and were therefore used in this study.

At the time this manuscript was written, a single study on OR was found in the literature review. Kharouf et al. [15] evaluated the amount of debris extruding apically after root canal preparation with OR in curved canals.

Today, reciprocation is presented as a safe and viable alternative to traditional continuous rotational motion. In addition, previous studies have reported that systems operating with reciprocal motion have a 0.13–0.26% lower incidence of fracture than systems with rotation [27,28]. On the other hand, there are two main topics for which the reciprocal movement is criticized. The first is that reciprocating instruments are more likely to promote the development or propagation of dentinal microcracks than conventional fully rotating rotary systems, while the other is likely to cause debris accumulation during preparation and the extrusion of debris from the apex [29].

In the present study, no significant difference was found in terms of volume change in the samples as a result of the preparation made with OR and PU in teeth with traditional and conservative access cavities ($p > 0.05$). According to this result, the type of access cavity does not have any effect on the volume change as in the study of Barbosa et al. [30]. Rover et al. did not find any effect of traditional and conservative access cavities on volume change after canal preparation in a similar study on upper molars [18]. According to these results, the nature of a cavity as a traditional or conservative access cavity does not affect the volume change in the root canals because of root canal preparation and agrees with the results obtained in this study.

No statistically significant difference was found among groups in terms of the change in surface area ($p > 0.05$). From the perspective of the two different types of access cavities prepared, this result agrees with Dos Santos Miranda et al.'s results obtained by investigating the effects of traditional and ultraconservative access cavities on the shaping of mandibular central teeth [31]. Additionally, Lima et al. evaluated the shaping efficiency of two different rotating and reciprocating files in traditional and ultraconservative cavities of mandibular molars. Similarly, no effect of different cavity types and files on the surface area change was found in this study either [14]. As a result of root canal shaping with OR and PU, no effect of the Ni–Ti file system used was found on the surface area change regardless of endodontic access cavity type ($p > 0.05$). The preparation was carried out by selecting files with similar apical diameters and similar tapers in both systems. Therefore, the morphological and geometrical changes in the root canals were similar.

A statistically significant difference was found between the groups in terms of non-instrumented area ($p < 0.05$). This difference arose independently of the Ni–Ti instrument system used. This result is in agreement with the results of the study obtained by Lima et al. [14]. In a separate similar study, Krishan et al. emphasized that conservative access cavities remain more non-instrumented than traditional access cavities [32]. The reason for this result may be that the files cannot provide a completely straight-line entrance to the root canals in CAC groups and touch more cavity and canal walls until they reach the apex. The Ni–Ti instrument system used in the aforementioned studies, similar to this study, did not affect the non-instrumented area.

Non-instrumented areas of the canal walls obtained by micro-CT analysis were observed, especially in the apical region and isthmus areas. However, these areas may contain pulp tissue, bacteria, and dentin residues. These data show that studies should continue to make irrigation and shaping protocols more effective [33]. In a minimally invasive treatment, the amount of non-instrumented area should be disinfected using complementary cleaning methods, not by increasing the taper of the Ni–Ti instrument used [34]. Thus, unnecessary dentin is not removed from the middle and coronal parts of the root canal, and the resistance of the endodontically treated teeth against root fractures is increased [35]. The percentage of unprepared areas is significantly reduced with the increase in the instrumentation size from 25 to 40, regardless of the system used. However, this situation may conflict with minimally invasive treatment principles, and by utilization of a modern system with a regressive taper, pericervical dentin can be preserved without compromising it [36].

One of the major points of mechanical damage as a result of excessive instrumentation in an already slim dentin wall under normal conditions is that it can lead to failure of root canal treatment [37]. Abou-Rass et al. stated in their study that such damage may cause perforations in the middle section of the root. This section is known as the danger zone and is located in the distal area of the mesial roots of mandibular molars. Additionally, the safety zone was defined as the mesial area of the mesial root with a thicker dentin layer [38]. The safety zone is usually minimally instrumented with endodontic instruments. In this study, no significant difference was found between the groups in terms of the thickness of the danger zone ($p > 0.05$). This result is consistent with the results of Peng et al.'s study on the danger zone [39]. In other words, these results demonstrate the reliability of both systems when preparing mesial root canals of mandibular molars because neither system excessively removed the dentin in the furcation area.

Canal transportation equals the deflection (in millimeters) of the root canal from its original axis after root canal preparation in comparison to before preparation [40]. Wu et al. showed that canal transportation of >0.3 mm causes adverse effects on root canal obturation [41]. In accordance with this, the canal transportation values obtained in all sections for all the groups in the present study were <0.3. In this study, canal transportation values were measured for all groups at distances of 2 mm, 5 mm, and 8 mm from the apical foramen. As a result of these measurements, a statistically significant difference was found among the groups in terms of canal transportation ($p < 0.05$). According to these results, regardless of the Ni–Ti instrument system used, more transportation was observed in the CAC-OR and CAC-PU groups compared to the TAC-OR and TAC-PU groups at all levels where the canal transportation value was measured. This result was in line with Rover et al.'s study, which compared the effects of traditional and conservative access cavities on shaping efficiency and transportation values in upper molars [18]. In another similar study, Lima et al. reported that access cavities with an ultraconservative approach in mandibular teeth cause more transportation than traditional cavities. The reason for obtaining these results is due to the presence of coronal interferences of access cavities with conservative and ultraconservative approaches, which causes the instruments to not provide a straight entry into the canals and results in an uneven force distribution [17,42]. In contrast, in Peng et al.'s study on the shaping efficiency of traditional and conservative access cavities and the preservation of dentin tissue in mandibular and upper molar teeth, no significant difference was found in terms of transportation post-instrumentation with WaveOne Gold in the two different access cavities evaluated [39]. Additionally, Kadhim et al. revealed that transportation and centering ability were not statistically affected by traditional versus conservative access cavities [43].

For conservative access cavities, the use of 3D imaging technology, an operating microscope, a good light source and illumination, ultrasonic tips, irrigation activation methods, and the use of new-generation Ni–Ti rotary instrument systems were found to minimize the negative effects on clinical procedures related to root canal treatment.

Within the limitations of this in vitro study, the effects of TACs and CACs on the shaping efficiencies of two different Ni–Ti instrument systems were evaluated using micro-CT. In this regard, future experiments should be encouraged with larger sample sizes, the use of irrigation activation methods, the incorporation of different shaping tools with fewer expansion angles, and over long-term clinical studies.

5. Conclusions

Treatments with a minimally invasive approach have emerged as a new perspective in endodontics in recent years. In this study, non-instrumented areas of the canal and canal transportation were statistically larger in the CAC groups than the TAC groups, but there was no statistical difference between the groups in terms of volume change, surface area, or the thickness of the dentin in the danger zone after root canal preparation. In general, this type of treatment is aimed at reducing the loss of healthy tissue in the teeth. This study demonstrated that the adverse effects of CAC can be mitigated by utilizing various techniques, such as irrigation activation, with the evaluated parameters in the study. With the proper methods, the use of CAC can be recommended within the limitations of the study since this method of treating cavities protects the healthy dentin more.

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Data Availability Statement: Data can be provided for academic purposes on request from the corresponding author.

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Comparative Analysis of Temperature Variation with Three Continuous Wave Obturation Systems in Endodontics: An In Vitro Study

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Abstract: The aim of this study was to assess temperature changes with different continuous wave obturation systems when downpacking to 2 mm and 4 mm from the apical foramen in an open system not simulating the surrounding biological structures at body temperature. Sixty single-rooted teeth were divided into three groups: (A) Dia-Duo® (DiaDent Group International, Cheongju-si, Korea), (B) Elements Free® (Kerr Corporation, Orange, CA, USA) and (C) Calamus® (Dentsply Sirona, Ballaigues, Switzerland). The root canals were instrumented with Protaper Gold (Dentsply Sirona, Ballaigues, Switzerland) to size F2 (25.08). The root canals were filled by a continuous wave using an AH Plus® sealer (Dentsply Sirona). Temperatures during the obturation procedure were measured by a thermal imaging camera (Testo 875-1®) perpendicular to a vice where the teeth were held at −2 mm and −4 mm from the apical foramen. Comparisons were made by applying Student's *t*-test and ANOVA ($p = 0.05$). The continuous wave technique at −2 mm with the Dia-Duo system® emitted average temperatures of 37.3 °C, Elements Free® emitted 39.85 °C and Calamus® emitted 40.16 °C. At −4 mm, the Dia-Duo system® emitted average temperatures of 34.81 °C, Elements Free® emitted 33.73 °C and Calamus® emitted 32.91 °C. There were significant differences between continuous waves at −2 mm and at −4 mm ($p < 0.05$). Dia-Duo® was the only system that did not present significant differences between the two lengths ($p = 0.197$). Regarding the heat emitted, the best system was Elements Free®, since, at −2 mm, it emitted the highest temperature without going above 47 °C. The Dia-Duo® system had lower temperatures. It could be concluded that not all systems transmit the same temperature to the apex and, therefore, to the periapical tissues. The surrounding conditions, such as temperature and humidity, have not been considered in this study.

Keywords: continuous wave technique; endodontic treatment; gutta-percha; heat packing; root canal obturation; temperature

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1. Introduction

The main purpose of root canal treatment is a good intracanal preparation followed by adequate filling. According to Schilder [1], the final objective of obturation should be the complete and three-dimensional filling of the root canal, achieving a hermetic seal [2–7]. Up to 60% of endodontic failures can be attributed to the inadequate filling of the root canal, which is a very important field to study in order to obtain new materials and methods for filling in endodontics [2].

Long-term sealing plays an important role in supporting healing of the periapical tissue and prevents intracanal contamination after treatment [8]. Different thermoplastic obturation systems aim to completely seal both the apical and coronal pathways from a possible leak and maintain the state of disinfection achieved to prevent reinfection [9]. Full root space debridement, the development of a fluid-tight seal of the apical foramen and total canal obliteration must be accomplished to ensure the long-term success of the treatment [8–10]. There are several methods of root canal filling, but none are perfect, and we can always expect complications. Therefore, choosing the most precise and safest root canal filling device is still a challenge for practitioners.

The thermal environment of teeth caused by eating or drinking hot or cold food and beverages or simply breathing cold air varies over a wide range of temperatures. Different studies have been carried out to address this challenge [11,12]. Jacobs et al. reported that thermal stresses induced by repetitive temperature cycling can lead to cracks in the teeth [11]. The structural changes that teeth undergo when subjected to heat will depend on the temperature and the exposure time, but also on the way in which these high temperatures are produced or applied [12]. When one tooth is suddenly subjected to a change in temperature, the surface does not change immediately; it takes time for heat transfer to occur, eventually resulting in the increased thermal energy of the tooth [11,12].

According to some authors, the maximum temperature increase tolerated by the external surface of the root without causing damage to the supporting tissues is 10 °C [3]. In this sense, exceeding this limit could cause bone resorption, ankylosis of the tooth, or postoperative pain. In addition, the resorption of alveolar bone tissue was observed without evidence of regeneration after an increase of 10 °C for 5 min and of 13 °C for 1 min [4]. The thickness of dentin walls is another factor, because the thinner the walls, the greater the damage to surrounding tissues [13–15].

Enamel and dentin have different thermal and mechanical properties. The differences in these properties can lead to thermal stresses and cracking within the tooth when subjected to thermal stimuli [16]. The thermal denaturation of dentin collagen often occurs in endodontic treatments, during which root canal dentin may be exposed to a temperature of ∼300 °C. The denaturation temperatures of the demineralised dentin matrix have been reported to be 65.6 °C, 148.5 °C and 166.8–172.7 °C for demineralised dentin saturated with water; dentin saturated with methanol, ethanol or acetone; and dentin bonded with resin, respectively [16,17].

Periapical disease is an inflammatory response around the root canal endings due to intracanal bacterial infection. The dental pulp and periodontium have different communication pathways such as the apical root canal, accessory (or lateral) canals and dentinal tubules [18]. Pathological communication between these structures includes the migration of microorganisms and inflammatory mediators between the root canal and the periodontium [18,19].

Although root canal sealers are very important in obturation, the amount used should be minimal. To achieve a high amount of gutta-percha, filling techniques have been developed to achieve this purpose. When the apical part of the canal is filled with the Buchanan continuous wave technique at −4 mm, the gutta-percha is not thermoplasticised in the last few millimetres, since the gutta-percha is a very poor conductor of heat [20–22]. For that reason, in this study, canals have been filled to different depths with three different systems, and the emitted heat has been observed. In a study performed by Lipski and Woźniak in 2003, the temperatures recorded at the tips of a continuous wave plugger varied with their taper and were lower than the temperature set on the System B LCD display [23]. There are other studies that have measured the real intracanal temperature during different obturation techniques [18,24] and studies that compare the same technique with different devices [25], but the temperature transmitted by new devices is not known. Furthermore, the actual temperature changes in periapical tissues are currently unknown.

The aim of this study was to calculate the heat emitted when downpacking at −2 mm and −4 mm with three different continuous wave obturation systems.

2. Materials and Methods

A randomized experimental in vitro trial was carried out conducted in accordance with the principles defined in the statement of the German Ethics Committee for the use of organic tissues in medical research (Zentrale Ethikkommission, 2003), and was approved by the University Ethics Committee (Process No. 07/2020). All patients who were asked to transfer teeth agreed and signed an informed consent document before entering the study. The sample size of the study was calculated on the basis of the EPIDAT 4.2 program (Dirección Xéral de Saude Pública, Galicia, Spain) and the article by Lipski and Woźniak [23] with statistical significance (p-value < 0.05). The randomization of the study sample was carried out with the EPIDAT 4.2 statistical package, which was used to generate a table of random numbers in three groups. Sixty single-rooted first upper premolar teeth extracted for orthodontic or periodontal reasons were selected at random in this study. The inclusion criteria consisted of patients 15–65 years of age, the absence of caries, cervical abfraction or root fracture, a curvature of less than 5° according to Schneider's technique [22], a root length of 14 ± 1 mm and rather similar mesiodistal and buccolingual dimensions (±10%). Furthermore, the teeth were submitted to a radiographic exam to analyse the number of root canals, and the absence of previous endodontic treatment, restorations and root resorption. The teeth were divided into 3 groups: Group A made with Dia-Duo® (DiaDent Group International, Cheongju-si, Korea), Group B with Elements Free® (Kerr Corporation, Orange, CA, USA) and Group C with Calamus® (Dentsply Sirona, Ballaigues, Switzerland). Each filling system had two groups of 10 teeth each. One group was performed at −2 mm and the other at −4 mm of the apical foramen.

The distance from the apex to the cementoenamel junction (CEJ) as well as the diameters in the oral–buccal and mesial–distal directions at the CEJ were measured. The teeth were randomized into 6 groups (n = 10) according to the distance from the apex to the CEJ (p = 0.999) and the ratio of the diameters in the oral–buccal and mesial–distal directions at the CEJ (p = 0.824). The homogeneity of the 6 groups with respect to the aforementioned parameters was assessed using analysis of variance and the post hoc Student–Newman–Keuls test.

The roots were cleaned with ultrasound to remove the plaque and adhering tissues. Teeth were then cut to 16 mm by means of a calliper and a disk bur.

Once the canals had been opened, they were permeabilized with #10 k-file (Dentsply Sirona, Ballaigues, Switzerland) and instrumented with Protaper Gold® (Dentsply Sirona, Ballaigues, Switzerland), up to the F2 file (25.08). The canals were irrigated with 5.25% hypochlorite after each instrument in 2 mL syringes. Canals were filled according to the Buchanan technique. The continuous wave of condensation obturation technique was applied to simplify warm gutta-percha downpacking of Schilder's warm vertical condensation; the continuous wave of condensation is an obturation technique that applies a heated "plugger" into a single custom fit master gutta-percha cone with a greater taper. The heat plugger is placed through the master cone to within 3–5 mm of the predetermined working length. Gutta-percha cones from Protaper Gold® F2 (25.08) (Dentsply Sirona, Ballaigues, Switzerland) were used. First, AH Plus cement® (Dentsply Sirona, Ballaigues, Switzerland) was introduced with a paper point and then the gutta-percha F2 (25.08) cone up to the working length.

All systems were adjusted to 200 °C and the downpacking was activated for 3 s, based on the article by Zhou et al. [24] in which they concluded that the plugger should not be activated at 200 °C for more than 3 s inside the tooth since it can reach temperatures higher than 47 °C, which is harmful to the tissues adjacent to the teeth.

Once the gutta-percha cone had been inserted into the tooth, the plugger was activated to cut away the excess gutta-percha and was left at the level of the canal entrance. Next, the plugger was activated, softening the gutta-percha until it reached the predetermined length (−2 mm or −4 mm from the apical foramen) at 3 s. The pluggers of three systems were different: #40.04 (Dia-Duo), #40.06 (System B) and #40.03 (Calamus).

When the working length had been reached, the apical pressure was maintained for 10 s, which allowed the gutta-percha to cool, then pressed for 1 s, and the plugger was extracted.

When it came to filling the canals and measuring temperatures, a vice was used so that the last 5 mm of the root was in the air. The temperatures were collected from the mesial face of the teeth using a thermographic camera (model Testo 875-1®, Testo SE & Co. KGaA, Titisee-Neustadt, Germany).

Measuring a temperature gradient across a boundary layer requires high accuracy. Typical values for the convective heat transfer coefficients for air and water are shown in Table 1.

Table 1. Typical values for the convective heat transfer coefficient in water and in air.

System	Heat Transfer Coefficient h (W/m^2 K)
Air (natural convection)	5−25
Water (forced convection)	300–600

The camera was mounted perpendicular to the tooth surface at about 11 cm. The experiment was carried out at a room temperature of 23.9 °C (Figure 1).

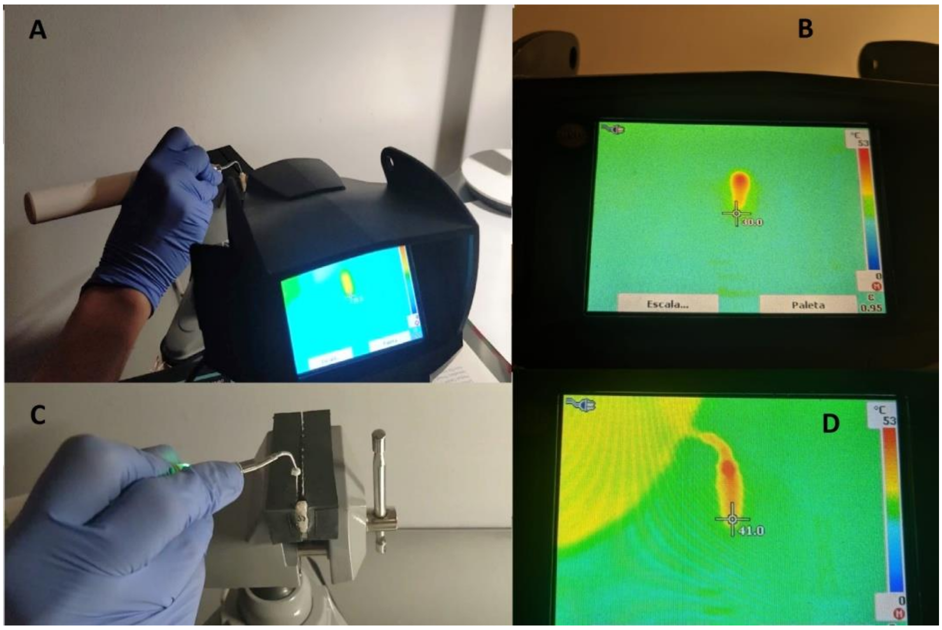

Figure 1. Experimental set up. (**A**,**C**) Positioning of the tooth with respect to the camera and location of the vice. (**B**,**D**) Images of the thermographic camera.

Descriptive statistics were obtained for each study variable with the corresponding normality tests. The statistical analysis was performed using Student's *t*-test and ANOVA to compare the three systems used, always assuming a confidence level for our study of 95%. When the data did not follow normality, the Mann–Whitney U test was carried out (in this case, when the plugger length of the systems was analysed).

3. Results

The results obtained are shown in Table 2. The mean temperature when the teeth were filled at −2 mm was higher with Calamus® than with the other systems (40.16 °C vs. 39.85 °C and 37.3 °C) while at −4 mm, the highest temperature was found with Dia-Duo® (34.81 °C) followed by Elements Free® (33.73 °C) and Calamus® (32.91 °C). The Calamus® system presented higher temperatures at −2 mm.

When we compared the different systems, there was no statistically significant difference between filling at −4 mm and −2 mm in the Dia-Duo System® (p = 0.213) (Figure 2). There were statistically significant differences between filling at −4 mm and −2 mm for the Elements Free System® (p = 0.001) (Figure 3) and the Calamus System® (p = 0.002) (Figure 4).

Table 2. System averages based on their sealing depth and minimum and maximum temperature values.

System	Length	Mean	>Temperature	<Temperature
Dia-Duo	−2 mm	37.3	42.4	33.4
	−4 mm	34.81	45.1	27.8
Elements Free	−2 mm	39.85	45.2	34.2
	−4 mm	33.73	40.3	29.8
Calamus	−2 mm	40.16	51.4	35
	−4 mm	32.91	42.1	29.8

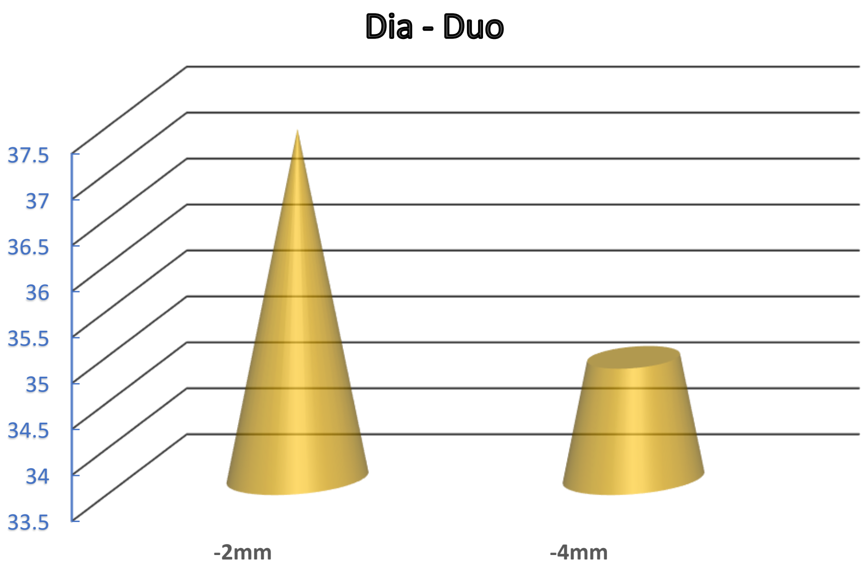

Figure 2. Graph of temperatures at −2 mm and −4 mm with Dia-Duo® (p = 0.213).

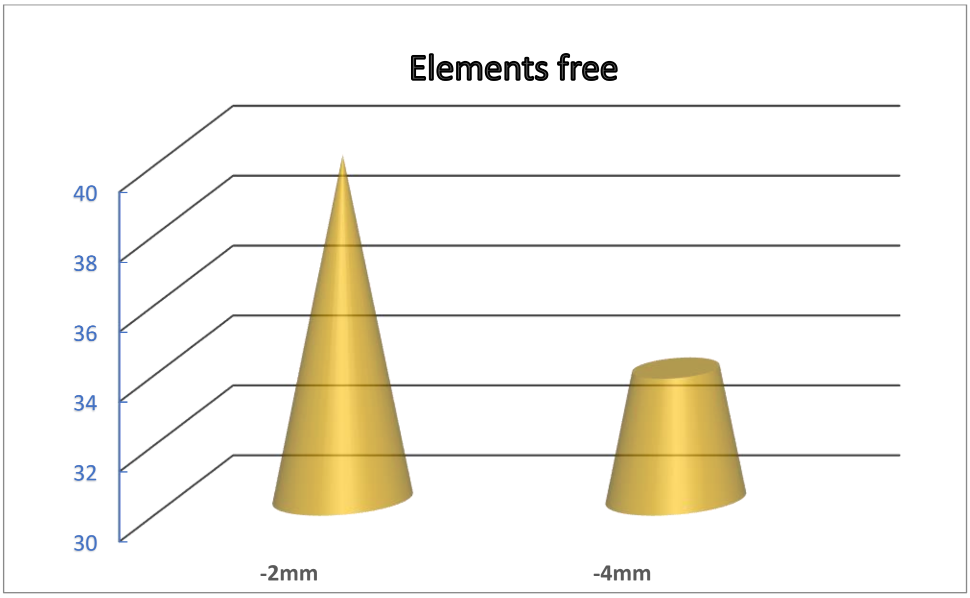

Figure 3. Average temperatures at −2 mm and −4 mm with Elements Free® (p = 0.01).

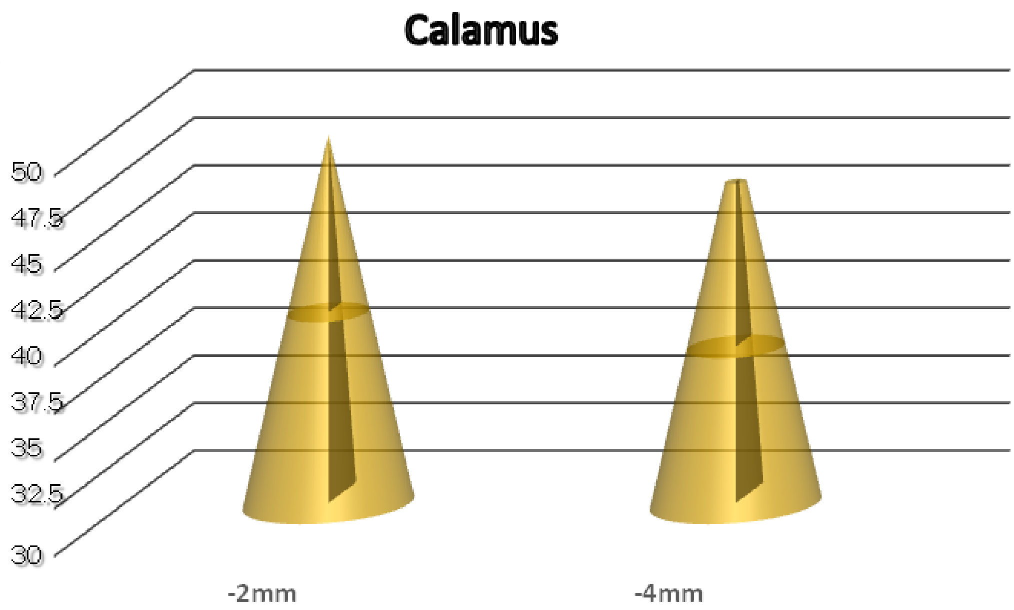

Figure 4. Average temperatures at −2 mm and −4 mm with Calamus® (p = 0.002).

If the different systems were compared according to the sealing depth, no statistically significant differences were found between the different systems at −2 mm (p = 0.197) (Figure 5) or −4 mm (p = 0.620) (Figure 6).

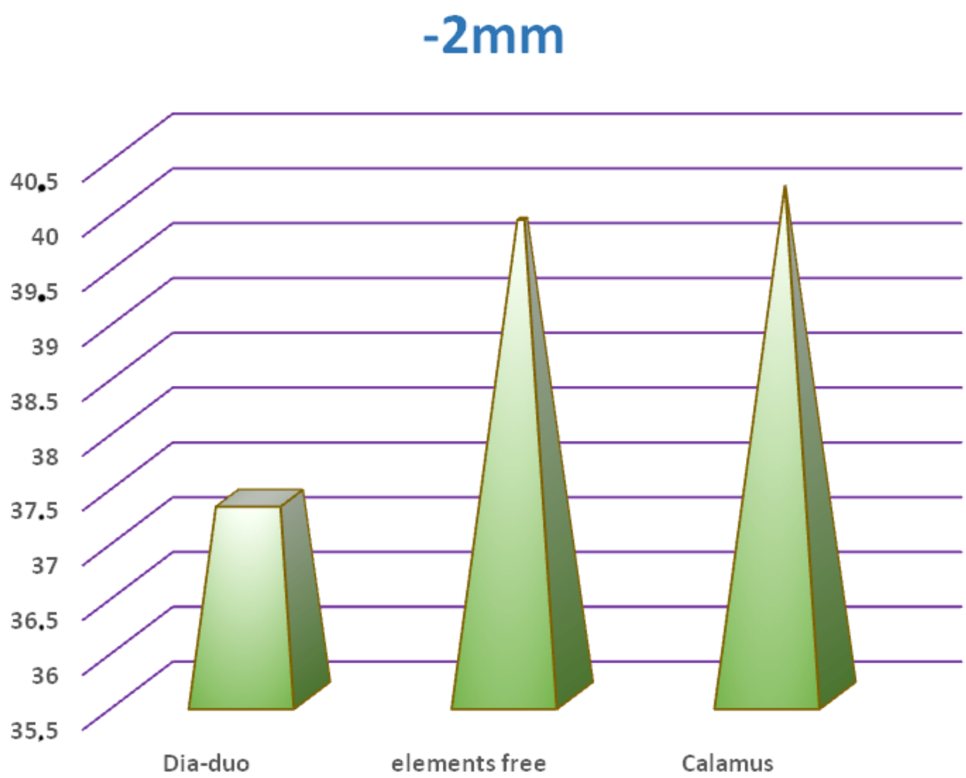

Figure 5. Average temperatures at −2 mm of the three systems compared. The data show no statistically significant differences (p = 0.197).

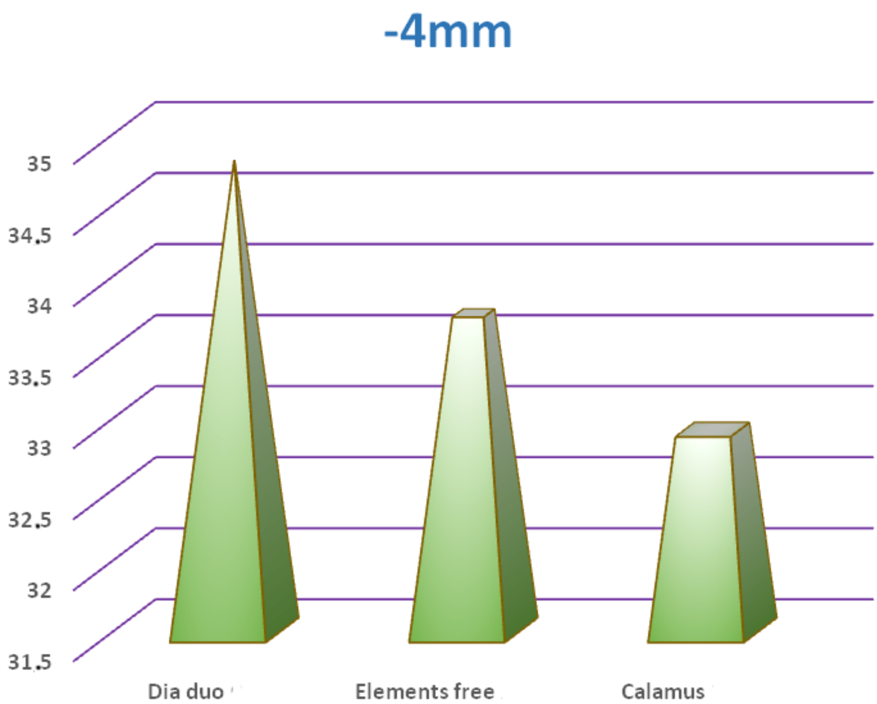

Figure 6. Average temperatures at −4 mm of the three systems compared. The data show no statistically significant differences ($p = 0.620$).

Finally, all systems were examined according to the depth of obturation, and a statistically significant difference was observed in the obturation at −2 mm and −4 mm ($p = 0.000$). A temperature difference of about 5 °C was also found between the obturation at −2 mm and −4 mm (Figure 7).

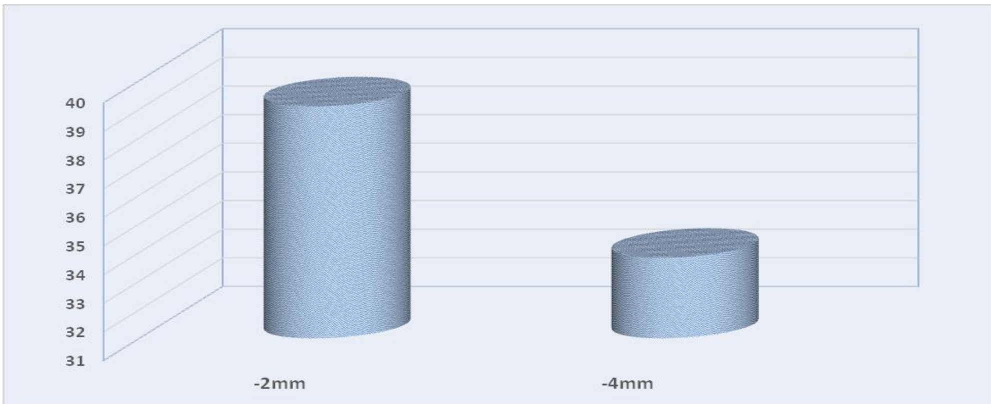

Figure 7. Comparison of mean values of temperature between all systems at −2 and −4 mm. There were significant statistical differences ($p = 0.000$).

4. Discussion

In this in vitro study, the temperature of the outer root surface was measured during root canal filling using the continuous heat wave technique. It has been suggested that an increase in temperature greater than 10 °C at the external root surface could be responsible for damage to the cementum, the periodontal ligament and the alveolar bone that may trigger resorption or ankylosis [26,27]. With the experimental technique applied in this study, the rise in temperature of the single-rooted teeth never exceeded 10 °C except in one sample (maximum temperature recorded: 51.4 °C).

The current findings contrast with the study by Romero et al. [26], which reported that the elevation of the root surface temperature during the continuous heat wave technique did not exceed 1−2 °C. In the abovementioned study, the extracted teeth (maxillary canines and mesial roots of mandibular molars) were imbibed in a water-containing medium (alginate), similar to the periodontal ligament. When selecting the present in vitro study model, the possibility of simulating the periodontal ligament and bone, similar to Romero et al. [26], was considered; however, it was decided not to include the samples in any medium but to keep the external conditions constant in all measurements. In addition, the aim was to know the increase in the external temperature, and this was not influenced by the environmental conditions, since the amount of bone, periodontal ligament or bone conditions that exist in the maxilla and mandible cannot be accurately reproduced in an in vitro study.

Venturi et al. [28] revealed that a negligible temperature rise (0.5–0.9 °C) was induced in the apical gutta-percha by the System B® obturation technique. These data indicated that the apical gutta-percha was often compacted at body temperature when using the continuous heat wave condensation technique, as described by Buchanan. In the present study, the heat of the plugger tip was not measured, but the increase in the external temperature was dissipated, meaning that it was actually transmitted to the patient, who sometimes feels that heat. Similar to the study of Venturi et al. [28], the measurements were performed in the air, unlike Dimopoulos et al. [21], who used a polytetrafluoroethylene (PTFE) cylinder. According to Nikolaev et al. [29], the current devices used to perform the continuous heat wave technique are completely safe and effective. In their study, they used GuttaEst® at −5 mm and observed a temperature difference of +3.8 ± 0.6 °C. Nevertheless, Lipski [30] examined the heat emitted by sealing with System B® on the maxillary and mandibular central incisors and maxillary canines; on mandibular incisors, they observed a temperature increase of more than 10 °C. McCullagh et al. [31] reported a temperature increase of 13.9 °C by measuring the heat with a thermoelectric couple and 28.4 °C with a thermographic camera, so their results were also higher than 10 °C.

Lipski and Woźniak [23], in another study, re-treated teeth obturated with Thermafil® and used the B-System® to seal the canals for 5 and 8 s, and observed that the temperatures ranged from 26.7 °C to 46.0 °C, with possible damage to the periodontal tissues. It is known that if heat is applied for more than 3 s, temperatures above 10 °C can be reached; therefore, all manufacturers recommend not applying heat for more than 3 s.

Romero et al. [26], Venturi et al. [28] and Nikolaev et al. [29] reported that with the continuous heat wave technique, there was almost no rise in temperature. The present study agrees with these authors, since we found an increase in temperature that exceeded 3.8 °C with the three systems used; nevertheless, no statistically significant differences were found. On the other hand, Lipski [30] and McCullagh et al. [31] explained that when the technique was applied to mandibular central incisors, temperatures exceeded 10 °C using System B®. In the present study, only one sample exceeded 10 °C. Lipski and Woźniak [23] reported an increase of more than 10 °C with the use of System B® as a shutter system, but their time of application was 5 s and 8 s. Zhou et al. [24] conducted a study on mandibular molars filled with System B® for 3 s and 4 s. They reported that when obturating for 3 s, the temperature of the periodontal ligament was 46,914 °C and when obturating for 4 s, the temperature increased by more than 10 °C; in conclusion, one should be careful not to extend the activation time beyond 3 s. In the present study, the plugger in all systems was set to 3 s.

Eriksson et al. [27] observed that bone resorption occurs when temperatures of 47 °C and above are reached during bone drilling. In a study on the temperature threshold for heat-induced bone tissue injury in rabbits, it was found that a temperature of 47 °C maintained for 1 min could cause microscopic evidence of bone remodelling and adipose tissue necrosis. Molyvdas et al. [15] showed a periapical inflammatory histological reaction in beagle dogs after an injection of thermoplasticized gutta-percha at a high temperature (160 °C) into the root canal. However, the tissue destruction was localized in the area around the apical foramen, while the periodontal ligament of the root surfaces remained normal. Saunders [32] performed a study on canine teeth filled with thermomechanical compacted gutta-percha in 17 ferrets, in which a histological evaluation of the root cementum, adjacent periodontal membrane and alveolar bone was performed at time intervals of 24 h, 20 days and 40 days. At 24 h, there was no inflammatory response and no evidence of hyperaemia. At 20 days, 20% of the teeth showed resorption of the cementum surface in the central section of the root. This resorption was not of an inflammatory nature, unlike resorptions due to trauma. All control teeth and their supporting tissues appeared normal. However, 40 days after thermomechanical compaction, 28% of the experimental teeth were affected by surface resorption; of these, 22% showed ankylosis of the alveolar bone. Therefore, these authors concluded that the heat generated by thermomechanical compaction stimulates surface resorption and long-term ankylosis.

Eriksson et al. [27], Molyvdas et al. [15] and Saunders et al. [32] observed alterations such as necrosis, destruction around the foramen and ankylosis in their studies. In all of these studies, temperatures above 47 °C or a time longer than 3 s were harmful to teeth. From these studies, it can be concluded that caution is necessary to avoid excessive heat when performing techniques with thermoplastic gutta-percha.

Cen et al. [33] conducted a study of a 3D-printed mandibular molar sealed by a computer system with System B® and Obtura II®. They made two models: one simulating blood flow and one without blood flow. The study concluded that in the model without blood flow, the temperature in the periodontal ligament was 50 °C along the distal canal and 52.5 °C in the mesiolingual canal. However, in the model simulating the blood flow in the periodontal ligament, the peak temperature was 47 °C. The conclusion of the study is that the blood flow of the periodontal ligament is one of the factors to be taken into account when investigating the heat emitted during thermoplastic filling.

Cumbo et al. [34] studied three obturation systems used for different times, namely 10, 15, 20 and 25 s, and the different systems used were System B®, Endo-Twin® and E-Fill®. The results obtained showed that System B® reached temperatures of 86.85 °C after 10 s, 94.9 °C after 15 s, 100.4 °C after 20 s and 104.5 °C after 25 s; with Endo-Twin®, similar results to System B® were observed but E-Fill® had temperatures lower than 69.9 °C. The study concluded that with System B® and Endo-Twin®, the temperatures reached at 25 s were sufficient for correct gutta-percha adaptation in the canals. This study did not take into account the possible damage to surrounding tissues that could occur at these temperatures.

In the studies by Zhou et al. [24] and Cumbo et al. [34], a temperature increase of more than 10 °C was observed if the heat application time with the plugger was longer than 3 s, but the temperatures still did not exceed 10 °C.

One limitation of this study was that only one heat carrier was assigned per manufacturer. Therefore, the quality of the heat carriers used for this study could have affected the results. Furthermore, not having included the samples in some medium that simulated the periodontal ligament could have affected the temperature measurements. Measurement with a thermographic camera instead of a thermocouple could also have yielded different results. In the future, finite element analysis (FEA) may use these data to reveal the actual effect of these temperature levels on the periodontal membrane and alveolar bone.

From a clinical point of view, this in vitro study presented the difficulties associated with all the clinically relevant factors, so the question of the possible implications of temperature increases in the periodontal tissues remains unsolved. However, knowing which system transfers the temperature most effectively allows better use of the technology.

5. Conclusions

The results of this study showed that Dia-Duo® was the only system in which there were no statistical differences between the two filling lengths (−2 mm and −4 mm). Regarding heat emission, the best system was Elements Free®, as at −2 mm, it emitted the highest temperature without exceeding 47 °C. It can be concluded that not all systems transmit the same temperature to the apex and, therefore, to the periapical tissues.

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Potential Synergistic Inhibition of *Enterococcus faecalis* by Essential Oils and Antibiotics

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Abstract: Recurrent infections after root canal treatments often involve *Enterococcus faecalis*, a microorganism closely associated with therapy failures due to its biofilm production, survival in nutrient-deprived conditions, and antibiotic tolerance. Essential oils (EOs), which display antimicrobial and antibacterial properties, exhibit inhibitory effects on the growth of many microorganisms including *E. faecalis*. This study assessed the in vitro efficacy of combining 5% antibiotics (kanamycin 2.5 mg/mL, streptomycin 2.5 mg/mL, gentamicin 1.5 mg/mL, and ampicillin 5 mg/mL) with cinnamon (1.25% to 5%) or clove (25% and 50%) EOs in inhibiting the growth of *E. faecalis*, using disk diffusion tests. Disks were treated with EOs-only, antibiotics-only, or EO–antibiotic combinations, placed on BEA agar plates, and incubated for 24 h, and the zones of inhibition were measured. Results showed that EOs (cinnamon and clove) and 5% antibiotics, by themselves, had robust growth inhibition of *E. faecalis* across all tested concentrations. Moreover, combining 5% aminoglycosides (kanamycin 2.5 mg/mL, streptomycin 2.5 mg/mL, and gentamicin 1.5 mg/mL) with 5% cinnamon EO produced significantly enhanced antimicrobial effect than the corresponding 10% antibiotic solution alone. These findings suggest that combining cinnamon EO with aminoglycoside antibiotics can achieve significant inhibition of *E. faecalis* at a lower concentration of antibiotics compared to using a higher dose of antibiotics alone. Further in vivo studies should determine the safety, efficacy, and treatment duration, with the potential to reduce antibiotic dosages and associated toxicity while preventing recurrent infections.

Keywords: cinnamon; clove; essential oils; *E. faecalis*; antibiotics; gentamycin; streptomycin; kanamycin; ampicillin; root canal infection

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1. Introduction

Since their discovery, antibiotics have become essential in successfully combating infectious diseases to improve human health [1,2]. In dentistry, antibiotics are selectively used for orofacial infections of odontogenic and non-odontogenic origins such as dental abscess, pulpal necrosis, periodontal diseases, dental caries, dental trauma, adenoiditis, otomastoiditis, and in prophylaxis [2–5]. Penicillin beta-lactam and amoxicillin, along with clindamycin, are the most widely prescribed oral antibiotic agents in dentistry, prophylactically and for therapeutic use, for their broad-spectrum antimicrobial activity of the former and as an alternate to penicillin allergic responses for the latter [1,6,7]. Along with their health benefits, however, the overprescription of antibiotics is a concern for clinicians and for patients. An analysis of outpatient prescription data from British Columbia showed the dental antibiotic prescription rate increasing by 71.6% between the years 1996 to 2013 [6]. Similar UK and US studies also found that over 65% of antibiotic prescriptions were issued when there was no evidence of spreading infection, and 73% to 85% of antibiotic prescriptions of penicillin beta-lactam and lincosamides for adult and children outpatients were either unnecessary or untimely [8–11].

Microorganisms, such as *actinomyces*, *streptococci*, and *enterococci* (*E. faecalis*), that produce dental biofilms, complex communities of microorganisms composed of bacteria that adhere to surfaces forming a protective matrix of extracellular substances, often diminish the effectiveness of antibiotics, increase their resistance, and contribute to the development of endodontic and periodontal diseases, ultimately causing failures of their therapies [12,13]. In teeth that are treated with root canal therapy, 77% to 90% of recurring infections and subsequent treatment failures are linked to *E. faecalis* [14–16]. Furthermore, *E. faecalis* is commonly recovered in teeth that were treated in multiple visits [12,17], likely due to its ability to form biofilms, persist in saliva, survive in nutrient-free environments, resist many antibiotics, and remain dormant as a facultative anaerobe [18–20]. In addition to root canal infections, enterococcal bacteria are also associated with endocarditis, bacteremia, urinary tract infections, intra-abdominal infections, and prostatitis [21,22]. Increased natural defense mechanisms acquired by the microorganisms, along with overprescription and increased prophylactic use of antibiotics, have elevated global concern for the emergence of antibiotic-resistant microorganisms and increased incidences of antibiotics-related secondary health issues such as dysbiosis, clostridium difficile infection, resistant urinary tract infection, and methicillin-resistant Staphylococcus aureus infections [6,23–26].

Essential oils (EOs) and their anti-inflammatory, antifungal, antimicrobial, and antibacterial properties are well elucidated [27–31]. Overall, EOs display increased sensitivity to Gram-positive bacteria, and their antimicrobial responses are mediated in part by ATP and inhibition of ATPases, disruption of membrane permeability, and inhibition of biofilm synthesis in the microbes [32–35]. In vitro studies report significant antibacterial effects of thyme, clove, sage, peppermint, lavender, cinnamon EOs, and their chemical components of thymol, eugenol, thujone, menthol, linalool, and cinnamaldehyde on caries-causing bacteria such as *streptococci* and *lactobacilli* spp. [36,37]. Among the phenylpropanoid EOs, thymol, eugenol, menthol, and cinnamaldehyde report potent antimicrobial properties in MIC (minimum inhibitory concentration), MBC (minimum bactericidal concentration), disk diffusion, and mouth rinse tests [38–41]. Moreover, combination of cinnamon, lavender, peppermint, oregano, and thyme EOs with antibiotics (β-lactam, penicillin, cephalosporin, and aminoglycoside) report enhanced and synergistic antimicrobial effects compared to when tested individually [42–46]. The enhanced antimicrobial benefits of combining antibiotics with EOs could further be examined as a novel strategy to lower the concentrations and use of antibiotics to mitigate the proliferation of antibiotic- resistant bacteria.

In this study, we assessed the presence of enhanced antimicrobial effect of cinnamon and clove essential oils when combined with penicillin (ampicillin) or aminoglycoside (kanamycin, streptomycin, gentamicin) classes of antibiotics in inhibiting the growth of *Enterococcus faecalis* using the Kirby–Bauer disk diffusion test.

2. Materials and Methods

2.1. Essential Oils and Antibiotics

Cinnamon (Cinnamomum Zeylanicum; bark) and clove (Eugenia Caryophyllus) essential oils (EOs) were purchased from Now Pure Essential Oils (Bloomingdale, IL, USA). According to the GCNS data by the manufacturer, the main chemical component of the cinnamon and clove EOs was trans-cinnamaldehyde and eugenol, respectively. The EOs were diluted with DMSO (20% v/v) to make a stock concentration of 10% for the cinnamon (CN) and 50% for the clove (CL) EOs. For the experiments, the CN oil was further diluted with DMSO and tested at 10%, 5%, 2.5%, and 1.25%, and the CL oil was diluted and tested at 50% and 25% concentrations. These EO concentrations were chosen as they produced a zone of inhibition that was below the CLSI Intermediate Breakpoint for *Enterococcus* spp. and when combined with an antibiotic would produce a zone of inhibition at or near the level of Intermediate Breakpoint [47].

Two classes of antibiotics, a penicillin class (ampicillin) and aminoglycoside class (kanamycin, streptomycin, gentamicin), were tested in this study. The stock concentrations of the antibiotics were: Kanamycin 50 mg/mL, Streptomycin 50 mg/mL, Gentamicin

30 mg/mL, and Ampicillin 100 mg/mL (Fisher Scientific, Waltham, MA, USA). For the experiments, the antibiotics were diluted with distilled water and tested at 10% (kanamycin 5 mg/mL, streptomycin 5 mg/mL, gentamicin 3 mg/mL, ampicillin 10 mg/mL) and at 5% (kanamycin 2.5 mg/mL, streptomycin 2.5 mg/mL, gentamicin 1.5 mg/mL, ampicillin 5 mg/mL) concentrations. These antibiotic concentrations were chosen as they produced a zone of inhibition that was below the CLSI Intermediate Breakpoint for *Enterococcus* spp., and when combined with an EO would produce a zone of inhibition at or near the level of Intermediate Breakpoint.

2.2. Bacterial Strain and Culture Conditions

For the study, the reference strain of *E. faecalis* (ATCC 29212) was grown and cultured on Bile Esculin Azide (BEA) agar plates. The reference strain ATCC 29212 and the BEA agar plates were purchased from Fisher Scientific (Waltham, MA, USA). Using an inoculation loop, the BEA plates were streaked with *E. faecalis* and incubated for 24 h under aerobic conditions (5% CO_2, 37 °C) to achieve an even growth. These cultures were used to inoculate fresh sets of BEA plates that were used for the antimicrobial susceptibility Kirby–Bauer disk diffusion test.

2.3. Disk Diffusion Test for EOs and Antibiotics

The Kirby–Bauer disk diffusion test was used to determine the antimicrobial susceptibility for EOs, antibiotics, and antibiotics combined with EOs on *E. faecalis*. The baseline antimicrobial effects of EOs were tested by placing 2 mL of freshly prepared CN (CN10%, CN5%, CN2.5%, and CN1.25%) and CL (CL50%, CL25%) EO solutions in individual culture tubes and vortexed for 30 s. Then, one sterile filter disk (6 mm diameter) was dropped in each tube, vortexed for another 15 s, and placed on BEA agar plates streaked with cultured *E. faecalis*. Two to three EO-soaked filter disks were placed firmly on the agar surface per each plate (n = 6–8/condition). To determine the baseline antimicrobial effects of the antibiotics, 2 mL of the 10% (kanamycin 5 mg/mL, streptomycin 5 mg/mL, gentamicin 3 mg/mL, ampicillin 10 mg/mL) and 5% (kanamycin 2.5 mg/mL, streptomycin 2.5 mg/mL, gentamicin 1.5 mg/mL, ampicillin 5 mg/mL) solutions of kanamycin, streptomycin, gentamicin, and ampicillin were added in individual culture tubes. One sterile filter disk (6 mm diameter) was dropped in each antibiotic solution, vortexed for 15 s, and placed on BEA agar plates streaked with cultured *E. faecalis*. Two to three antibiotic-soaked filter disks were placed firmly on the agar surface per each plate (n = 4/condition).

For the combinational antimicrobial effects of antibiotics and EOs, the solutions were prepared as follows: a 10% concentration of an antibiotic solution was combined with an equal volume of each of the four concentrations of EOs (CN10%, CN5%, CN2.5%, and CL100%). These pairings would yield a 5% concentration antibiotic solution containing a half dilution of EO solution that individually would produce a zone of inhibition that was below the CLSI Intermediate Breakpoint for *Enterococcus* spp., but when combined would produce ZOI at or near the CLSI Intermediate Breakpoint. For ampicillin, a 1.5 mL of 10 mg/mL ampicillin (10% v/v) was placed into four culture tubes that contained one of the following EO solutions: 1.5 mL of CN10%, 1.5 mL of CN5%, 1.5 mL of CN2.5% or 1.5 mL of CL100%. This 1:1 ratio of combination yielded a combined solution with final concentration of 5 mg/mL ampicillin + CN5%, 5 mg/mL ampicillin + CN2.5%, 5 mg/mL ampicillin + CN1.25%, and 5 mg/mL ampicillin + CL50%. The above steps were repeated for kanamycin 5 mg/mL (10%), streptomycin 5 mg/mL (10%), and gentamicin 3 mg/mL (10%), where 1.5 mL of antibiotics was combined with 1.5 mL of CN10%, 1.5 mL of CN5%, 1.5 mL of CN2.5%, or 1.5 mL of CL100% (Table 1). The culture tubes were vortexed for 30 s, followed by placing a sterile filter disk in each culture tube. The culture tubes were vortexed for an additional 15 s, and the filter disks were placed on BEA agar plates streaked with cultured *E. faecalis*. Two to three antibiotic-soaked filter disks were placed firmly on the agar surface per each plate (n = 8/condition).

Table 1. Treatment groups by combining antibiotics and EOs.

Treatment Groups	Ampicillin 10 mg/mL	Kanamycin 5 mg/mL	Gentamycin 3 mg/mL	Streptomycin 5 mg/mL
Cinnamon 10% EO	Ampi5 + CN5%	Kana2.5 + CN5%	Genta1.5 + CN5%	Strep2.5 + CN5%
Cinnamon 5% EO	Ampi5 + CN2.5%	Kana2.5 + CN2.5%	Genta1.5 + CN2.5%	Strep2.5 + CN2.5%
Cinnamon 2.5% EO	Ampi5 + CN1.25%	Kana2.5 + CN1.25%	Genta1.5 + CN1.25%	Strep2.5 + CN1.25%
Clove 100% EO	Ampi5 + CL50%	Kana2.5 + CL50%	Genta1.5 + CL50%	Strep2.5 + CL50%

All BEA agar plates with filter disks were secured with lab tape, inverted, and incubated at 37 °C for 24 h. The size of zone of inhibition was measured from the smallest clearings using a ruler at 1 mm scale (Figure 1). The filter disks soaked in DMSO for 15 s. were used as the control.

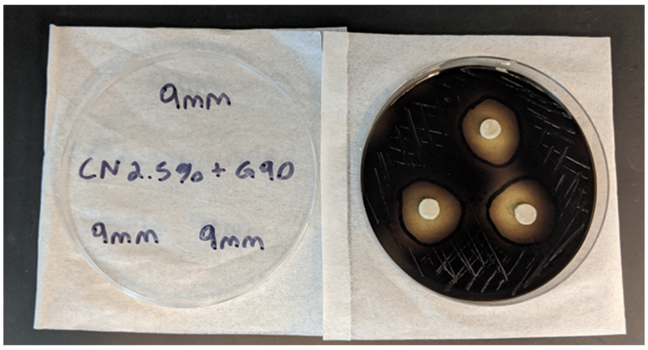

Figure 1. An image of BEA agar plate inoculated with *E. faecalis* showing the zones of inhibition of 5% gentamicin (1.5 mg/mL) combined with cinnamon 2.5% EO. The plate was incubated at 37 °C for 24 h and the size of zone of inhibition was measured using a ruler. BEA, Bile Esculin Azide; EO, essential oils.

2.4. Statistical Analysis

To determine the statistical significance in antimicrobial effects when antibiotics were combined with EOs or tested separately, the ZOIs of treatment groups were analyzed by one-way ANOVA (by treatment condition) followed by a Tukey's HSD post hoc test (GraphPad, La Jolla, CA, USA). All results were considered statistically significant at $p < 0.05$.

3. Results

3.1. Antimicrobial Effects of Cinnamon and Clove EOs

The antimicrobial efficacies of the cinnamon (CN10%, CN5%, CN2.5%, and CN1.25%) and clove (CL50% and CL25%) EO solutions were quantitated using the Kirby–Bauer disk diffusion test. The CN and CL EOs showed a concentration-dependent growth inhibition of *E. faecalis* at all tested concentrations compared to the control ($F(6, 24) = 227.8$, $p < 0.0001$, Figure 2A). At the lowest concentration of CN1.25%, the zone of inhibition (ZOI) was 4.67 ± 0.21 mm. For the CN2.5% and CN5%, the ZOIs were 7.50 ± 0.22 mm and 9.00 ± 0.40 mm, respectively. At the highest concentration of CN10%, the ZOI was 11.67 ± 0.33 mm. Across all concentrations of CN EO examined, there was a significant increase in ZOI by 36% ± 1.2% as the concentration of CN EO doubled. For CL EO, the ZOIs for CL25% and CL50% were 6.00 ± 0.00 mm and 8.33 ± 0.33 mm, respectively. Similar to CN EO, the CL EO also showed a significant increase of about 38% in ZOI as the concentration increased by two-fold from 25% to 50%. When comparing the antimicrobial effects between the CN and CL EOs, the CN5% solution produced much stronger antimicrobial effect than those observed in CL 50% solution at about a 10-fold lower concentration. The diffusion disks immersed in control DMSO solution did not produce any inhibitory growth responses on *E. faecalis*.

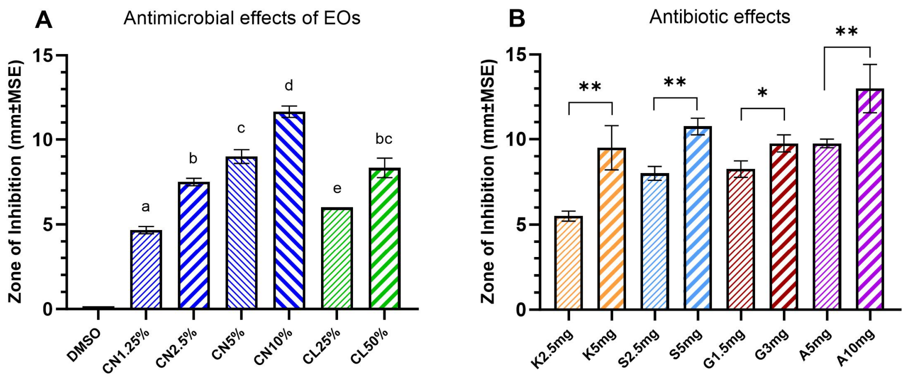

Figure 2. Antimicrobial effects of EOs ((**A**), cinnamon and clove) and antibiotics (**B**) on *E. faecalis* were quantitated using the disk diffusion test. The cinnamon (1.25% to 5%) and clove (25% to 50%) EOs showed significantly graded responses in inhibiting the growth of *E. faecalis* (Panel (**A**)). The cinnamon EO showed stronger antimicrobial effect than clove EO at much lower concentrations. Each antibiotic was diluted to 5% and 10% concentrations that produced a ZOI similar to that of EOs (**B**). This paired antibiotic concentration was selected for the Antibiotic + EO combination experiment. All antibiotics showed significant increase in ZOI between the 5% and 10% concentrations. CN = cinnamon, CL = clove, EO = essential oil, K = kanamycin, S = streptomycin, G = gentamicin, and A = ampicillin. Different letters (a to e) above the columns indicate significant difference between the groups ($p < 0.005$). * $p < 0.05$ and ** $p < 0.005$.

The Tukey post-hoc test showed significant paired differences between the cinnamon EOs as the concentration increased by two-fold (Figure 2A; $p < 0.005$). A similar statistical response was observed between the clove EOs where the CL50% had significantly larger ZOI than the CL25% (Figure 2A; $p < 0.001$).

3.2. Antibacterial Effects of Antibiotics

The effectiveness of four antibiotics (kanamycin, streptomycin, gentamicin, and ampicillin) in inhibiting the growth of *E. faecalis* was evaluated using the disk diffusion test. For each antibiotic, the stock solution was diluted in distilled water to 10% and 5% since these concentrations produced ZOI values that were similar in range with the ZOIs observed for CN and CL EOs tested previously (between 4 mm and 12 mm). The ZOI for 5% antibiotic solution of kanamycin (2.5 mg/mL), streptomycin (2.5 mg/mL), gentamicin (1.5 mg/mL), and ampicillin (5 mg/mL) were 5.50 ± 0.28 mm, 8.00 ± 0.40 mm, 8.25 ± 0.47 mm, and 9.75 ± 0.25 mm, respectively. The ZOI for 10% antibiotic solution of kanamycin (5 mg/mL), streptomycin (5 mg/mL), gentamicin (3 mg/mL), and ampicillin (10 mg/mL) were 9.50 ± 0.64 mm, 10.75 ± 0.25 mm, 9.75 ± 0.25 mm, and 13.00 ± 0.70 mm, respectively (Figure 2B). All antibiotics showed significant increase in ZOI between 5% and 10% concentration where kanamycin, streptomycin, and ampicillin showed robust increases (34% to 72%). There was significant difference among the antibiotic treatment groups ($F (7, 24) = 24.16$, $p < 0.0001$), and the Tukey post-hoc test showed significant paired differences between 5% and 10% for each antibiotic solution ($p < 0.01$).

3.3. Selection of Antibiotics and EOs Solution for the Combination Study on Inhibiting E. faecalis

All concentrations of cinnamon (CN10%, CN5%, CN2.5%, and CN1.25%) and clove (CL50% and CL25%) showed ZOIs that were comparable to the ZOIs of antibiotics (10% and 5%), and only CN10% solution had ZOI that was near the CLSI Intermediate Breakpoint for *Enterococcus* spp. Therefore, for the antimicrobial susceptibility test of combining EOs with antibiotics, we chose to combine the CN 5%, 2.5%, 1.25%, and CL50% for EO solutions with 5% antibiotic solutions as individually they produced the ZOIs that were below the CLSI Intermediate Breakpoint values.

3.4. Combined Antibacterial Effects of Antibiotics with EOs

There were significant increases in antimicrobial effects when 5% antibiotics (kanamycin (2.5 mg/mL), streptomycin (2.5 mg/mL), gentamicin (1.5 mg/mL), and ampicillin (5 mg/mL)) were combined with EOs (CN 5%, 2.5%, 1.25%, and CL50%). These enhanced antimicrobial effects were primarily observed when 5% antibiotics were combined with CN5%, where the combined solution produced ZOI that was larger than that of the corresponding 10% antibiotic solutions as well as their individual component solutions of 5% antibiotics and CN5% (Figure 3). For kanamycin, the ZOI of K2.5 mg + CN5% (13.00 ± 0.07 mm; $F (7, 36) = 19.44$, $p < 0.0001$) was significantly larger than the ZOI of K5 mg/mL (10% antibiotic; 9.50 ± 0.64 mm; $p < 0.05$) and CN5% (7.50 ± 0.22 mm) and K2.5 mg/mL (5% antibiotic; 5.50 ± 0.28 mm) individually ($p < 0.05$). For streptomycin, the ZOI of S2.5 mg + CN5% (12.38 ± 0.56 mm; $F (7, 36) = 47.67$, $p < 0.0001$) was significantly larger than the ZOI of S5 mg/mL (10% antibiotic; 10.75 ± 0.25 mm; $p < 0.05$), and CN5% (7.50 ± 0.22 mm) and S2.5 mg/mL (5% antibiotic; 8.00 ± 0.40 mm) individually ($p < 0.05$). For gentamicin, the ZOI of G1.5 mg + CN5% (11.75 ± 0.49 mm; $F (7, 36) = 44.14$, $p < 0.0001$) was significantly larger than the ZOI of G3 mg/mL (10% antibiotic; 9.75 ± 0.25 mm; $p < 0.05$), and CN5% (7.50 ± 0.22 mm) and G1.5 mg/mL (5% antibiotic; 8.25 ± 0.47 mm) individually ($p < 0.05$). For ampicillin, however, the ZOI of A5 mg + CN5% (13.00 ± 0.39 mm; $F (7, 35) = 63.19$, $p < 0.0001$) was not significantly different than the ZOI of A10 mg/mL (10% antibiotic; 13.00 ± 0.70 mm), but was significantly larger than its component solutions CN5% (7.50 ± 0.22 mm) and A5 mg/mL (5% antibiotic; 9.75 ± 0.25 mm) individually ($p < 0.05$).

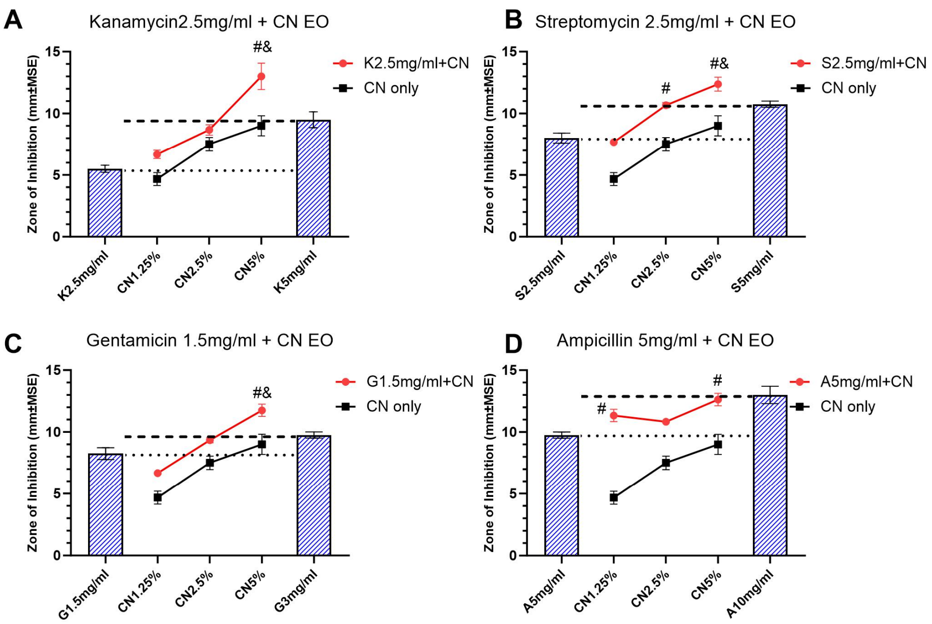

Figure 3. Enhanced and additive antimicrobial effects of antibiotics combined with cinnamon EO (solid red circle lines) in inhibiting the growth of *E. faecalis* in disk diffusion test. The dotted horizontal lines and shaded blue bars represent the average ZOI for 5% and 10% concentrations of antibiotics, and the solid squared lines represent ZOI for different concentrations of cinnamon EO alone. The kanamycin 2.5 mg/mL (5%) with CN5% (**A**) had significantly larger ZOI than its individual components (kanamycin 2.5 mg/mL or CN5% separately; dotted line and right solid square box) and kanamycin 5 mg/mL (10%). The streptomycin 2.5 mg/mL (5%) with CN5% (**B**) solution and gentamycin 1.5 mg/mL (5%) with CN5% (**C**) solution also produced significantly larger ZOI than their corresponding 10% antibiotics, CN5%, and 5% antibiotics separately. The ampicillin 5 mg/mL (5%) with CN5% (**D**) solution showed significantly larger ZOI only to its individual components but not to ampicillin 10 mg/mL (10%). #: $p < 0.05$ vs. 5% antibiotic concentration and corresponding CN EO individually; &: $p < 0.05$ vs. 10% antibiotic concentration alone.

For 5% antibiotic solutions combined with CN2.5%, all antibiotics failed to produce ZOI that was larger than their corresponding 10% antibiotic solutions on *E. faecalis*. Only streptomycin showed that the S2.5 mg + CN2.5% (10.67 ± 0.21 mm) had significantly increased ZOI compared to its individual component solutions of S2.5 mg (8.00 ± 0.40 mm) and CN2.5% (7.50 ± 0.22), but not to the 10% antibiotic solution. For 5% antibiotic solutions combined with CN1.25%, all antibiotics failed to produce ZOI that was larger than one of their corresponding component solutions.

For 5% antibiotic solutions combined with CL50% EO, there were no enhanced antimicrobial effects as the combined solutions did not produce ZOI that was significantly different from its individual component solutions nor from the 10% antibiotic solutions against *E. faecalis* (Figure 4).

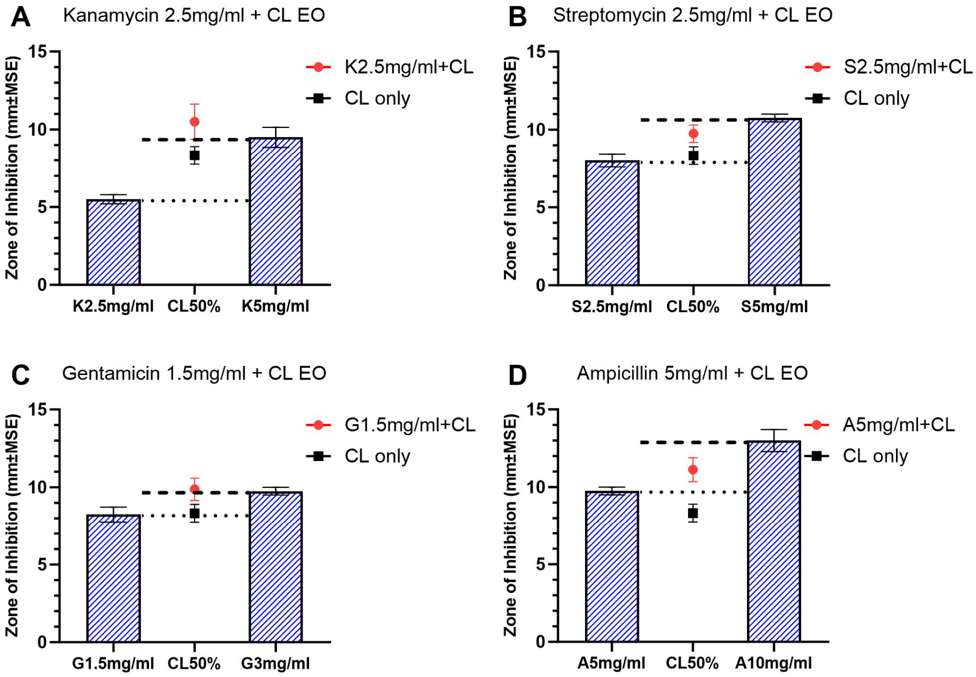

Figure 4. Antimicrobial effects of kanamycin (**A**), streptomycin (**B**), gentamicin (**C**) and ampicillin (**D**) combined with clove EO (solid red circles) in inhibiting the growth of *E. faecalis* in disk diffusion test. The dotted horizontal lines and shaded blue bars represent the average ZOI for 5% and 10% concentrations of antibiotics, and the solid squares represent ZOI for clove50% EO alone. Combining 5% concentration of antibiotics with CL50% did not produce any significant increase in ZOI compared to its individual components nor to 10% concentration of antibiotics against *E. faecalis*.

4. Discussion

The increased rate of prescription for antibiotics in recent decades, their prophylactic use, and the subsequent rise of antibiotic-resistant pathogens are changing prescription protocols for the use of antibiotics in medical and dental clinical settings and the search for alternate therapeutic medicinal compounds such as essential oils. In dentistry, endodontic diseases of periapical and intraradicular infections and their root canal treatments are becoming increasingly difficult to manage due to *E. faecalis*, a facultative aerobe that forms biofilms, survives in low-nutrient environments, and can resist antibiotics, in the root canal space. EOs, with their anti-inflammatory, antifungal, antimicrobial, and antibacterial properties, have been shown to enhance the antimicrobial effects against *E. faecalis* when combined with antibiotics and antiseptics. Given this, the examination of synergistic effects of antibiotics with EOs becomes highly relevant. In this study, we investigated the growth-inhibiting effects of penicillin and the aminoglycoside class of antibiotics when combined with cinnamon and clove essential oils in *E. faecalis*.

All concentrations of cinnamon and clove EOs were effective in inhibiting the growth of *E. faecalis* in a dose-dependent manner. The cinnamon EO produced greater antimicrobial effects than clove EO at about 10-fold lower concentration. For clove EO, the ZOI of CL25% was 7.5 ± 0.2 mm and increased by 139% when the concentration of clove EO was increased to CL50%. For cinnamon EO, increasing the oil concentration two-fold from 1.25% to 10% also increased the size of ZOI by about 36 ± 1.2% for each doubling of CN EO concentration. Our data support previous reports showing 1% to 10% of cinnamon and 50% of clove EOs were effective in inhibiting the growth of *E. faecalis* to almost 100% within 15 min of exposure, and with the cinnamon EO, the inhibitory effect was maintained for up to 10 days [48–50]. Marcoux et.al (2020) [46] reported that cinnamon EO (MIC 1.56 µg/mL) was equally effective on *E. faecalis* embedded in biofilm, killing over 90% within 15 min and outperforming chlorhexidine wash, which only showed 31% effectiveness.

For the antibiotics examined in the present study, all antibiotics were equally effective in inhibiting the growth of *E. faecalis* at 10% concentration with ampicillin having the largest ZOI. When the concentration was lowered to 5%, the streptomycin, gentamicin, and ampicillin still maintained strong antimicrobial effects against *E. faecalis*. Kanamycin 5% showed the least effectiveness with ZOI of 5.50 ± 0.28 mm. Our data are in line with a previous report where the MICs for ampicillin, gentamicin, and streptomycin were in a similar range of 8–14 µg/mL (MIC for kanamycin was 32 µg/mL) on an antimicrobial test against *E. faecalis*, and gentamicin was much more effective than kanamycin on an in vivo *E. coli* meningitis bacteremia test [51–53].

There were enhanced and additive synergistic antimicrobial effects against *E. faecalis* where 5% cinnamon EO combined with 5% antibiotics produced significantly larger ZOI than 10% antibiotics alone, as well as its individual components of 5% antibiotics and CN5% separately. Such additive synergistic effects were observed only with the aminoglycoside class of antibiotics tested (kanamycin, streptomycin, and gentamicin). There was also an enhanced antimicrobial effect when CN2.5% cinnamon EO was combined with 5% streptomycin (2.5 mg/mL), where the combined solution produced significantly increased antimicrobial effect comparable to that of 10% streptomycin (5 mg/mL) solution alone. Such enhancements were not observed with kanamycin/gentamicin/ampicillin combined with CN2.5% EO solution. The CN1.25% combined with 5% antibiotic solutions did not produce enhanced antimicrobial effects as their ZOI values were not significantly different than those for either one or both or their individual component solutions. For CL50% EO combined with 5% antibiotic solutions, there was no improvement in the antimicrobial effects of the combined solution beyond the effects observed for their individual component solutions.

The enhanced antimicrobial effects of combining antibiotics with cinnamon or clove EOs have been reported previously in Escherichia coli, pseudomonas aeruginosa, and in methicillin-resistant Staphylococcus aureus [54,55]. In our study, using the Kirby–Bauer disk diffusion test, we report that 5% concentration of aminoglycoside-class antibiotics (kanamycin, streptomycin, gentamicin) combined with 5% cinnamon EO produced significantly enhanced antimicrobial effect than 10% concentration of corresponding antibiotics alone against *E. faecalis*. The limitation of the present study is that since the MIC and the MBC values were not measured, it is not possible to define whether our enhanced antimicrobial observations show strictly synergistic or additive effects. However, based on our data, it may be reasonable to infer the presence of additive-like synergism where the 5% antibiotic + CN5% EO shows a significant increase in antimicrobial response compared to the 10% concentration antibiotic alone.

Acquisition of antibiotic resistance by *E. faecalis* is reported to be associated in part with its ability to synthesize β-lactamase, incorporate aminoglycoside-resistant genes aac(6′)-Ie-aph(2′′)-Ia and aph(2′)-Ib, and upregulate expression of low-affinity penicillin-binding protein Pbp5, and with the presence of ATP-binding cassette multidrug efflux pump EfrAB [56–60]. These adaptations, along with its ability to survive in biologically inhospitable environments, make *E. faecalis* an ideal candidate to thrive and persist at sites in and around endodontic infections. The antimicrobial and antibacterial properties observed in EOs, such as cinnamon and clove oils, involve disruption of bacterial genes, non-specific permeabilization of the cell membrane, and inhibition of transmembrane proton motif force and ATPase via anti-quorum sensing effects [32]. Our in vitro data show that the enhanced antimicrobial effects observed against *E. faecalis* by combining the antibiotics with cinnamon and clove EOs, presumably by interfering with the antibiotic-resistant cellular mechanisms, may be a suitable and practical approach to reduce the prevalence and incidence of persistent dental infections and treatment failures. Exploration of such strategies in in vivo and clinical studies to assess the efficacy, safety, and duration of their effects should be examined in future studies.

5. Conclusions

In conclusion, our study highlights the enhanced effectiveness of combining essential oils (EOs) with antibiotics in inhibiting the growth of *E. faecalis*, a pathogen associated with persistent dental infections and treatment failures. Our results show that both cinnamon and clove EOs, when tested alone, exhibited dose-dependent growth-inhibiting effects on *E. faecalis*, with cinnamon EO displaying superior efficacy at lower concentrations. Moreover, enhanced antimicrobial effects were observed when 5% cinnamon EO was combined with 5% aminoglycosides (kanamycin 2.5 mg/mL, streptomycin 2.5 mg/mL, gentamicin 1.5 mg/mL), where their combined effects were significantly stronger than the antimicrobial effects of corresponding 10% antibiotics alone (kanamycin 5 mg/mL, streptomycin 5 mg/mL, gentamicin 3 mg/mL) against *E. faecalis*. Our results demonstrate that the enhanced antimicrobial effects achieved by combining essential oils with antibiotics may be an effective strategy to maintain high antimicrobial effects while using a lower concentration of antibiotics. The antimicrobial properties of EOs by disruption of bacterial genes and cell membrane permeabilization may offer novel strategies to combat antibiotic-resistant pathogens such as *E. faecalis*. Future research should explore these strategies in in vivo and clinical settings to assess their safety, efficacy, and duration of action.

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First Experience of an Undergraduate Dental Student with a Reciprocating System in Simulated Root Canals—A Pilot Study

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Abstract: Rotary instrumentation has been proposed in undergraduate teaching. The aim of this study was to evaluate student's performance, through the obturation quality and treatment time, in a sequential range of L-simulated root canals. A senior undergraduate dental student sequentially prepared randomly numbered canals from 1 to 40, with the WaveOne Gold glider and primary file, according to the manufacturer instructions. A gutta-percha cone matched with the finishing instrument and epoxy resin-based sealer (AH Plus) was selected for the obturation. Three independent observers evaluated the obturation quality according to both density and length. Active, total instrumentation and obturation times were also measured. Statistical analysis was obtained by Mann–Whitney and Kruskal–Wallis tests with a significance level of $p < 0.05$. The quality of the obturation was independent of the number of prepared canals with adequate length and density in 87.5% of the prepared canals. Both active and total instrumentation, as well as obturation times, reduced significantly as the number of the prepared canals by the student increased ($p < 0.05$). The use of WaveOne Gold instrumentation and matched cone obturation by an inexperienced operator provided an adequate obturation quality in most of the curved simulated canals. The working time was significantly reduced through a short learning curve.

Keywords: dental education; root canal preparation; root canal obturation; root canal therapy; undergraduate student

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1. Introduction

With increased life expectancy, root canal treatment and novel endodontic therapy proposals have emerged with a great impact, enabling the retention of natural permanent teeth that otherwise would be lost. The aim of endodontic treatment (ET) is to remove pulp tissues, dentinal debris, and microorganisms, achieving the adequate conditions that will enable a proper filling and sealing of the root canal system. To reach this purpose, effective cleaning and shaping is essential. Over the years, mechanized instruments have been developed and commercialized to overcome the difficulties inherent to the conventional and rigid stain-steel (SS) manual instrumentation, simplify procedures, reduce the time required for root canal preparation, and offer a safer approach associated with less risk of errors and complications [1]. Amongst a myriad of new instruments and devices, the reciprocating movement is one of the most recently reported. It is a single-file system producing faster and centered shaping procedures, when compared to the traditional rotary instruments [2]. Not less important, for student's safety, is the single-use (reciprocating) file's concept, preventing cross-infection or reducing cyclic and torsional fatigue, as well as instrument's corrosion by irrigating solutions [3]. Thus, it is suggested as one of the most suitable systems for inexperienced operators, namely, undergraduate students [4]. There have also been reported contradictory findings, claiming that shaping procedures with ProTaper Next instruments, for instance, removed less resin from the curvature, showing a better centering ability than the reciprocating WaveOne Classic system [5]. The authors emphasized the different taper of the instruments (WaveOne 0.08, ProTaper Next 0.06) as the main factor for the different outcome. Nevertheless, improvements in the instrument designs to obtain greater flexibility are still emerging. WaveOne Gold (WOG; Dentsply Sirona) uses the same kinematics as its predecessor, WaveOne (classic) (WO; Dentsply Sirona, Ballaigues, Switzerland), but has some differences in design, size, and taper. The Wave One Gold instrument has a parallelogram cross-sectional design with two cutting edges incorporating four instruments: 21/06 (small), 25/07 (primary), 35/06 (medium), and 45/05 (large). A new reciprocating glide path instrument, the WaveOne Gold Glider, has also been introduced, with 0.15-mm tip diameter and variable 2%–6% taper. The innovative gold treatment increases the flexibility and resistance to cyclic fatigue and improves cutting efficiency [6,7]. It also allows a more conservative preparation, while maintaining the original shape of the root canal [8]. It is reported to be less prone to dentin removal during instrumentation [9], even in canals with high curvatures [10]. In addition, it enabled a faster canal preparation compared to other nickel–titanium (Ni–Ti) instrumentation systems, such as ProTaper Next [11].

Studies evaluating non-surgical ET, performed by specialists, report success rates of over 90%, with differences between initial/primary root canal treatments compared to non-surgical retreatments [12]. Regarding undergraduate students, it has been reported that the quality of endodontic treatment is poor, with unsatisfactory success rates, particularly in multirooted teeth [13,14]. The authors were unanimous in stressing the importance of introducing new teaching methodologies, techniques, and instruments into educational pre-clinical and clinical undergraduate curricula to improve general practitioners' confidence in root canal treatments. There is a growing trend of Ni–Ti rotary techniques taught in dental schools, with reports appearing nowadays of the clinical performance of Ni–Ti instruments operated by undergraduate students. Sonntag et al. [15] observed that undergraduate dental students could achieve better canal preparations with Ni–Ti, although file separations may still be the main concern for beginners that avoid using Ni–Ti rotary systems. Results from an audit at Queen's University Belfast on technical quality of root fillings performed by undergraduate students [16] concluded that in most of the teeth the technical quality of the root filling was acceptable, and students were exposed to an appropriate case mix for endodontic training. The use of machine-driven systems for instrumentation, the selection of primary ET, as well as the exclusion of ET with post-root filling or where only manual instrumentation was used, might have influenced the results. Hence the outcomes of similar audits have conflicting results [17–19].

The European Society of Endodontology (ESE) and the Association for Dental Education in Europe (ADEE) have published curricular guidelines to define and harmonize the skills of students by the end of graduation, emphasizing the requirement of a minimum pre-clinical and clinical training that allows future professionals to achieve the necessary quality in ET performance [20–22]. Several studies on the effect of operator experience using mechanized systems concluded that, despite the good performance obtained by inexperienced operators, the associated learning curve should be highlighted; they considered it a crucial factor for establishing the minimum amount of work required for students to obtain the necessary competence for independent professional practice [16,23–26]. Regarding the use of reciprocating systems, characterized by a simple and safer protocol [4,11], there are still few studies that evaluate the quality of the treatments performed by inexperienced operators, such as, undergraduate students, and the extension of its learning curve.

In this regard, this pilot study aimed to evaluate the quality of the endodontic treatments performed by an undergraduate dental student using the reciprocating motion (WOG) and obturating with a matched gutta-percha cone and epoxy-resin-based sealer, along the sequential preparation of 40 L-simulated root canals. It also had the purpose to determine the time required for each of the following stages: active instrumentation, total instrumentation and obturation, as the number of treated canals increased. Additionally, it had the purpose of tracing a learning curve of total instrumentation times/filling quality across the sequential preparation of the 40 canals.

The null hypothesis was that there would be no differences in quality of the fillings or treatment time of students' ET, using reciprocating instrumentation and filling with matched gutta-percha cones and sealer, as the number of treated canals increased.

2. Materials and Methods

2.1. Sample Selection and Preparation

A total of 40 transparent cubes with artificial L-shaped acrylic canals were used. Before treatment, each cube was photographed using a digital camera (Canon EOS 450D + EF-S 18–55 mm IS Lens, Tokyo, Japan). The total length of the canals was determined using a 10 K file, adjusting the stop when the file's tip was visible in the apical foramen. The only operator was a final-year student of the integrated Master's in dental medicine program, University of Porto, with no previous experience in continuous rotary or reciprocating instrumentation (his curricular pre-clinical teaching only included manual files). Before the study, the student received a short training that included written instructions about the reciprocating instrumentation system WOG. He also had to learn how to check patency, create a glidepath and do an ET in an L-shaped simulated acrylic canal, using the WOG glider instrument and the primary WOG file with all the WL filling it with the matched gutta-percha point. In the experimental assay, the cubes were randomly numbered from 1 to 40, sequentially prepared and obturated, following this order (1–40). To minimize the effect of operator's fatigue, one group of ten simulated canals were prepared in each session. After obturation, each cube was photographed, again using a digital camera (Canon EOS 450D + EF-S 18–55 mm IS Lens, Tokyo, Japan).

2.2. Canal Instrumentation

Canal instrumentation was performed using the WOG reciprocating instrumentation system according to the manufacturer's instructions. The patency of the canals was previously checked by progressing with a 10 K file (Dentsply Sirona, Charlotte, NC, USA) until it was visible at the apical exit ("apex")—patency length (PL). A mechanized glidepath was then created using the WOG glider file (Dentsply Sirona, Charlotte, NC, USA) with the working length (WL), which was determined reducing 1 mm to the PL. Between each file, the canals were irrigated with 96% alcohol using a 5 mL plastic syringe and a 27 G needle. The canals were instrumented with a WOG Primary file (25.07) (Dentsply Sirona, Charlotte, NC, USA), with pecking motion, advancing the file in an apical direction until resistance was offered. The file was then removed and wiped with a damp gauze, and the canal was irrigated with alcohol. According to the instructions for use, cleaning the file is essential for maintaining the cut's effectiveness and avoiding increased pressure exerted at the apical level, which increases the risk of fracture. This process was repeated until reaching the WL. A patency file (10 K) was used between the files with the PL. The final irrigation was performed with 1 mL of alcohol, and the canal was dried with paper cones.

2.3. Canal Obturation

A calibrated gutta-percha cone, WOG Conform Fit gutta-percha Primary (Dentsply Sirona, Charlotte, NC, USA), matching the taper and size of the last file used, a WOG Primary file (25.07) with the total WL, was selected and checked, visualizing its fitting through the transparent cube. In addition, the cubes were photographed with the correspondent gutta-percha cone inside each simulated canal; then, AH Plus Jet epoxy resin sealer (Dentsply Sirona, Charlotte, NC, USA) was introduced into the canal with a paper cone. The primary gutta-percha cone was inserted with slow movements, from coronal to apical until reaching the WL. A heated burner was used to cut and remove excess coronal material (gutta-percha and sealer), and a vertical condenser (Dentsply Maillefer, Tulsa, OK, USA) compacted the gutta-percha inside the root canal. Subsequently, all excess filling material outside the canal was removed with cotton wool soaked in 96% ethyl alcohol. Figure 1 shows a schematic view of the process.

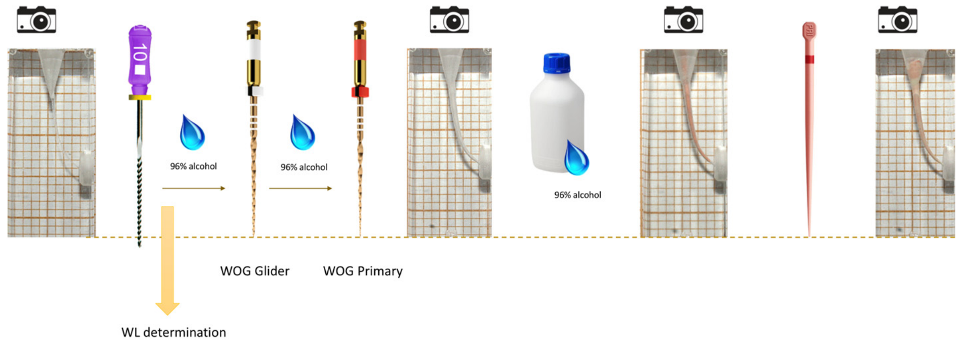

Figure 1. Schematic view of the experimental protocol.

2.4. Time Accounting

Each canal's treatment time was measured and recorded by order of preparation (1–40) in seconds, using a digital stopwatch with the help of an assistant operator. The treatment time was divided into two phases: a first phase corresponding to the biomechanical preparation (instrumentation and irrigation) and a second phase corresponding to the obturation. In the first phase, two parameters were recorded: the total and active instrumentation times. The total instrumentation time included from when the instruments began working inside the canal, to the changing and cleaning of the files and time for canal irrigation. The active instrumentation time referred only to when the instruments (10 K file, WOG Glider, and WOG Primary) were working inside the simulated canal. In the second phase of the treatment, the obturation time was recorded, including all the procedures from the main gutta-percha cone's selection and adjustment to the cut of excess gutta-percha/sealer, and canal entrance cleaning.

2.5. Obturation Quality Assessment

The obturation quality was evaluated according to the standard-of-care, density and length parameters. Density was classified as adequate in the absence of vacuoles and inadequate in the presence of vacuoles or heterogeneous canal filling. The obturation length was classified as short when more than 2 mm away from the apical exit, adequate when 0–2 mm from the apical exit, and long when material extended beyond the limits of the acrylic canal. Strict criteria were applied; that is, the obturation quality was only considered adequate when both length and density filled the requirements, and inadequate when any or both conditions were inadequate. Three independent observers assessed the obturation quality: two undergraduate senior students (final year students) and an experienced Endodontics professor. The inter-observer correlation, as well as the global agreement, were determined.

2.6. Statistical Analysis

Statistical data analysis was performed using IBM® SPSS® Statistics (SPSS® Version 26, Armonk, NY, USA). The following variables were analyzed: quantitative variables in the descriptive data study, including profile graphs and summary statistics tables; and differences in instrumentation and obturation times in the comparative study, including the Mann–Whitney and Kruskal–Wallis tests.

For the Mann–Whitney and Kruskal–Wallis tests, the sample was divided into four groups of ten canals: Group 1, canals 1 to 10; Group 2, canals 11 to 20; Group 3, canals 21 to 30; Group 4, canals 31 to 40. The decision rule consisted of detecting significant statistical evidence for probability values lower than 0.05.

3. Results

The obturation quality was adequate in 87.5% of the treatments performed, according to the established strict quality criteria (length and density) (Table 1). Table 2 shows the inter-observer and global agreement values.

Table 1. Analysis of the obturation length, density, and quality parameters.

Density (%)		Length (%)			Quality (%)	
Adequate	**Inadequate**	**Short**	**Adequate**	**Long**	**Adequate**	**Inadequate**
37 (92.5%)	3 (7.5%)	1 (2.5%)	38 (95%)	1 (2.5%)	35 (87.5%)	5 (2.5%)

Table 2. Inter-observer and global agreement.

	Global Agreement	(Obs1, Obs2)	(Obs1, Obs3)	(Obs2, Obs3)
Density	0.767	0.850	0.750	0.700
Length	0.917	0.950	0.900	0.900

The time variable showed statistically significant differences between the four groups ($p < 0.05$) (Table 3).

Table 3. Instrumentation and obturation time in seconds (s).

Time (s)		Group 1	Group 2	Group 3	Group 4	Total	*p* Value
Total instrumentation	Median	418.0	309.5	209.0	171.5	254.0	$p = 0.001$
	Min–Max	300–689	225–351	163–267	145–274	145–689	
Active instrumentation	Median	247.0	168.5	90.5	78.0	130.5	$p = 0.001$
	Min–Max	109–377	84–196	70–158	62–139	62–377	
Obturation	Median	137.5	104.5	85.0	76.5	96.5	$p = 0.001$
	Min–Max	114–167	86–135	75–98	68–83	68–167	

Multiple comparison tests between pairs of groups showed statistically significant differences between groups 1 and 3, 1 and 4, 2 and 3, and 2 and 4 regarding the total instrumentation, active instrumentation, and obturation times ($p < 0.05$) (Figures 2 and 3). As no differences were detected between groups 1 and 2, and between 3 and 4, it can be assumed that the time decreased from group 2 onwards and stabilized from group 3 onwards. Thus, the null hypothesis regarding the instrumentation time differences between the groups of prepared cubes was rejected.

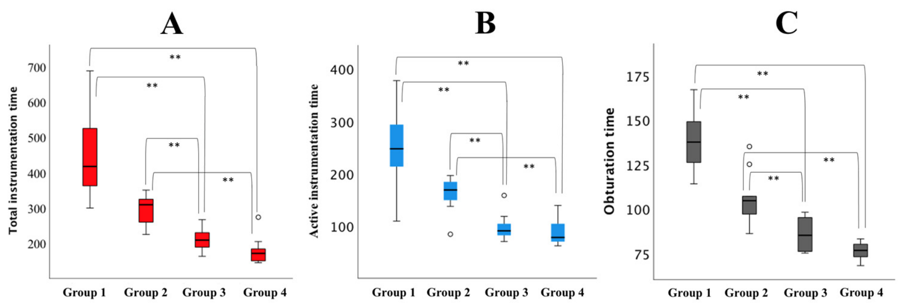

Figure 2. Multiple time comparisons between groups of 10 canals organized by preparation order. (**A**)—Total instrumentation time; (**B**)—active instrumentation time; (**C**)—obturation time. (** significant differences $p < 0.05$; "○"—outlier).

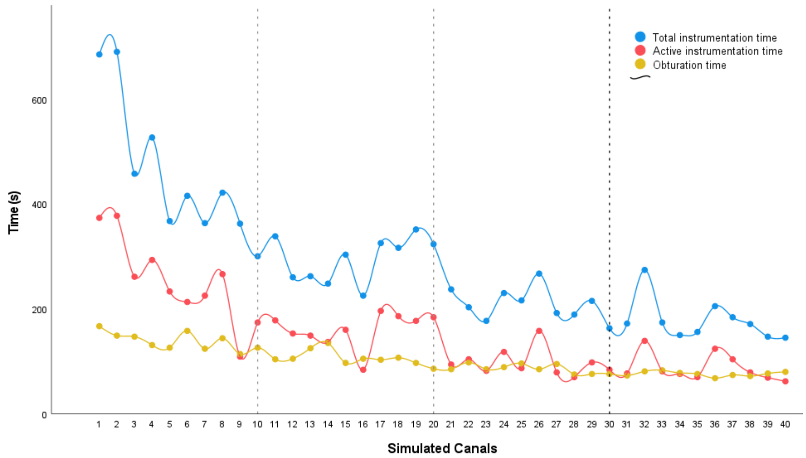

Figure 3. Profile graph of total instrumentation, active instrumentation, and obturation times per group (10 canals) by preparation order.

Concerning the obturation quality, there were no significant differences between the four groups of prepared simulated canals (1–10; 11–20; 21–30; 31–40) ($p > 0.05$), even though inadequate fillings were recorded, essentially in groups 1 and 2, associated with a longer preparation time (Figure 4). Thus, the null hypothesis regarding obturation quality differences between groups of acrylic cubes was verified.

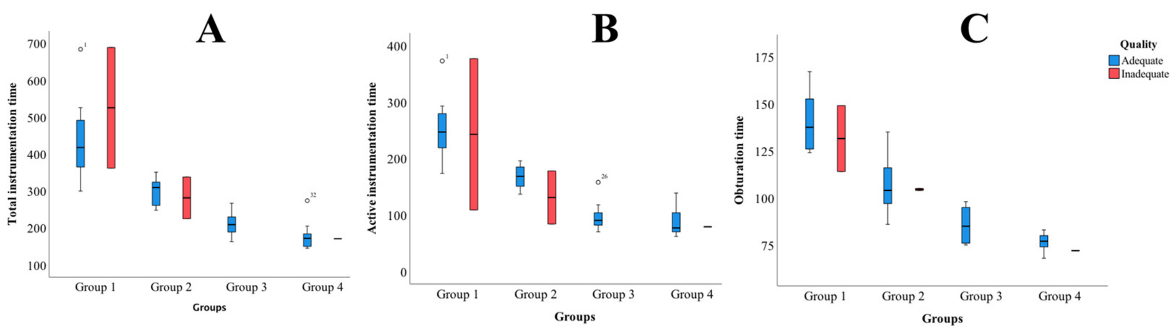

Figure 4. Multiple comparisons between treatment time and quality per group, by preparation order. (**A**)—Total instrumentation time; (**B**)—active instrumentation time; (**C**)—obturation time. ("○"—outlier).

4. Discussion

According to the guidelines provided by ESE, the assessment of the technical quality of root canal treatment focus on the length of the filling material (between 0.5 to 2 mm from the radiographic apex) and the homogeneity of filling density, with the absence of empty spaces, that must be interpreted radiographically [22]. Considering these strict criteria, 87.5% of the filling's quality performed by an undergraduate student were considered "adequate", with 95% and 92.5% presenting the required length and density, respectively. These values are relevant because the quality of the root canal fillings is associated with the root canal treatment's prognosis. Moreover, studies report that a poorer obturation quality is associated with a lower success rate, i.e., more prone to persistent post-treatment disease [27,28].

However, epidemiological evaluations of the technical quality and result/outcome of treatments performed have recognized the need to improve the performance of students and general dentists in endodontics' clinical practice [14,29,30]. Studies stress that, before graduation, students should be exposed to various clinical cases adapted to their level of training, to prepare for a future independent, but responsible, clinical practice. This will allow the acquisition of the necessary skills to adequately perform simple procedures. It is also essential that every agent know their limits and be aware of the need for continued learning throughout their lifetime [16,31]. Thus, introducing new materials and techniques in undergraduate teaching, as well as monitoring the results obtained, is imperative for a better outcome for future professionals, to provide the best and safest clinical practice for patients [14,29].

In this sense, the present investigation had the purpose of assessing novel strategies, such as the introduction of a mechanized instrumentation system within undergraduate teaching, through a pilot study, evaluating its performance in curved simulated canals. It was, thus, the specific purpose of the present investigation to evaluate ET's quality and time required for an inexperienced operator to perform root canal treatments using the reciprocating motion (WOG) and a matched gutta-percha cone associated to an epoxy-based sealer. The outcome of the 40 simulated canals, sequentially prepared, were compared. They were divided into four groups of 10, ordered from the first to the last (40th) canal prepared. With the results obtained, a profile graph was presented. It enabled an estimate of the minimum number of treated canals needed to stabilize the performance outcome of undergraduate students with reciprocating instrumentation. This could be traced only based on ET time spent by the student, as there were no differences regarding filling quality.

To increase the reliability of the results, three observers were considered, including two other final-year students of the integrated Master's in dental medicine and an Endodontics professor (observer 3). In this study, the simulated canals facilitated visualization, through photographs, evaluated by three previously calibrated observers. There was a high overall agreement between the observers, which was expected. This is because the evaluation was done through photographs of the transparent cubes, without the limitations of conventional periapical radiographs. Similar to other studies, the present investigation used artificial canals [11,26,32]. Although allowing a better standardization of shape, length, and degree of curvature, their properties are not identical to dentin, which may have influenced the results, and thus, be a limitation of the investigation. Other authors used extracted teeth and assigned scores to determine density by visualizing digital radiographs [33].

Concerning ET's evaluation, in the present study there were no significant differences regarding the obturation quality over the 40 sequentially prepared canals. Although the reduced number of canals with inadequate obturation was essentially recorded in the first two groups, i.e., amongst the first 20 filled canals, these was no significant impact on the overall obturation quality, between groups. Other authors [33] reported an increasing proportion of adequate density and length of the fillings as the number of treated canals increased. This divergence may be explained by the type of samples, such as natural extracted teeth, with the inherent anatomical variability, whereas, in the present study, the ET was performed in standard simulated curved root canals. Accordingly, Fong et al. [16], in a retrospective evaluation of clinical cases performed by students, attributed the worst result regarding lateral adaptation and obturation length to anatomical variations.

The main finding herein was that, as the number of treated canals increased, the total and active treatment and filling times decreased, showing a corresponding profile as a "learning curve". Similarly, in a previous study [11] with one senior student, regardless of the instrumentation system used and continuous or reciprocating movement, a significant decrease was reported in the time spent in active instrumentation between sequential groups of 10 teeth. In fact, in both studies, the relationship between training and learning showed a significant initial improvement (i.e., a time decrease with relevance after the first 20 canals) that stabilized along the remaining ones. This minimum number of 20 canals prepared to achieve the required competency for ET, performed by graduating students, has also been corroborated in a very controlled study with four fourth-year undergraduate dental students [33]. Using naturally extracted teeth, the results reinforced that machine-driven files result in an overall better quality of root canal treatments, independent of the Ni–Ti system used. In addition, the total treatment time was clearly reduced with increasing number of ETs, due to shorter time spent not only on preparation, but also on filling procedures. Similar to our findings, the reduction in the operation time was primarily seen from the first to the second session, i.e., from the first 20 extracted teeth treated. Additionally, before the experiment, operators participated in only one calibration session concerning the manuals of the treatment systems. Results from ex-vivo studies have also already emphasized the shorter treatment time associated with rotary instrumentation compared to manual instrumentation, particularly when using systems with fewer files (e.g., WaveOne, Reciproc) [2,25,33].

It must be emphasized that the senior student herein had a previous short training of root canal treatments in his pre-clinical curricular path, similar to other studies where they are referred to as novice [4]. Although it is advisable to expose students in their pre-clinical stage to new developments in Endodontics, such as rotary instrumentation, due to several factors (e.g., high number of students, difficulty in natural extracted teeth selection or COVID-19 impact), senior students actually are left with a short pre-clinical experience, anxious to have the opportunity to experience all sorts of advancements in Endodontics. They realize that they will need these tools in the short term, as future professionals.

In that sense, it can be assumed that the findings of the present pilot study are in line with the literature and should be considered for implementing mechanized instrumentation in undergraduate curricula in Endodontics.

Contrary to the present study, in the referred study by [33], the proportion of adequate ratings of length and adequate seal of root canal fillings also improved with increasing experience. Nevertheless, differences in the methodology in respect to the evaluation of the filling seal through radiographs and scores, as well as the use of naturally extracted teeth, might have influenced the results. It was also reported that systems using fewer files were less time-consuming. Instead, a previous study of our group did not find differences between single-file or multi-instrument systems, emphasizing that the creation of a glidepath, manual (10 K SS-file) or mechanized (ProGlider), can influence the results, [11]. Furthermore, differences in methodology, such as considering manual (K-flex stainless steel files) and several machine-driven systems, such as ProTaper Universal, ProTaper Next and Wave One, may account for the differences [16]. Additionally, apart from reporting that the manufacturer's manual was followed, there is no mention of the creation of a glidepath or maintenance of patency during the root canal preparation, as stressed in the present work. The promising findings of this pilot study justify implementing the experience with a larger number of students, to evaluate the impact of several factors, such as teaching methodology and training levels, as well as different assessment criteria for evaluation.

The existence of a short learning curve, using a reciprocating system, was evidenced in the present study, with significant time-reduction in instrumentation, after the preparation of 20 L-simulated root canals. In addition, a good filling quality was generally registered. This has been also corroborated by other investigations, either with experienced operators or students [32]. Other characteristics inherent to these systems, such as, being "single-use" systems, with a reduced risk of cross-infection and instrument fracture, improves the safety of the procedures [3,4]. Moreover, students seem to prefer the "single-file" systems with a smaller number of instruments due to their ease of learning [4].

Despite the recommended "single use" of some instrumentation systems, mechanized files are generally used, in clinical settings, in more than one canal, such as in the case of multirooted teeth, being reported as very safe instruments [6,34]. In the present investigation, the instruments, according to similar studies, were discarded after five uses, which means five simulated canals [2,34], its use reported by some studies until five teeth [33]. In any case, the lack of variability between the simulated canals anatomy did not allow to infer other factors that could influence a potential wear that could impact the treatment quality [34].

Technological advances in materials and the greater predictability of endodontic nickel–titanium instruments compared to stainless steel instruments have reduced procedural errors and complications in endodontic treatments [2,32,35]. In the present work, no iatrogenic errors were detected. Other novel materials recently proposed for root canal filling, such as the hydraulic calcium silicate-based endodontic cements [36], represent a new challenge to search for the interaction of specific materials and proper clinical protocols. The need for standardization prevented its study in the present undergraduate setting.

In the present pilot study, the emphasis on the short learning curve was directly corelated with an improvement in treatment (instrumentation and filling), concerning time. This aspect alone should not be the primary aim in the search for ET quality, even though improving any technical procedure implies training periods to acquire the needed skills. In this sense, the stabilization of the treatment time after 20 preparations supports the previous curricular guidelines for the undergraduate teaching of Endodontics [37], recommending a minimum of 20 root canal treatments before graduation, clinical or pre-clinical, to achieve the minimum competences in the ET of simple cases. Although the current guidelines [22] still value a minimum skill acquisition, they specifically emphasize that each student may require a different work effort to achieve the same skills. Thus, it may not be possible to strictly suggest an exact number, or the same number of acts required in a standardized way.

The present results have the major limitation of reflecting only the path of a single operator, besides the unique use of simulated canals in resin blocks. Therefore, the results cannot be directly extrapolated to determine a minimum number of canals prepared to achieve the stabilization of the learning curve. However, the corroboration of a previous investigation with a similar methodology [11], and the former ESE guidelines suggesting a minimum of 20 canals prepared, highlight the present pilot test results to implement mechanized instrumentation in the pre-clinical teaching of undergraduate students. Additionally, the positive clinical performance in ET by undergraduate students with mechanized systems reinforces this teaching [16].

The low quality of ET in populations may lead to the speculation that the conventional manual instrumentation needs a longer undergraduate training, not being able to produce the desired learning outcomes at the end of graduation. Thus, the introduction of more predictable instrumentation and obturation techniques should be further implemented and assessed for a better prognosis of root canal treatments in populations.

5. Conclusions

In the present conditions, the quality of the obturation performed by an inexperienced operator after NiTi reciprocating instrumentation in simulated curved canals was adequate, in most of the cases. This quality was not particularly affected by the number of canals prepared. A short learning curve translated into a decrease in total treatment time, including instrumentation and filling procedures, stabilizing after the first 20 prepared canals. Thus, the WaveOne Gold system and the obturation with a matched gutta-percha cone and epoxy resin-based sealer were presented as safe and predictable procedures, independent of the experience of the operator. Pre-clinical and clinical practice for undergraduate students should be further implemented and investigated, to improve the technical quality and outcome of the endodontic treatments performed by future professionals for the general population.

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Validation of a Suggested Pre-Operative Protocol for the Prevention of Traumatic Dental Injuries during Oroendotracheal Intubation: A Pilot Study

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Abstract: The aim of this study was to develop a protocol for oral pre-operative registration and dental risk assessment by the anaesthesiologist, determining its reliability through the inter-observer agreement between a senior dental student and an internal physician specializing in anaesthesiology. A convenience sample consisting of 35 patients was selected. These were observed during the anaesthesiology consultation, at Hospital de São João, Porto, Portugal. The protocol included a self-administered questionnaire and a brief clinical examination by the two observers. A descriptive analysis (qualitative and quantitative variables) was performed. The Fleiss Kappa index was used to measure the degree of agreement between the two observers. In most of the parameters defined, the agreement presented Kappa index values between 0.6 and 1, corresponding to good and excellent correlation, respectively. The general oral status was considered "poor", with a great number of missing teeth, namely the upper central and lateral incisors. The proposed pre-anaesthetic protocol can be a reliable tool for the anaesthesiologists, which suggests the relevancy of incorporating interdisciplinary training between future health professionals. Further research is needed to assess its implementation, providing information about the pre-operative oral status, preventing intraoperative damage and potential medicolegal litigation.

Keywords: dental trauma; dental injury; endotracheal intubation; general anaesthesia; pre-anaesthetic protocol

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1. Introduction

The Association for Dental Education in Europe (ADEE) has recently reclassified four major domains or competences, namely: (i) Professionalism, (ii) Safe and effective clinical practice, (iii) Patient-centred care and (iv) Dentistry in Society. Hence, periodic curriculum reviews are advised to incorporate realistic environments for students with assessable criteria developed for undergraduate and post-graduate education. As an example, pilot studies can have a particular role, highlighting the need to improve awareness and implement preventive measures in a model of "holistic, evidence-based patient care to support the oral and general health of patients" [1]. To bridge between dental and medical practices, mutual undergraduate training can be an instrument to improve practitioner competence. Aside from mutual recognition, this might lead to reduced morbidity and litigation due to clinical procedures in their autonomous practice, such as intubation anaesthetic procedures. These main principles in dental education should extend to both pre- and post-graduate programs, and the mission must not only focus on training achievements but also enabling the direct provision of patient care [2]. However, dental students have been, in the last few decades, allocated to dental institutions, which work independently from hospital environment. This prevents a regular discussion of clinical cases with emphasis on oral health as an integral part of patients' global welfare.

Scientific literature is scarce respecting dental trauma in patients undergoing general anaesthesia. Besides being a relevant cause of litigation, it can be enlightening of the lack of awareness between different professionals, such as dentists and anaesthesiologists. The incidence of dental and hard tissue injuries undergoing oroendotracheal intubation, for instance, have an estimated prevalence of 0.02% to 0.7% [3–8]. Other studies, however, report values of 12.1% [9]. These include enamel or crown fracture, damage to restorative or prosthetic rehabilitations, luxation or avulsion, as well as soft tissues injuries [8]. Despite the eventual low incidence, they are one of the main causes of medical negligence against the anaesthesiologists, occurring mainly during the act of classical laryngoscopy. Moreover, their aesthetic and/or functional consequences, as the social impact, are extremely important [3,4,7]. The anterior sextant of the maxillary region, more specifically the central maxillary incisors, is the most affected [8]. In fact, the left central incisor is reported to be the tooth with the highest risk of dental injury, due to the direct contact of the laryngoscope blade as well as its use as a fulcrum in order to position the laryngoscope [4,7,8,10]. Although the most frequent situation is that only one tooth is affected, traumatic injuries often occur simultaneously in two or more teeth [3,4,10]. The dental condition and the difficulty inherent to the intubation procedure are well known risk factors listed in the literature. Poor dentition stands out, including the absence of a great number of teeth, the huge presence of caries, extensive fillings, crown fractures, tooth mobility and limited degree of mouth opening [3,7,8,11,12]. Furthermore, a higher Mallampati score is generally correlated with an increased incidence of operative dental trauma [8,10,11].

In order to avoid possible medicolegal disputes, several authors have suggested a systematic documentation of the pre-operative patients' dentition and of the associated accidents [4,7,8,11–13]. Corroborating this need is the awareness that dental factors increase the susceptibility to trauma, accounting for 40% of injuries [14]. However, there are few publications on registration strategies that support the implementation of effective pre-operative measures to prevent damage, namely through the laryngoscopy procedures. A succinct protocol of the pre-operative registration that allows a more objective assessment of the oral status, which is susceptible to be regularly used by the anaesthesiologists in patients scheduled to general anaesthesia, could contribute to reducing the risk of dental injury and potential medicolegal disputes. The objective of the present study was thus to develop a dental risk assessment protocol for regular pre-operative anaesthesiologists' registration. For this purpose, specific parameters of the oral status were selected and a correlation between two observers, a final-year student of the Integrated Master of Dental Medicine and an internal physician specializing in anaesthesiology, were analysed.

2. Materials and Methods

The study was approved by the Ethics Committee of the Hospital Center of São João/Faculty of Medicine of the University of Porto, Portugal. Written informed consent was obtained from all participants after an oral explanation of its objectives and respective procedures. The investigation took place at a university hospital. The data collected refer to patients who attended the anaesthesiology consultation between the 24th of January and the 27th of February 2020, and who met the inclusion criteria. These included patients over 18 years of age who had scheduled surgeries and underwent general anaesthesia requiring tracheal intubation. All patients with an inability to provide informed consent and patients who refused to participate in the study were excluded.

The elaborated protocol included a self-administered questionnaire to patients, and a form for a clinical record of oral-dental evaluation outside of the dental setting. After a brief training on observation and oral registration given by the senior dental student to the anaesthesiologist intern, the data collection was carried out by both the dental student and by the anaesthesiology trainee, filling the clinical record form in duplicate. The training, given by the dental student, comprised didactic lectures and the required maxillary and mandibular teeth nomenclature and identification, enabling a comprehensive knowledge of relevant dental and periodontal diseases. In the same sense, normal and pathological features of soft tissues that could be wrongly perceived as injuries due to intubation procedures were stressed. Additionally, a hands-on simulation was performed with a small sample of patients, with a demonstration by the dental student, of basic diagnostic procedures such as inspection of dental structure integrity, caries detection and periodontal probing. These two observers, with different pre-graduate backgrounds, were used to assess the agreement between each set of data to investigate if this form could be an adequate tool for an autonomous and reliable oral examination registration by the intern. The self-administered questionnaire was based on a published survey [15], which included four questions related to sex, age, weight and height, and 13 questions grouped into different categories: oral hygiene behavioural habits, specific systemic diseases and factors of dental risk fracture, such as a recent dental trauma or the presence of implants. It also addressed the sociodemographic characterization and medical history, oral hygiene routines, main reasons for dental medicine consultations and date of the last visit to the dentist. The questions related to the patients' self-perception regarding their oral health focused on the presence of pain on chewing, gingival bleeding, tooth mobility and number of missing teeth.

The clinical examination was performed and registered in the form, including the evaluation of ´index teeth´ considered to be at higher risk of injury during the intubation procedure—that is, the upper teeth 12, 11, 21, 22, 23 and one of the following teeth of the lower jaw, 32/31/33 [4,7,8,10,11,16]. From the latter, the tooth with the greatest mobility and therefore of the greatest periodontal involvement was chosen. When only one of these three lower teeth was present in the arch, that same tooth was then elected to be assessed. The evaluation included the following parameters: mobility, bleeding on probing, presence of neighbouring teeth, caries, restorations, malformations, removable or fixed prosthesis, presence of deciduous tooth, implant, orthodontic treatment, dental fracture, and history of trauma. Two other parameters were also evaluated: the protrusion of the upper incisors and the Mallampati score. This parameter is usually assessed in the anaesthesiology consultation, given the importance of airway valuation in order to predict the difficulty of intubation, which may be also reflected in dental trauma [4,7,8,11,13]. The protrusion of the upper incisors was assessed, due to its association to a higher risk of dental injury, by measuring the overjet—the distance between the upper and lower incisal edges of occluded maxillary and mandibular incisors—with a ruler [8,10,11]. Finally, the protocol also included the odontogram registration with the identification of missing teeth for a better understanding of the patient's general oral condition.

In order to classify the general oral status of the sample, only the data from the clinical examination forms collected by the dental student were used.

Statistical analysis of the data was performed using IBM® SPSS® Statistics (Version 27.0). A descriptive analysis (qualitative and quantitative variables) was performed. The Fleiss Kappa index was used to measure the degree of agreement between the two observers regarding the studied clinical parameters. The concordance results were classified according to the levels presented in Table 1 [17].

Table 1. Classification, by levels, of the agreement results [17].

Kappa Value	
0–20	Null or slight agreement
0.21–0.40	Considerable
0.41–0.60	Moderate
0.61–0.80	Good
0.811–1	Excellent

3. Results

3.1. Analysis of the Questionnaire

Regarding the sociodemographic data of the sample, 54.3% of the patients were male and 45.7% female. The sample had an average age of 62.78 years with a standard deviation of 10.46, with ages between 35 and 89 years. The average weight was 77.49 KG, with a standard deviation of 23.69, and the average height was 161.97 cm, with a standard deviation of 18.65. Table 2 shows the percentages and frequencies corresponding to each answer given to the 13 questions in the questionnaire.

Table 2. Patients' responses to the pre-anaesthetic evaluation questionnaire.

	n	%
How frequently do you use mouthwash?		
More than once a day	0	0
Once a day	3	8.6
Sometimes	5	14.3
Never	27	77.1
How often do you brush your teeth?		
3 or more time a day	2	5.7
1\2 times a day	22	62.9
Sometimes	4	11.4
Never	7	20
When was the last time you went to the dentist?		
Less than 1 year	6	17.1
1 year ago	12	34.3
More than 1 year ago	17	48,6
More than 2 years ago	0	0
What was the reason for your last visit to the dentist?		
Check-up/cleaning	6	17.1
Fillings/root canal treatment	5	14.3
Tooth extraction	18	51.5
Placement of a crown, bridge or prosthesis	6	17.1
How many teeth do you have missing?		
None	3	8.6
1\2 teeth	1	2.8
More than 2 teeth	14	40
Most teeth	17	48.6
Do you experience gum bleeding?		
Never	13	37.1
Sometimes	21	60
Very often	1	2.9
Always	0	0
Do you experience pain on chewing?		
Never	25	71.4
Sometimes	10	28.6
Very often	0	0
Always	0	0
Do you experience tooth mobility?		
No	27	77.1
Only 1 tooth	8	22.9
2\5 teeth	0	0
Almost all teeth	0	0
Do you have a recent dental trauma?		
No	35	100
Yes, in the posterior teeth	0	0
Yes, In the anterior teeth	0	0
Do you have any implant?		
No	35	100
Yes, in the posterior teeth	0	0
Yes, In the anterior teeth	0	0

3.2. Correlation between Clinical Examination by the Two Observers

The results regarding the statistical evaluation of the agreement between the two observers, according to the Fleiss' Kappa index, are listed in Tables 3 and 4. The greatest discrepancies between the two observers were found in the following parameters: presence of "caries lesions" and "restorations". Regarding the parameters "malformations", "removable prosthesis", "deciduous tooth" and "implant", there was no variability and therefore it was not possible to calculate the Kappa index (marked in the Table 4 as NA—Not applicable). Regarding the registration of the number of missing teeth in the odontogram, Table 4, a moderate agreement was obtained, whereas for the protrusion parameters of the upper incisors and the Mallampati scale, the agreement was excellent.

Table 3. Results of interobserver agreement according to the Fleiss' Kappa index for each of the analysed parameters. (CI—Confidence interval; NA—Not applicable).

Tooth	11	21	22	12	23	31/32/33
Mobility	1.000 (CI 95%: 0.989–1.000)	1.000 (CI 95%: 0.986–1.000)	0.738 (CI 95%: 0.724–0.751)	0.778 (CI 95%: 0.767; 0.789)	1.000 (CI 95%: 0.986–1.000)	1.000 (CI 95%: 0.990–1.000)
Bleeding	0.827 (CI 95%: 0.817–0.838)	0.672 (CI 95%: 0.662–0.682)	0.915 (CI 95%: 0.905–0.925)	0.918 (CI 95%: 0.907–0.928)	0,847 (CI 95%: 0.837–0.857)	0,887 (CI 95%: 0.879–0.896)
Missing teeth	1.000 (CI 95%: 0.986–1.000)	1.000 (CI 95%: 0.986–1.000)	1.000 (CI 95%: 0.989–1.000)	0.770 (CI 95%: 0.755–0.784)	1.000 (CI 95%: 0.986–1.000)	0.910 (CI 95%: 0.901–0.920)
Dental caries	0.553 [0.542; 0.564]	0.506 [0.495; 0.517]	0.250 [0.239; 0.261]	0.101 [0.088; 0.113]	−0.026 [−0.040; −0.012]	0.565 [0,554; 0.575]
Fillings	−0.027 (CI 95%: −0.041; −0.014]	0.510 (CI 95%: 0.499–0.520)	0.780 (CI 95%: 0.769–0.791)	0.638 (CI 95%: 0.624–0.652)	0.779 (CI 95%: 0.768–0.790)	0.523 (CI 95%: 0.513–0.533)
Malformations	NA	NA	NA	NA	NA	NA
Removable prosthesis	NA	NA	NA	NA	NA	NA
Fixed prosthesis	1.000 (CI 95%: 0.986–1.000)	NA	NA	1.000 [0.986; 1.000]	NA	NA
Deciduous teeth	NA	NA	NA	NA	NA	NA
Presence of implants	NA	NA	NA	NA	NA	NA
Orthodontic treatment	1.000 (CI 95%: 0.986–1.000)	1.000 (CI 95%: 0.986–1.000)	1.000 (CI 95%: 0.986–1.000)	1.000 (CI 95%: 0.986–1.000)	1.000 (CI 95%: 0.986–1.000)	1.000 (CI 95%: 0.988–1.000)
Dental fracture	1.000 (CI 95%: 0.989–1.000)	1.000 (CI 95%: 0.989–1.000)	1.000 (CI 95%: 0.986–1.000)	1.000 (CI 95%: 0.986–1.000)	NA	NA
History of trauma	1.000 (CI 95%: 0.986–1.000)	1.000 (CI 95%: 0.986–1.000)	NA	NA	NA	NA

Table 4. Results of interobserver agreement according to the Fleiss' Kappa index in relation to the protrusion of the upper incisors, Mallampati scale and registration of the number of missing teeth (odontogram). (CI—Confidence interval).

	Kappa de Fleiss	CI 95%
Protrusion I sup.	0.960	(0.953–0.966)
Mallampati scale:	0.870	(0.862–0.877)
Odontogram	0.480	(0.477–0.483)

3.3. Assessment of Oral Status

The data presented was collected from the clinical examination form registered by the dental student for pre-operative oral status assessment:

A. Protrusion: Through the measurement of the overjet, a percentage of 37% corresponded to the measurement of 2 mm; 6% of the patients had an overjet greater than 4 mm.

B. Mallampati scale: 46% of the patients presented a score 1, 26% a score 2 and 29% a score 3.
C. Missing teeth: With regard to the upper incisors, 46% of the patients observed did not have both teeth 11 and 12 and 40% did not have teeth 21 and 22. Tooth 23 was absent in 43% of patients. In the lower jaw, 26% of patients did not present any of the three teeth selected for evaluation (teeth 31, 32 or 33).
D. Mobility: No vertical mobility was registered—that is, grade 3 mobility was registered.
E. Bleeding: The observed patients, presented in the great majority, bleeding shortly after probing.
F. Odontogram: An average of 17 isolated teeth were registered. In total, 43% of the patients had an average of 26 missing teeth.
G. Dental caries: In total, 60% of patients presented caries on tooth 11, 51% on teeth 21 and 22, 54% on tooth 12, 46% on tooth 23 and 34% on at least one of teeth 31/32/33.
H. Malformations: No malformed teeth were detected, such as teeth with dentinogenesis and amelogenesis.
I. Removable prosthesis: All patients who presented removable prosthesis in the 'index teeth' were informed by the anaesthesiologist that on the day of surgery, the prosthesis would be removed. In this case, the respective teeth were included in the parameter "removable prosthesis on that tooth".

Thus, the following percentages were obtained, corresponding to the filling option "removable prosthesis in this tooth": 50% (tooth 11), 60% (tooth 21), 60% (tooth 22), 54% (tooth 12), 57% (tooth 23), 74% (tooth 31\32\33).

J. Fixed prosthesis: The presence of fixed prostheses, namely ceramic crowns, were observed in tooth 11 (3%) and tooth 12 (3%).
K. Presence of deciduous teeth: The presence of deciduous teeth was not observed.
L. Presence of implants: The presence of implants was not observed.
M. Orthodontic treatment: A percentage of patients with brackets, accounted for as the presence of orthodontic treatment, was registered in 3% of the cases.
N. Dental fracture: In total, 3% of the teeth observed were registered as 'dental fracture': enamel fracture in teeth 11, 21, 22 and 12, and enamel fissure in teeth 11 and 21. The history of trauma with dental injury was recorded in teeth 11 and 21, in a total percentage of 3%.

The general oral status of the sample was considered "poor" based on the criteria reported in the literature, mainly justified by the number and type of the missing teeth, namely upper central and lateral incisors, gingival bleeding after probing, high number of caries lesions and marked mobility in the index teeth.

4. Discussion

The present investigation made it possible to meet the defined objectives, namely the development of a succinct clinical evaluation protocol of registration strategy for a routine dental examination in every patient scheduled for general anaesthesia. The reliability of this protocol was demonstrated by the high correlation values obtained between the two observers, a senior dental student and an internal physician specialised in anaesthesiology. The "presence of orthodontic treatment", "dental fracture", "history of trauma" and "presence of fixed prosthesis" are examples that stood out due to the excellent agreement achieved. It should be noted that the interobserver accordance was only lower in the situations of "restorations detection" and "caries lesions"; it is understandable that caries lesions without enamel destruction, for example, are hardly detectable without the ideal conditions of lighting in a dental clinic. Furthermore, changes in colour or indirect signs such as swelling or bleeding in the adjacent gingiva to the carious lesion are difficult to detect by a professional without dental experience. The same is true for small restorations with aesthetic materials. Contrary to the presence of isolated or fractured teeth, which can be clearly perceived, major aesthetic restorations and periodontal involvement were also reported as risk factors for dental injury in the anaesthetic act and may present higher diagnosis difficulties for professionals without dental training or the ideal logistical conditions [3,7,8,11–13]. Thus, it is imperative to define clear oral parameters representative of the potential risk that endotracheal intubation can imply and of reliable application by the anaesthesiologists prior to surgical procedures. For this, the brief training of the intern anaesthesiologist on oral observation and registration proved to be essential, as well as the selected strategy of identification and evaluation of 'index teeth'. These are mentioned as representative of the risk of dental injury during endotracheal intubation procedures [4,7,10,11,16].

Patients with worse dentition status, with prosthetic work or more difficult airways, are reported as having a higher risk (approximately 20× higher) of dental damage than those who present "good dentition" and an "easier" airway [18]. Moreover, among patients classified as "easy to intubate", in those with "worst dentition", the probability of suffering dental injuries related to anaesthesia was three to four times higher [18]. Other authors highlight the pre-existing periodontal disease as the most likely cause of dental injury. It is also mentioned that, in patients with Angle Class II, Division 1 malocclusion, in which there is a pro-inclination of the central maxillary incisors, the risk of dental injury may increase. Recent studies reveal that in 90% of the patients with class I malocclusion, Angle division II and Mallampati score 3, there is contact between the blade and the teeth [8]. Thus, pre-surgical anaesthetic evaluation is reported as one of the relevant approaches that should be taken to minimize the risk of dental injury.

Through the data collected by the questionnaire, 20% of the patients who underwent surgery never brushed their teeth, the majority (77.1%) never used a mouthwash and nearly 50% had only been to the dentist as recently as 1 year ago, many of them for tooth extraction. Dental mobility was not a complaint reported by most patients, which can be explained by the reduced number of teeth present in the mouth. These data allowed us to anticipate that we were present to a population with a critical/poor oral status. This was confirmed by the clinical observation and reflected in an average of about 18 missing teeth per individual, a generalized bleeding on probing and a significant number of isolated teeth. A pre-existing poor oral condition, corroborated by the present findings, is considered to increase the risk of dental damage in the general anaesthesia procedure by 12×, leading the authors to emphasize the need for a careful risk assessment and pre-operative guidelines for the most critical teeth [18]. In this sense, the effect of a pre-operative protocol on the increased awareness of the patients is considered crucial in dental trauma prevention and consequently in the grade of litigious situations. Patients can still be advised to consult their dentist whenever severe dental pathology is detected, which eventually may require changes in the planned intubation route [4,6,7,15]. Apart from caries lesions treatment, mobile teeth splinted or extracted, and the use of protective devices such as mouthguards, although controversial, may be recommended. Despite this, none of these isolated preventive measures will guarantee the absence of oral or dental injury. Although all the professionals involved are advised to give the adequate information about the risks of dental trauma to the patient, it is the responsibility of the anaesthetist to ensure the safety of the procedure.

It is also claimed that anaesthesiologists must have a detailed knowledge about anatomy and dental development and be aware of the main risk factors for dental injury [16,19,20]. The moderate correlation in the odontogram registration, founded in the present study, emphasizes this issue. It would not be expected, at the outset, that anaesthesiologists would not recognize the number of missing teeth, but it can in fact be challenging in some situations whether the toothless space corresponds to one or more teeth, especially if the tooth loss occurred long ago.

These aspects do not preclude the validity of the protocol, but they warn of the need for more detailed and frequent exchange training between different health professionals, before its general use; a theme has been highlighted by other authors and advised by the recent education guidelines for a holistic patient care [1,6,16,19].

In the available literature, the evaluations of dental injuries associated with anaesthetic acts are most often retrospective. This may justify the lack of standardization in the way of recording pre-operative dental condition, as well as the distinct emphasis of each study attributed to this factor.

Although the literature is not coherent on which is the most common post-surgical accident, namely regarding dislocation or tooth avulsion, common in the upper incisor teeth the importance of a careful oral/dental evaluation is recognized by specialists of anaesthesiology [7,8,12]. The literature is, at the same time, scarce about a registration protocol that is simple but reliable, which is to be used by a medical professional without training in dentistry. In the present study, it was possible through a literature review to identify dental risk factors that could help the anaesthesiologists to classify specific oral conditions, as well as teeth with a higher risk of injury, to inform the patient and prevent iatrogenic injuries. It was essential to assess the correlation between both dental students and anaesthesiologist interns' evaluations in order to confirm or reject some of the selected variables, or even to reinforce the extension of the prior dental training. For this purpose, a correlation analysis was carried out and, in general, a good interobserver correlation was found, contributing to the validation of the pre-operative protocol suggested [10,18,21]. However, to be able to suggest clinical recommendations, the assessment of the oral status through this protocol needs to be correlated to the post-operative dental injuries.

A limitation of the study may be the sample size. A convenience sample was selected based on the greater ease of reconciling a larger number of patients and the availability of both observers in the referred period. Therefore, even though the "poor" oral condition has been generally reported, with a reduced number of remaining teeth—in particular, missing 'index teeth'—there are few investigations presenting a detailed chart to implement in view of a harmonization of the oral registration by the anaesthesiologists. However, further research is needed to address its role concerning pre-operative inspection and litigation about tooth injury following intubation.

5. Conclusions

The developed pre-operative protocol presented acceptable correlation criteria, allowing it to be used by an anaesthesiologist, after a brief training on clinical oral examination in dentistry. Exchange programs might be implemented to fulfil the requirements of patient-centred care amongst health students/professionals with different backgrounds.

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Chronic Obstructive Pulmonary Disease and Apical Periodontitis and Other Oral Health Variables: A Case-Control Study

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Abstract: Background: The relationship between chronic inflammatory diseases and their comorbidities and correlation with periodontal diseases has become an increasing focus of research. Objectives: The aim of this case-control study was to conclude if patients suffering from COPD (Chronic Obstructive Pulmonary Disease) tend to have more AP (Apical Periodontitis) than non-COPD patients. Materials and Methods: The study was conducted on 30 patients assigned as cases, associated with 30 control patients linked by age (+/−5 years) and sex. Results: A total of 60 patients were recorded, and a total of 12 radiographic variables were analyzed. A total of 43 (71.7%) patients were registered with PAI (Periapical Index) ≥ 3, and there was a slightly tendency in the patients from the control group 22 (73.3%) compared to those from the cases 21 (70%), respectively ($p > 0.05$). Conclusions: It was concluded that there was not a significant association between the levels of PAI (Periapical Index) ≥ 3 per patient in those suffering from COPD. In fact, it could be concluded that patients diagnosed with COPD tend to have more teeth with PAI ≥ 3, more endodontic treatments and their periodontitis tended to accumulate more caries. Clinical Significance: This study establishes, in a case-control study, some specific aspects of oral health in patients with COPD, as well as analyzing the importance of oral health in this disease.

Keywords: apical periodontitis; endodontics; oral epidemiology; oral medicine; root canal treatment; COPD

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1. Introduction

1.1. Apical Periodontitis (AP)

AP is a worldwide prevalent infectious disease, and it is characterized by an inflammatory response and bone destruction in the periapical tissues caused by microbial infection in the dental pulp. It is characterized radiographically by the presence of a Radiolucent Periapical Lesion (RPL) [1]. The bone destruction is caused by both microbial infection and the immune response as part of the defense reaction [2].

It is the most frequent pathological lesion in the jaws, mainly manifested as periapical granulomas and cysts [3]. Root canal treatment is the elective treatment for teeth with

AP that must be preserved [2]. It is essentially an inflammatory disease of microbial etiology, and there is clear evidence that microbial interaction plays an important role for the pathogenesis of AP [3].

1.2. Periapical Index (PAI)

There are different indices to evaluate AP, but one of the most-used is the PAI proposed by Ørstavik et al. in 1986 [4]. The scoring system for the registration of AP in radiographs is presented. The system is called PAI and provides an ordinal scale of five scores ranging from one (healthy) to five (severe periodontitis with exacerbating features). Its validity is based on the use of reference radiographs of teeth with verified histological diagnoses. The system may be suitable for the analysis of periapical radiographs in epidemiological studies, clinical trials and the retrospective analysis of treatment results in endodontics [4].

1.3. AP and Systemic Diseases

Chronic AP develops as a chronic inflammatory process characterized radiographically by the presence of periapical radiolucency, a radiolucent image surrounding the apex of the affected tooth [5]. In recent years there has been a biological basis for suggesting that different systemic diseases may influence BP (Blood Pressure). One of the most studied is DM (diabetes mellitus). DM can affect the periapical immune response, causing a delayed healing process, and, consequently, there could be expected to be a higher prevalence of periapical lesions and a higher rate of post-treatment disease in diabetic patients than in control subjects without diabetes. Different studies have clinically investigated in humans the possible association between DM and AP; Falk et al. in 1989 [6], Bender and Bender in 2003 [7], Britto et al. in 2003 [8], Fouad and Burlesson in 2003 [9] and Pérez-Losada et al. in 2020 [10].

Other studies support the concept that smoking is associated with an increase in the prevalence of AP (Aleksejuniene et al. in 2000 [11], Kirkevang and Wenzel in 2003 [12], Kirkevang et al. in 2007 [13], Segura-Egea et al. in 2008 [14] and 2011 [15], and López-López et al. in 2011 [16]) and is able to act as a risk factor for AP [17].

It has also been related to a lesser degree with other systemic diseases, such as: hypertension [18], osteoporosis [19–21], multiple myeloma [21], cardiovascular diseases [22,23], metabolic syndrome [24], and transplanted [25] and coagulation disorders [5]. There is a significant association between ICD (Inherited Coagulation Disorders) and the presence of radiographically diagnosed periapical lesions, an aspect that shows that coagulopathies hardly can be regarded as a primary cause of root canal infection and AP [10]. It can also be stated that there is an association between AP and MetS (Metabolic Syndrome) and ACVD (Atherosclerotic Cardiovascular Disease) [7].

During these last two years of the pandemic, it has been hypothesized that periodontopathic bacteria are also involved in COVID-19 aggravation, and, therefore, the management of good oral hygiene potentially contributes to its prevention [26–28].

1.4. Chronic Obstructive Pulmonary Disease (COPD) and Periodontal Diseases

In recent years, it has been recognized that periodontitis is linked to the severity of COPD through the microbial colonization of the respiratory system by dental plaque or airway inflammation caused by periodontal pathogens. Oral pathogens may be related to elevated circulating levels of cytokines and systemic inflammation, which has been implicated in the pathogenesis of COPD [29]. Both chronic periodontitis and COPD have a common element of host susceptibility to environmental factors [30].

It commonly appears associated with other comorbidities that are thought to share an underlying inflammatory process, whether systemic or organ-specific, including chronic inflammatory oral diseases such as periodontitis [31]. Recognized as a heterogeneous disease with different contributions from airway and parenchymal abnormalities, the diagnosis and monitoring of patients are currently made using airflow measurements. It is a chronic lung condition characterized by progressive, irreversible airway obstruction [29].

COPD is characterized by an abnormal inflammatory response of the lungs to noxious particles or gases, particularly cigarette smoke, and is defined by the presence of airflow obstruction. It consists of several pathological subtypes, such as emphysema, small-airways disease and chronic bronchitis, which are distinct entities, although often combined in a single patient. There has been growing interest in the hypothesis that COPD forms part of a 'chronic systemic inflammatory syndrome'. Patients with COPD have higher levels of circulating inflammatory cytokines including C-reactive protein, IL-8 and TNF α, which have been shown to relate to disease severity [30,31].

1.5. Justification

Despite all the revised literature exposed previously, it cannot be concluded from the scientific evidence that COPD is correlated with chronic AP; in fact, this study sets up a hypothesis: due to the lack of scientific evidence on a possible correlation between AP and COPD, a comparative work analyzing if COPD patients tend to suffer more AP than non-COPD patients was set up. Based on the hypothesis, the comparative work was classified as a clinical study of cases and controls to contrast it.

2. Materials and Methods

This case-control study was formed by 30 panoramic radiographies from COPD patients assigned as cases, which were associated with 30 panoramic radiographies from patients assigned as controls linked by age and sex without diagnosed COPD or any other confusing disease with inflammatory responses, such as DM or CVD (Cardiovascular Diseases), among others.

The panoramic radiographies assigned as cases were facilitated by the Department of Pulmonary Medicine from the Hospital Universitari de Bellvitge, and the controls were taken from patients attending the Hospital Odontològic Universitat de Barcelona.

The aim of this study was to discover if patients with diagnosed COPD tend to suffer more AP than non-COPD patients. In order to give more consistency to the study we settled on a list of several radiographic variables to analyze as secondary objectives: I—the calibration of the investigator in the PAI's evaluation, II—the analysis of the Klemetti Index in the panoramic radiographies in both groups, III— the analysis of the CW (Cortical Width) in both groups, IV—the analysis of the periodontal loss observed in the radiographies from both groups, V—the analysis of the reduction in the number of teeth in both groups, VI—the analysis of the number of endodontic teeth in both groups and VII—the analysis of the correlation between PAI and endodontic teeth in both groups.

According to the panoramic radiographies, the level of AP was settled, calibrating it with the PAI described by Ørstavik et al. [4]. It settled in the following way; 1: normal status, 2: minor change of the bone structure, 3: more change of the bone structure and some mineral lost, 4: radiolucide area well-defined and 5: severe radiolucide area well-defined with exacerbation signs. When in doubt of two stages, the higher stage was used as the settled one. PAI $\geq$ 3 was defined as AP [4].

With all the obtained data, we correlated different clinical values based on COPD established by the Department of Pulmonary Medicine.

2.1. Study's Design

The project consisted of a case-control study following the statements of the STROBE guide [32]. As limitations of the study, we delimited; the sample's size, the lack of previous studies involving COPD and AP and the measures used to collect all the data.

2.2. Subjects of the Study

The group of cases was formed by patients with COPD diagnosis, according to GOLD (Global Initiative for Chronic Obstructive Lung Disease), a global consensus on strategy for the diagnosis, management and prevention of this disease. GOLD reports stratified patients considering three risk variables: FEV_1 (Forced Expiratory Volume in one second), symptoms and exacerbations. For the diagnosis of COPD, a FEV_1/FVC (Forced Vital Capacity) ratio of <0.70 assessed by spirometry after bronchodilator use was required [33]. These patients attended the COPD pneumology service from the Hospital Universitari de Bellvitge.

The panoramic radiographies from the cases subjects and the anonymous data of the patients were given by the Department of Pulmonary Medicine from the Hospital Universitari de Bellvitge, corresponding to patients that were visited for other medical reasons.

The control group was formed by non-COPD patients (also not suffering from any other disease that could be confused with COPD), and these panoramic radiographies were taken by the Hospital Odontològic de la Universitat de Barcelona from patients that had visited the Medicine, Surgery and Implantology Master from the UB (Universitat de Barcelona) and from patients that were visited at the "practicum" from the 5th course of the dentistry degree program at the UB.

The groups were divided as follows: Group 1 [cases]: 30 subjects with a medical history of COPD, who were the study group and Group 2 [controls]: 30 additional subjects who accepted and understood the inclusion criteria of the study, with non-referring clinical signs and no medical history of COPD or any other confusing disease, paired by age and sex, who were the control group.

Both groups were paired by age (+/−5 years) and sex with the obtained data from the cases group.

2.3. Inclusion and Exclusion Criteria

The inclusion criterion was the following: patients suffering from COPD, including the different existing types and stages. The exclusion criteria were: I—patients suffering from systemic diseases that had similar inflammatory responses and characteristics that could be confused with COPD, such as poorly controlled DM towards others, like CVD (both comorbidities frequently associated with COPD), and II—patients suffering from similar respiratory diseases that were confused with COPD (asthma, congestive heart failure, bronchiectasis, tuberculosis, bronchiolitis obliterans or diffuse panbronchiolitis).

2.4. Sample Size Calculation

According to López-López et al. [34], there is an existing evidence association between smoking and the presence of at least 1 periapical lesion, radiographically detected.

This case-control study was considered a pilot study; assuming that the prevalence of AP in patients with COPD has not been proved or studied, we shared the same equivalence of tobacco and AP from previous studies by López-López et al. [34], wherein the smoker patients had at least 1 tooth with PAI ≥ 3 (74.7%) in comparison to (12.7%) of the control group. Applying $p = 0.05$, we would obtain a sample size of 11 patients from the group (Figure 1). It was a descriptive study analyzed by the chi-square test and the quantitative variables according to central tendency measures and dispersion, following the Kolmogorov–Smirnov test accepted with a value of ($p < 0.005$) and defined with the statistical average and standard deviation or statistical median and IQR (interquartile range).

$$n = \frac{\left[Z_\alpha * \sqrt{2p(1-p)} + Z_\beta * \sqrt{p_1(1-p_1) + p_2(1-p_2)}\right]^2}{(p_1 - p_2)} \quad p = \frac{p_1 + p_2}{2}$$

Figure 1. Calculation of the study's sample. According to the calculation proposed by López de Ullibarri et al. [35], n = subjects needed in each one of the samples; Z_p = Z value assigned as the wished risk; p_1 = proportion of the value of the reference group, placebo, control or usual treatment; p_2 = value of the proportion in the group of the new treatment, intervention or technique; p = average of both proportions p_1 and p_2.

2.5. Description of the Treatment or Intervention

First, the investigator (A.C.-C.) examined 87 randomly chosen dental radiographies (periapical and panoramic), analyzing the PAI index, following the Ørstavik et al. [4] criteria and also the radiographic variables KI (Klemetti Index) and CW (Cortical Width) described by López-López et al. in 2011 [36]. Once all the data had been analyzed, a concordance with the two co-investigators was established (J.L.-L. and J.J.S.-E.). The process was repeated with different panoramic radiographies until the obtention of a Kappa Index of a $K \geq 0.61$ according to López de Ullibarri Galparsor and Pita Fernández [35].

During the process we obtained a Kappa Index with a value of a $K \geq 0.81$. Once we had obtained the value, we proceed to analyze the panoramic radiographies corresponding to the descriptive study. For that, we studied 30 panoramic radiographies from anonymous patients facilitated by the Department of Pulmonary Medicine of the Hospital Universitari de Bellvitge. We also selected the 30 control patients (paired by age and sex) from those at the Hospital Odontològic de la Universitat de Barcelona, All the panoramic radiographies were analyzed in an HP screen of 21 inches with environmental light and always between 4 p.m. and 6 p.m.

2.6. Description of the Variables

The following variables were extracted from the patient's medical history and/or the panoramic radiographies with a scale of 1:1: I—sex (qualitative variable); II—age (nominal variable); III—COPD level (qualitative variable), noted following the data facilitated from the Department of Pulmonary Medicine and using the classification proposed by the Global Strategy for the Diagnosis, Management and Prevention of COPD; IV—radiological values: CW (mandibular cortical in mm), KI (values 1, 2, 3); these 2 parameters were analyzed following the proposed indication by López-López et al. [36], the Klemetti et al. [37] criteria and Horner and Devlin [38]; V—number of present teeth; VI—number of endodontic teeth; VII—PAI data was noted as (0, 1, 2, 3, 4, 5), as described by Ørstavik et al. [4]; VIIII—mesial and distal bone loss (mm) of each one of the present teeth; for that, the distance from the enamel–junction line to the cortical bone was measured, observing how many teeth show mesial or distal loss > 4 mm, and the global average loss is sum of the mesial and distal loss divided by 2 and the number of present teeth.

2.7. Statistical Analysis

For the concordance of the study, the KI (Kappa Index) was used. All data was collected in an Excel table from the Microsoft Office Package (2013) and was analyzed with the SPSS program 17.0 for Windows (SPSS, Chicago, IL, USA, 2011).

A descriptive analysis of the qualitative variables was realized, according to their frequency and percentage, and the quantitative variables according to their central tendency measures and dispersion.

The quantitative variables, depending on their normal distribution following the Kolmogorov–Smirnov test ($p > 0.05$), were defined with the statistical average and standard deviation if they are not in accordance with the statistical median and IQR.

2.8. Specification of the Acceptance of National and International Ethical Standards

This study was conducted under the ethical principles of the Helsinki Declaration and according with the requirements of the *Real Decreto* 1090/2015, 4th December, to regulate clinical trials with medical products, the Ethics Committee of the medical investigation and the Spanish Register of Clinical Studies and article nº 32 of the Ethics Code and Medical Deontology of Spanish of Collegiate Medical Organization (OMC).

The study had the approval of the Ethics Committee on the investigation of medical products and medical devices (CEIm) with the code 2/2021 from version 2 of the Hospital Odontològic de la Universitat de Barcelona studies' protocol.

Personal data employed in the study was kept anonymous all of the time. The study was carried out according to the General Rules of Data protection (EU) 2016/679 and the Organic Law 3/2018, 5th December, of Data Personal Protection and Digital Rights Guarantee.

The names of the patients did not appear in any document of the study, and on no occasion were the identities revealed in any publication or communication. The panoramic radiographies were transferred in an anonymous way.

3. Results

3.1. Sample's Description

The cases group formed by 52 male patients (86.6%) and 8 female patients (13.3%) were recorded. There was no significant difference in sex and age, since the cases were paired by age (+/−5 years) and sex. The total average age was 71.20 ± 8.947 ($p > 0.05$), and the range of age was 50–88.

3.2. Case and Control Groups

The cases group was 4 females (13.3%) and 26 males (86.7%). All of them were diagnosed with severe or very severe (post-bronchodilator FEV1 < 50% predicted) COPD, making them the studied group. The total average age was 71.83 ± 8.292 ($p > 0.05$). Patients' ages ranged from 50 to 87. A total of 24 of these 26 patients were former smokers, and the rest ($n = 4$) were active smokers. One-third of the patients had well-controlled type 2 diabetes mellitus ($n = 10$) without target organ involvement.

The control group was 4 females (13.3%) and 26 males (86.7%). The total average age was 70.57 ± 8.791 ($p > 0.05$). Patients' ages ranged from 52 to 88. In this group, there was no existence of COPD history or any other disease that could be confused with it (such as DM or CVD among others).

Table 1 shows the presence of all the diseases and medical conditions from the control group. A total of 22 patients from the control group were current or former smokers.

Table 1. Medical conditions of the control group.

Code	Medical Conditions
2	Early Alzheimer's disease
3	Coronary stent carrier
4	NR
6	Gout/hypotension/arthritis/osteoarthritis/BPH/intermittent claudication
7	NR
8	BPH/slow digestion/bile duct stones/occasional headaches/deafness/difficulty urinating
10	Mild cholesterol/duodenal ulcer
11	NR
12	Hereditary hemochromatosis/diverticulitis/lithiasis/BPH
13	Hepatitis B (already healed)/malignant prostate cancer
14	NR
16	NR
17	HIV + (since 1986 with retroviral treatment)
18	Tubal ligation
19	Osteoarthritis
20	NR
21	NR
24	NR
25	Levothyroxine sodium/hiatal hernia/NHL/bilateral inguinal hernia

Table 1. *Cont.*

Code	Medical Conditions
26	Anxiety/depression
27	Essential tremors
28	NR
31	NR
32	Digestive problems
33	NR
35	NR
36	CCL
37	HIV + (>20 years with undetectable viral load)/MM
38	Hepatitis B
42	NR

NR: non-referring/BPH: Benign Prostatic Hyperplasia/HIV: Human Immunodeficiency Viruses/NHL: Non-Hodgkin Lymphoma/MM: Multiple Myeloma/CCL: Chronic Lymphocytic Leukemia.

3.3. Study's Variables

Table 2 shows the distribution of the study factors in both control and case groups. There was no significant difference in age, gender and number of teeth between both groups.

Table 2. Distribution of the study factors in both control and case groups.

	Control, *n* = 30 (50%)	**Cases, *n* = 30 (50%)**	**Total, *n* = 60 (100%)**	***p* Value**
Age, y Mean ± SD	70.57 ± 8.791	71.83 ± 8.292	71.20 ± 8.947	*t* student >0.05
Gender Female Male	 4 (13.3%) 26 (86.7%)	 4 (13.3%) 26 (86.7%)	 8 (13.3%) 52 (86.7%)	 >0.05 χ^2
No. of teeth Mean ± SD Median	 18.60 ± 8.245 20.5	 18.63 ± 7.545 18	 18.62 ± 7.835 19	*t* student >0.05
PAI ≥ 3/patient Yes No	 22 (73.3%) 8 (26.7%)	 21 (70%) 9 (30%)	 43 (71.7%) 17 (28.3%)	 >0.05 χ^2
PAI ≥ 3/teeth Mean ± SD Median	 1.60 ± 1.429 2	 1.93 ± 1.837 2	 1.77 ± 1.633 2	*t* student >0.05
No. of RFT Mean ± SD Median	 1.30 ± 1.393 1	 1.43 ± 2.079 0.5	 1.365 ± 1.736 1	*t* student >0.05
RFT with PAI ≥ 3 Yes No	 8 (26.7%) 22 (73.3%)	 7 (23.3%) 23 (76.7%)	 15 (25%) 45 (75%)	 >0.05 χ^2
Stage of periodontitis I II III IV	 10 (33.3%) 16 (53.3%) 0 4 (13.3%)	 12 (40%) 14 (46.7%) 0 4 (13.3%)	 22 (36.7%) 30 (50%) 0 8 (13.3%)	 >0.05 χ^2

Table 2. *Cont.*

	Control, n = 30 (50%)	Cases, n = 30 (50%)	Total, n = 60 (100%)	p Value
No. of caries				
Mean ± SD	2.33 ± 2.02	4.47 ± 3.53	3.4 ± 2.775	t student <0.05
Median	3	3	3	
CW				
Mean ± SD	3.183 ± 0.675	3.867 ± 0.73	3.525 ± 0.702	t student <0.001
Median	3	4	3.5	
Klemetti Index				
C1	20 (66.7%)	23 (76.7%)	43 (71.7%)	
C2	10 (33.3%)	7 (23.3%)	17 (28.3%)	>0.05
C3	0	0	0	χ^2
Mattila Index				
Yes	21 (70%)	28 (93.3%)	49 (81.7%)	<0.05
No	9 (30%)	2 (6.7%)	11 (18.3%)	χ^2
Tobacco				
Yes	22 (73.3%)	26 (86.7%)	48 (80%)	>0.05
No	8 (26.7%)	4 (13.3%)	12 (20%)	χ^2

PAI ≥ 3: Periapical Index > 3 (described as pathological)/RFT: Root-filled teeth/CW: Cortical Width (mm)/C1: Klemetti Index (endostic margin uniform)/C2: Slightly or moderately eroded/C3: severely eroded.

3.3.1. Age and Gender

For all subjects, the average age was 71.20 ± 8.947 (p > 0.05), 71.83 ± 8.292 (p = 0.568), in the case group and 70.57 ± 8.791 in the control subjects. A total of 60 patients were recorded; from these, 8 (13.3%) were female and 52 (86.7%) males. Both case and control groups contained 4 females (13.3%) and 26 males (86.7%) (p = 1).

3.3.2. Number of Present Teeth

The total number of present teeth in the 60 patients was 1070 teeth, 522 (48.7%) in the case group and 548 (51.2%) in the control group. The average number of teeth for all subjects of the study was 18.62 ± 7.835, a total of 18.63 ± 7.545 in the studied group and 18.60 ± 8.245 in the control group, respectively (p = 0.987).

3.3.3. Periapical Index ≥ 3 and Population

A total of 43 (71.7%) patients were found to have PAI ≥ 3. Not many differences were found between both groups; regarding teeth with PAI ≥ 3, we found a slightly increased number in the control group, 22 (73.3%), compared to the case subjects, 21 (70%) (p = 0.774).

3.3.4. Periapical Index ≥ 3 and Number of Present Teeth

Despite the finding between the number of PAI ≥ 3 per patient, we found that, if we analyzed the PAI ≥ 3 teeth, we found, in total, an average of 1.77 ± 1.633 from these; 1.93 ± 1.837 were part of the cases group and 1.60 ± 1.429 from the control subjects, respectively (p = 0.436).

3.3.5. Number of Root-Filled Teeth

The total average of RFT in the 60 patients was 1.365 ± 1.736; from these, 1.43 ± 2.079 were from the cases and 1.30 ± 1.393 from the controls, respectively (p = 0.771).

3.3.6. Root-Filled Teeth with Periapical Index ≥ 3

From the total recount, 15 RFT (25%) were recorded; there were not many differences between the groups. From these, 8 (26.7%) belonged to the control subjects and 7 (23.3%) to the cases. We found that there was a higher prevalence of healthy RFT 45 (75%) than RFT with PAI ≥ 3 15 (25%), respectively (p = 0.766).

3.3.7. Stage of Periodontitis (Bone Loss)

Patients were categorized according to the Classification of Periodontal and Peri-Implant Diseases and Conditions based on the AAP classification [39]. Stage I: bone loss 1–2 mm, Stage II: bone loss 3–4 mm, Stage III: bone loss $\geq$ 5 mm ($\leq$4 of lost teeth), Stage IV: bone loss $\geq$ 5 mm ($\geq$5 of lost teeth).

A total of 22 (36.7%) patients were categorized into Stage I, 10 (33.3%) in the control group and 12 (40%) in the cases group. A total of 30 (50%) patients were categorized into Stage II, 16 (53.3%) from the control subjects and 14 (46.7%) from the cases group. None of the patients were categorized into Stage III. A total of 18 (13.3%) were categorized into Stage IV, 4 (13.3%) in each group (p = 0.854).

3.3.8. Number of Caries

The total no. of caries found in all of the subjects was 3.4 $\pm$ 2.775, a total of 2.33 $\pm$ 2.02 for the control group and 4.47 $\pm$ 3.53 for the case group (p = 0.007).

3.3.9. Cortical Width

The CW was measured in mm, and the average for the 60 patients in the study was of 3.525 $\pm$ 0.702, a total of 3.183 $\pm$ 0.675 for the control patients and 3.867 $\pm$ 0.73 for the case group ($p < 0.001$).

3.3.10. Klemetti Index

The KI was categorized into three different stages; for all subjects, 43 (71.7%) were categorized into C1, 20 (66.7%) for the control group and 23 (76.7%) for the cases. C2 was recorded in a total of 17 (28.3%) patients; of these, 10 (33.3%) were from the control group and 7 (23.3%) from the cases (p = 0.390).

3.3.11. Mattila Index

The Mattila Index was recorded in order to discover a correlation between caries and periodontitis. From the total sample, 11 (18.3%) patients did not present an association between caries and periodontal disease; from these, 9 (30%) were from the control patients and 2 (6.7%) from the cases. A total of 49 (81.7%) patients were found to be suffering from both caries and periodontitis; of these, 21 (70%) were from the control population and 28 (93.3%) from the cases ($p < 0.05$).

4. Discussion

The aim of this study was to investigate the possible correlation between AP and COPD using a case-control study design.

4.1. Age and Gender

Because control subjects were (+/−5 years) paired by age and sex matched with case subjects, there were no significant differences between control and case patients in age or in gender.

4.2. Number of Present Teeth

According to Lang et al. [40], this study concluded that missing teeth (as well as number of teeth per patient) performed well as an indicator of oral health status. The average number of teeth per patient was similar in both groups; it can be considered that the oral health status of the control and case subjects was comparable.

4.3. Periapical Index $\geq$ 3 and Population

The PAI scoring system has been modified and applied to epidemiologic [40,41] clinical comparative studies of treatment outcome [41]. PAI was first described for periapical radiographs [4], but numerous epidemiologic studies have used this index for panoramic radiographies [41–46]. It has been reported that panoramic digital images find significantly higher percentages of teeth with PAI ≥ 3 [45–49]. On the other hand, other investigators have found that the underestimation of lesions occurred when panoramic radiographies were used [50–53]. As an outcome, it can be concluded that the number of teeth with PAI ≥ 3 per patient is very similar between both groups, with an increased slightly tendency among the control subjects. We can conclude that the controls from the study had a higher tendency to suffer PAI ≥ 3 than the cases.

4.4. Periapical Index ≥ 3 and Number of Teeth

According to the study of Ridao-Sacie et al. [47], a total of 2088 teeth were examined, and from that, 14.7% were found to be periapically diseased teeth in digital panoramic images compared to 3.1% analyzed with periapical radiographs. The frequency of teeth with AP in other studies varies from 0.6% in Eriksen et al. [48] to 9.8% in Allard et al. [49]. This range is large, probably due to the variation among the examined population. It can be stated that these results tend to change depending on the examined population. In our study, despite finding almost the same results between both groups analyzing the PAI ≥ 3 per patient, when we analyzed the number of PAI ≥ 3 per teeth, we observed that the tendency had changed. We found that the number of PAI ≥ 3 (described as pathological) per teeth was higher among the cases. As a result, we can conclude that the controls from the study had a higher tendency to suffer from PAI ≥ 3; instead, each one of the cases had more tendency to be suffering from PAI ≥ 3.

4.5. Number of Root-Filled Teeth

According to the study of Dydyk et al. [54] executed in an adult population of 435 patients from (Lviv, Ukraine), the overall percentage of RFT in adult dentition is 12.08%, and an increase of treated teeth with age was identified. A total of 82.5% examined adults had one or more endodontically treated teeth in this study. In our case-control study, we found a total of 82 RFT per 1070 present teeth from a total of 60 patients, estimating an average of 7.66%. As a result, the average number of RFT was higher among cases. With this fact, it can be reported that the case subjects required more endodontic treatments due to pathological and medical conditions, among other possibilities.

4.6. Periapical Index ≥ 3 and Root-Filled Teeth

It has repeatedly been demonstrated that the preoperative presence or absence of AP is one of the most important prognostic factors for a root-canal-treatment tooth [55–58]. According to Kirkevang et al. [56], the difference between the PAI score distribution of RFT and of teeth without root filling was statistically significant on all occasions, concluding that teeth without root filling had a much lower PAI score. It was finally concluded that each one of the five score categories had a distinct prognostic value for the course of periapical disease over a 5-year period, for both non-root-filled and root-filled teeth. Moreover, the higher the baseline PAI, the higher risk for the tooth to be extracted, which could explain the fact that, in our study, the case group had a smaller number of PAI ≥ 3 per endodontic tooth; this could be because of the final extraction of the tooth, due to the increased progression of the AP. It was also found that there were more RFT without PAI ≥ 3 than with PAI ≥ 3; with this, we can conclude that endodontic treatment could have cured and healed a possible tooth with a periapical lesion.

4.7. Cortical Width

On the panoramic radiographies, some anatomical and pathological structures can be found, and information provided by these is helpful to establish a proper osteoporosis diagnosis, to propose a treatment plan and to evaluate the results of the treatment (Maćkowiak et al. [58]). Different studies use these radiographies for the estimation of different mandibular parameters, mainly the distances between elements on the body of the mandible. The measure was done on the panoramic radiographies measuring the mandibular cortical thickness below the mental foramen. According to Abhyankar et al. [59], panoramic digital

X-rays are used for the diagnosis of osteoporosis, measuring the distance of CW; after this, the patients are classified as having a low bone-mineral density or normal bone-mineral density. Abnormalities in the CW as imaged on the panoramic radiography may be indicative of osteoporosis (especially in postmenopausal women). Some authors like, Karayianni et al. [60], concluded that, if the CW is <3 mm, an individual should be referred for further osteoporosis investigation.

As a result, we found similar results among the cases and the controls; the average from all the patients together from our study present a CW > 3 mm and no pathological signs.

4.8. Klemetti Index

The KI, according to López-López et al. [36], is based in a simple visual check of the trabecular pattern that enables us to observe if there is evidence of the loss of bone mass. The visual exams tend to be a bit subjective, as they depend on the level of training of the examining doctor. In our study, we could observe that the majority of the 60 patients present a KI according to a C1, regardless of the many differences between both groups. This could be because most of the patients from our sample were men (86.6%), and, as we have mentioned before, most the osteoporotic patients are female. Another reason is that, in Europe and more specifically in Spain, there is not much prevalence of severe bone pathologies compared to other parts of the world that could be more affected by them, so we can conclude that, in our geographic area, it is more common to find categories according to C1.

From the study of López-López et al. [31], we can extract C1: the endostic margin is uniform and marked on both sides; C2: slightly or moderately eroded, the endostic margin appears to have semi-lunar defects (lacunar resorption), or there appear to be forms of cortical residues; C3: cortex severely eroded, the cortical bone is clearly porous, and there is a significant amount of residue.

4.9. Stage of Periodontitis (Bone Loss)

According to Helmi et al. [61], the global burden of oral conditions in 2010 affected nearly 4 billion people; severe periodontitis was ranked the sixth most-prevalent condition, with about 744 million individuals affected globally. The use of radiographs to assess alveolar bone loss appears frequently in the literature. In this study, a total of 1131 individuals were included in the analysis; overall periodontitis prevalence for the sample was 55.5% (±1.4%); the moderate periodontitis prevalence was 20.7% (±1.2%), while 2.8% (±0.5%) of the whole sample had severe periodontitis. All three case definitions were highest among 65+ year-old, males, former smokers, those with CVD and stage 2 hypertension subjects. Mean increase in bone loss was higher for older age groups; males had higher amounts of bone loss than females, who have a higher risk of developing periodontal diseases with significantly higher alveolar bone loss. The existence of different predictive factors has different risks for the progression of periodontal diseases. That is why further analysis of the social determinants of health is a necessity to understanding all factors participating in the development of diseases and their associated risk factors.

Focusing on our study, there was no significative difference between both groups. It was reported that all patients had at least some beginnings of bone loss. All of them were staged according to (Stage I, Stage II, Stage III, Stage IV), and we can extract that the prevalence of bone loss could be directly correlated to the fact that the 86.6% of our sample was men. Furthermore, we can correlate the facts for patients in a range of age from 50 to 88 years old. The existence of risk factors and the medical conditions of each subject can also be directly correlated with the existence of bone loss and subsequently an instauration of periodontitis.

4.10. Number of Caries

According to Jepsen et al. [62], the prevalence of dental caries and periodontitis is high, with untreated dental caries being the most-common disease affecting humans worldwide

(GBD 2016). C. Heng [63] concluded that the prevalence of dental caries in permanent teeth was of 58% and that about 90% of adults aged ≥20 years had dental caries. In our study, there was a much higher prevalence of caries in the cases; this could be due to the existing correlation between caries and periodontitis. As an outcome, there could also be an association between the presence of the medical conditions from the cases and the prevalence of caries.

4.11. Mattila Index

The study carried out by Mattila et al. [64] concluded that severe periodontal diseases and dental caries tend to accumulate in the same subjects. According to Mattila et al. [64], 64% of subjects had periodontal disease; of these, 33% had significantly more dental caries compared with those without periodontal disease (23%). As a result, in our study, it can be considered that patients tend to have more caries if they present any sign of periodontal disease.

Finally, as part of the study, we delimited a series of limitations exposed before. In addition, panoramic radiography has been used to assess cortical thickness and bone loss [65–67]; however, the data obtained should be evaluated with caution because the technique used is not the best.

5. Conclusions

In conclusion, the results of the present study show that, after adjusting for age, gender, number of teeth, endodontic status and medical conditions, it can be concluded that: there is not a significative association between the number of PAI $\geq$ 3 per patient among those suffering from COPD. In fact, it can be concluded that patients with diagnosed COPD tend to have more teeth with PAI $\geq$ 3. Furthermore, the population suffering from COPD tends to need more endodontic treatments; these patients also have a smaller number of PAI $\geq$ 3 per endodontic tooth because of the final extraction of the tooth due to the increased progression of the AP.

Cases and controls from this study reported the same average of CW, >3 mm, in those without any pathological sign. The studied patients were all categorized as C1, according to the KI, regarding their gender and geographic conditions. All subjects participating in the study reported some beginnings of bone loss that can be directly associated with the presence and instauration of periodontal diseases. As evidence it can be concluded that patients with COPD present more teeth with caries and that those who have COPD and periodontal disease tend to accumulate more caries.

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Data Availability Statement: If additional data is desired, they can be requested from the authors.

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Investigating the Ability of the Tooth and Surrounding Support Tissues to Absorb and Dissipate Orthodontic Loads during Periodontal Breakdown—Finite Elements Analysis

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Featured Application: For an orthodontist practitioner, quantification of the absorption–dissipation ability of dental tissues is of extreme importance since there is a constant danger when applying loads to produce ischemia and further resorptive processes (e.g., 0.6–1.2 N vs. 2.4 N). Orthodontic treatment is not always performed in intact periodontium; thus, it must be emphasized that in reduced periodontium, there are biomechanical changes that if not acknowledged could compromise treatment prognosis. The study is the first to analyze not only the absorption–dissipation issues, but also to investigate how the biomechanical behavior is affected by bone loss. For researchers, this study not only approaches the technical issues related to the numerical studies methodology, but also clears some aspects that could significantly influence the results accuracy. Herein, the analysis proves that the tooth absorbs and dissipates most of the stress due to applied forces (high percentage variability), in both intact and reduced periodontium, acting as a single-stand continuum structure, with enamel having a similar absorption–dissipation ability as dentine (both behaving in a similar way to ductile materials). All other tissular components have a constant absorption–dissipation ability, that changes very little during periodontal breakdown. It has also been proven that some of the movements are more stressful (i.e., rotation and translation) than the others (tipping, intrusion, and extrusion). The analysis herein showed that the assumed boundary conditions (linear elasticity, isotropy, and homogeneity) widely used in dental studies, are correct up to 2.4 N of loads when Tresca failure criterion is employed.

Abstract: Herein, the finite elements analysis (FEA) numerical study investigated the absorption–dissipation ability of dental tissues under orthodontic forces, during orthodontic movements and the periodontal breakdown process. Additionally, we investigated the correctness of FEA boundary assumptions up to 2.4 N of loads. Eighty-one models of the second lower premolar were subjected to 810 FEA numerical simulations using Tresca failure criterion under 0.6 N, 1.2 N, and 2.4 N and five movements: intrusion, extrusion, rotation, tipping, and translation. The results showed that both coronal dentine and enamel components had comparable high absorption–dissipation abilities, allowing for only a limited fraction of stresses to reach the circulatory sensitive tissues. Isotropy, linear elasticity, and homogeneity are correct when Tresca is employed up to 2.4 N. Forces of 0.6 N, 1.2 N, and 2.4 N displayed similar qualitative results for all movements and bone levels, while quantitative results doubled for 1.2 N and quadrupled for 2.4 N when compared with 0.6 N. FEA simulations showed 0.6–1.2 N to be safe for application in intact periodontium, while for reduced periodontium more than 0.6 N are prone to resorptive and ischemic risks. For reducing these risks, after 4 mm of bone loss, 0.2–0.6 N are recommended. Rotation and translation were the most stressful followed by tipping.

Keywords: tooth; enamel; dentin; periodontal breakdown; finite elements analysis; failure criteria selection; orthodontic movements

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1. Introduction

Dental tissues are subjected to various levels of stress during the application of orthodontic loads. The first tissues that are subjected to orthodontic stresses are bracket, enamel, and dentine. Some of the stresses are absorbed and dissipated through these components, and only a part reaches other tissular components (i.e., periodontal ligament, PDL; neuro-vascular bundle, NVB; dental pulp; cortical and trabecular bone). This absorption–dissipation ability is theoretically and clinically recognized, but not yet quantified or sufficiently studied [1–4].

Some of the dental tissular components are more sensitive to the orthodontic pressures due to a better circulatory vessel system (i.e., PDL, NVB, and dental pulp); thus, the physiological maximum hydrostatic pressure MHP of 16 KPa (approx. 80% of systolic pressure) is exceeded and both ischemic and resorptive risks are inevitable [5,6].

Tissular absorption–dissipation ability is based on the internal micro-architecture of the tooth and supporting tissues, individual components, and the way they biomechanically behave under stresses.

The material of bracket (i.e., stainless steel) is a ductile material that can deform under loads and recover its initial shape when the loads ceased [7–9].

From a mechanical point-of-view (according to the material failure theory), a ductile material (e.g., steel and rubber) can suffer from various elastic deformations (higher tensile resistance) before its failure and could return to its original form when the force ceased. On the other hand, a classical brittle material (e.g., concrete and glass), when subjected to loads, suffers from limited deformations, closely followed by cracking and fracture, but with a higher compression resistance [9,10].

Enamel, considered as the hardest tissue, has an internal micro-architecture that is made of hexagonal-prism-shaped rods [1,11,12]. As a brittle material, when subjected to loads, the enamel should not deform, but rather should crack/fissure. However, clinically, this type of behavior is neither confirmed nor studied. Therefore, based on only clinical acknowledged behavior, the internal micro-architecture seems to suffer from some recoverable deformations, making it a ductile-like material with a certain brittle flow mode [10].

The dentine component (which resembles a ductile material) is the largest component in the tooth structure, with an internal micro-architecture made of oriented tubules surrounded by a highly mineralized cuff of peritubular dentin and inter-tubular matrix of type I collagen fibrils reinforced with hydroxyapatite [11,13]. Dentine is recognized for having the ability to absorb–dissipate both orthodontic and bite loads, while its physical properties vary depending on topography (i.e., anisotropy) [1,11,13].

Dental pulp and neuro-vascular bundle (NVB) are ductile-like materials [2], being highly vascularized tissues, with NVB being more sensitive to circulatory disturbances due to its topographical position in the apical third PDL [2,5].

Cementum (which resembles a ductile material) has similar physical properties to dentine and ensures the support and absorption–dissipation of both tissues [1,2,10,11,14].

The internal micro-architecture of PDL (which resembles a ductile material) comprises collagen fibers displayed as variously orientated dense fiber bundles that fill the spaces between the bone and cementum by 0.4–1.5 mm [3]. PDL along with NVB are the most sensitive to circulatory disturbance tissues due to a well-represented vascular support including apical vessels, perforating vessels, and gingival vessels. The outward-facing blood vessels are involved in biomechanical suspension and absorption–dissipation ability, while those facing inwards are involved in nutritional metabolism [3].

Bone (cortical and trabecular/cancellous components) behave as a continuum and single-stand structure with high adaptation ability of changing the shape to provide the strongest structure with a minimum of volume and resemblance to ductile materials [15–20].

All dental tissular components are anisotropic and non-homogenous materials (i.e., variable physical properties on different directions depending on circumstances) [16,19], do not obey Hooke's law [1], and with non-linear elastic behavior. These issues must be clearly addressed in every numerical study due to their significant influence over the results accuracy.

The acknowledged dental components' physical properties are: cortical bone—16.7 GPa of compressive modulus and 157 MPa of compressive strength; trabecular/cancellous bone—0.155 GPa of compressive modulus and 6 MPa of compressive strength [19–27]; enamel—62.2 MPa of compressive stress [1], 11.5–42.1 MPa of maximum tensile strength [11], and 53.9–104 MPa of maximum shear stress [13]; dentine—29–73.1 MPa of maximum shear stress; enamel–dentine—53.9–104 MPa of maximum shear stress [13].

If the tooth's surrounding support system is intact, common orthodontic forces up to 1.2 N [4] are safely applied. However, when there are various levels of bone loss, due to mechanical changes, the stress absorption–dissipation ability changes, with higher ischemic and resorptive risks and altered treatment prognosis [5,6,10]. When analyzing small movements under light forces, the issue related to the loading conditions must be carefully addressed. For predictable results, both intensity of the force and orthodontic strength must be addressed, as well as time and amplitude. The intensity is the power transferred per unit area (extremely important for small loads applied on small surface areas), while the strength is the capacity of an object—to withstand force/pressure, particularly the maximum load a material can sustain before yielding. To keep the intensity of the force constant for each orthodontic movement, the surface of the applied loads must be carefully measured, as well as the direction of the force (i.e., X-Y-Z spatial directions) since it directly influences the discharged area. The orthodontic strength of the materials and dental tissues must be higher than the stress manifested during the orthodontic movement. The time of the applied load is also important to simulate, as closely as possible, the biomechanical behavioral response of human dental tissues.

There is only one single available method for the study of stress distribution in dental tissues, the finite elements analysis (FEA), which individually investigates the stress distribution and biomechanical behavior of each dental component [5,6,10]. In dental field, the numerical studies' results are regarded with care since they often contradicted clinical knowledge and displayed various results from one report to another [19–47]. This issue was not addressed, except for our previous research [5,6,9,10,16,48].

FEA accuracy depends on the selection of proper failure criteria which are suitable for the analyzed material, anatomically correct models, and proper boundary conditions. Our previous studies [5,6,9,10,16,48] reported that since dental tissues are ductile-like materials (with a certain brittle flow mode) only a failure criterion specially designed for ductile materials is suitable (i.e., Von Mises overall stress and Tresca shear stress). Moreover, for validation, the quantitative results must be correlated with MHP, while qualitative results with acknowledged clinical data. Despite many FEA studies [19–47,49,50] investigating PDL and bone–implant interface, none approached these vital issues, thereby supplying questionable and contradictory results [5,6,9,10,16,48]. Only one older numerical FEA study [30] was found to make a limited distinction between brittleness and ductileness for the root canal filling, but without further development.

Most of the recent FEA studies [28–41,43,44,49–51] employed the hydrostatic pressure criterion (specially designed for liquids, with no shear stress), maximum principal S1 tensile stress, and minimum principal S3 compressive stress (for brittle-like materials) for the study of PDL (a ductile-like material), while the reported results invariably exceeded MHP even for light orthodontic forces (suggesting ischemic and resorptive risks that contradict clinical data).

The bone–implant [19–26,44] and bone–tooth [29,43,44] FEA studies employed S1–S3 (brittle-like) and Von Mises (ductile-like) failure criterion but without any discussion about the above-mentioned issues.

Most of the above FEA studies employed included boundary conditions isotropy, linear elasticity, and homogeneity, despite the anatomical tissues being none of these, without addressing their suitability, adequacy, and influence over the results accuracy issues. Moreover, the applied forces were higher than 1.2 N (despite the above boundary conditions being correct only for small displacements and applied forces), while the investigated models were artificially created and anatomically simplified (i.e., lower number of nodes and elements with a higher global element size) [19–47,49,50]. It must be emphasized that, from a biomechanical point-of-view, linear elasticity and isotropy assumptions are correctly employed only if the applied forces are up to 1 N, and the non-homogeneity of materials is considered and addressed using the Tresca criterion. There is no available data on the above issues for higher applied forces in dental tissues and no studies, except for our previous studies [2,3,5,6,9,10,16,48], acknowledged and addressed these issues.

Herein, the study aimed to individually assess the biomechanical absorption–dissipation ability of dental tissues in intact periodontium under 0.6 N, 1.2 N, and 2.4 N during the five most used orthodontic movements, as well as the changes produced by the reduction in supporting tissues during a gradual horizontal periodontal breakdown of 1–8 mm. Additionally, we investigated the suitability and biomechanical behavioral correctness of the frequently employed boundary conditions (isotropy, linear elasticity, and homogeneity) under increasing loads.

2. Materials and Methods

This numerical FEA analysis is part of a larger step-by-step research project [2,3,5,6,9,10,16,48] (with clinical protocol no. 158/02.04.2018) investigating the FEA methodology to improve the accuracy of numerical study results and to assess the biomechanical behavioral changes in dental tissues produced by the periodontal breakdown.

Herein, the study performed 810 numerical simulations on 81 models of the lower premolar from nine patients (with mean age 29.81 $\pm$ 1.45 years, four males, five females, oral informed consent); thus, a sample size of nine. Nevertheless, it must be emphasized that all FEA numerical studies mentioned above used a sample size of one (one patient, one model, and a few numerical simulations). Thus, the sample size used here was found to be acceptable.

Initially, more patients were examined, but only nine met the inclusion criteria (intact mandibular arch, intact teeth, no malposition, non-inflamed periodontium, no advanced bone loss, orthodontic treatment indication, regular follow-up availability, good oral hygiene). The exclusion criteria were in opposition to the above.

The mandibular region with two molars and premolars received a CBCT examination (cone beam computed tomography, ProMax 3DS, Planmeca, FI-00880 Helsinki, Finland, 0.075 mm voxel size). The DICOM slices containing various shades of gray were loaded in Amira 5.4.0 (Visage Imaging Inc., 300 Brickstone Square, Suite 201, Andover, MA 01810, USA) reconstruction software. The reconstruction process was manual since the automated software function did not correctly identify all tissular components, thereby enhancing the anatomical accuracy of the models.

Only the second lower premolar was reconstructed, while the alveolar bone socket of the other three teeth was filled with bone (cortical and trabecular). All dental tissue components were identified and reconstructed: enamel, dentine, dental pulp, neuro-vascular bundle (NVB), periodontal ligament (PDL), cortical and trabecular bone (Figure 1).

Cementum could not be separated from dentine; thus, due to the similar physical properties, it was reconstructed as dentine. PDL had a variable thickness of 0.15–0.225 mm and included NVB in its apical third. The base of a stainless-steel bracket was reconstructed on the enamel component. Since the models had various but small levels of bone loss, limited to cervical third, the missing bone and PDL were reconstructed, thus obtaining nine models with intact periodontium of 5.06–6.05 million C3D4 tetrahedral elements, 0.97–1.07 million nodes, and a global element size of 0.08–0.116 mm (high anatomical accuracy, when compared with the above-mentioned numerical studies). Due to the manual reconstruction process, all models displayed a small number of surface irregularities in non-essential areas (the stressed areas were quasi-continuous). All internal mesh testing for verifying the algorithm base processes in both software resulted in no mesh/element errors, with only a limited number of element warnings (Figure 2, yellow dots). Thus, for one of the intact periodontium nine models from a total number of 5.06–6.05 million elements, there were only 264 element warnings (representing 0.0043%): 201 (0.0039%) of 5,117,355 bone structure elements, 63 (0.00677%) of 930,023 of tooth, bracket, and PDL elements, 39 (0.00586%) of 665,501 tooth and bracket elements, 26 (0.00459185%) of 566,221 radicular dentine–cementum and coronal dentine elements, and 17 (0.0141469%) of 120,168 enamel and bracket elements (Figure 2).

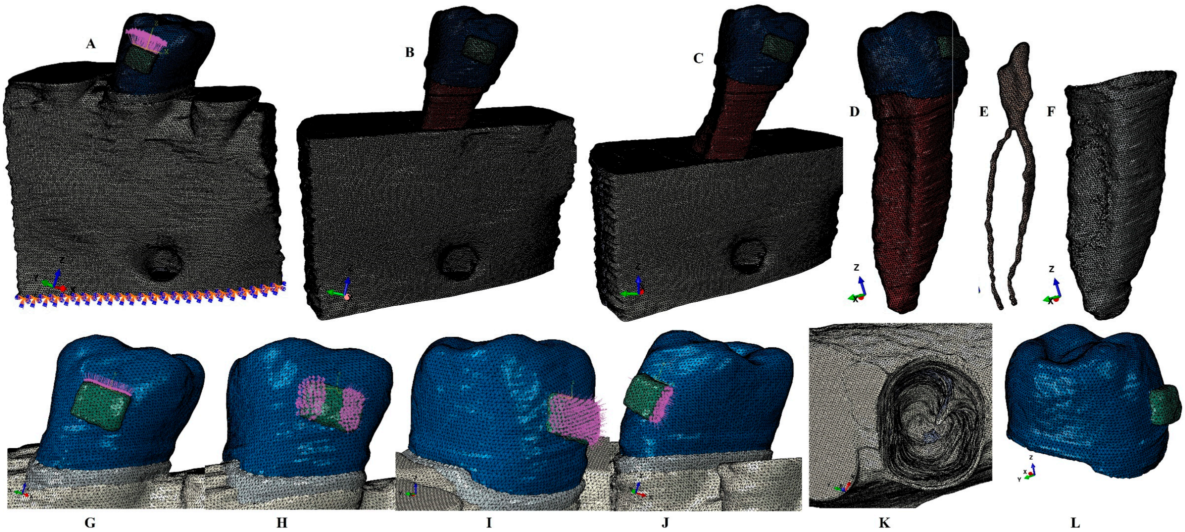

Figure 1. Mesh model: (**A**)—second lower right premolar model with 0 mm bone loss and applied vector for extrusion, (**B**)—second lower right premolar model with 4 mm bone loss, (**C**)—second lower right premolar model with 8 mm bone loss, (**D**)—second lower right premolar model, (**E**)—dental pulp and NVB, (**F**)—PDL, applied vectors: (**G**)—intrusion, (**H**)—rotation, (**I**)—tipping, (**J**)—translation, (**K**)—alveolar bone socket, (**L**)—enamel with stainless-steel bracket base.

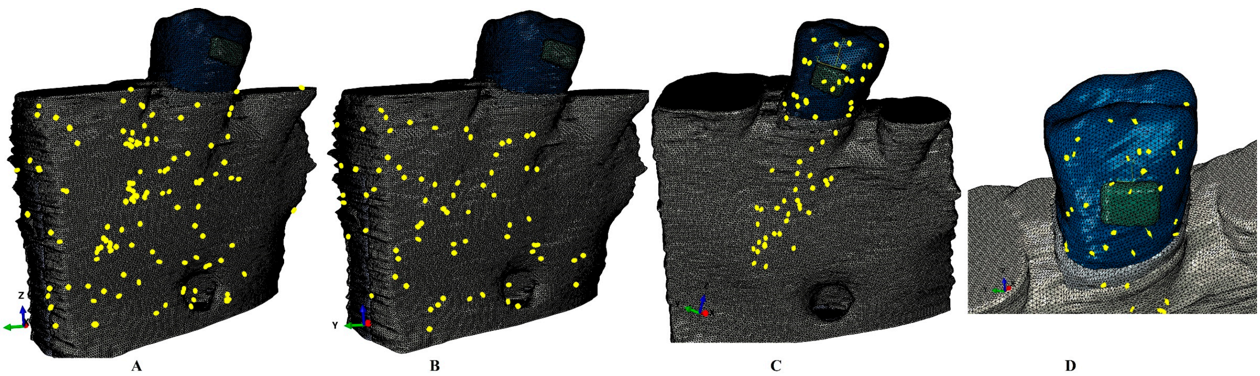

Figure 2. Element warnings in one of nine intact periodontium models: (**A**)—cortical component, (**B**)—trabecular component, (**C**)—tooth structure, (**D**)—tooth structure (details).

A gradual horizontal periodontal breakdown process (0–8 mm) was simulated by reducing the bone and PDL by 1 mm, thus obtaining eighty-one models.

All numerical simulations were performed using Abaqus 6.13-1 software (Dassault Systèmes Simulia Corp., Stationsplein 8-K, 6221 BT Maastricht, The Netherlands). The boundary conditions were isotropy, linear elasticity, and homogeneity (similar to the above-mentioned numerical studies, Table 1). Tresca failure criterion (maximum shear stress, specially designed for non-homogenous ductile materials with a certain brittle flow mode) was employed.

Table 1. Elastic properties of materials.

Material	Young's Modulus, E (GPa)	Poisson Ratio, υ	Refs.
Enamel	80	0.33	[2,3,5,6,9,10,16,48]
Dentin/Cementum	18.6	0.31	[2,3,5,6,9,10,16,48]
Pulp	0.0021	0.45	[2,3,5,6,9,10,16,48]
PDL	0.0667	0.49	[2,3,5,6,9,10,16,48]
Cortical bone	14.5	0.323	[2,3,5,6,9,10,16,48]
Trabecular bone	1.37	0.3	[2,3,5,6,9,10,16,48]
Bracket (Stainless Steel)	190	0.265	[2,3,5,6,9,10,16,48]

Three orthodontic forces, 0.6 N (approx. 60 gf), 1.2 N (approx. 120 gf), and 2.4 N (approx. 240 gf), were applied at the stainless-steel bracket base (on various surfaces) for simulating the five most used orthodontic movements: extrusion, intrusion, rotation, tipping, and translation. To keep the intensity of the force constant, the surface of the applied area was carefully considered for each movement (i.e., magnitude). In particular, the loads were adapted depending on each surface-measured area to keep the load constant and uniform. Abaqus load manager conditions include step procedure: static, general; load type: pressure; load status: created in step; distribution: uniform; magnitude: depending on the surface area; amplitude: ramp. Abaqus boundary manager conditions include step procedure: static, general; boundary condition type: symmetry/antisymmetry/encastre; boundary condition status: created in step. When editing the load appliance, the chosen distribution was uniform and the amplitude was "Ramp" (i.e., default)—the load was applied with increasing small increments up to the total amount of force. The load appliance was continuous with a small incremental progressive increase. Since deformations were extremely small as well as the amount of loads, with or without activating "follow the nodal rotation function", the results will be similar.

The first two loads were selected since they are considered safe for use in intact periodontium and for being able to correlate them with our previous research and the above-mentioned numerical studies. The third load (2.4 N) was chosen since it is higher than the mechanical limit of 1 N, to investigate the differences between the results (to assess the assumed boundary conditions), and to be able to correlate them with the above-mentioned numerical results.

The numerical simulation results were both qualitative (color-coded projections of various colors of the maximum shear stress with high stress—red-orange, moderate stress: yellow-green, and low stress: blue) and quantitative (average numerical values in KPa). These results were then correlated with the 16 KPa of physiological MHP, mechanical knowledge, acknowledged clinical data, and other similar numerical analyses.

3. Results

Herein, the numerical simulation analyzed 81 3D models in 810 simulations. No visible influence of age, periodontal status, or gender was seen. The results were both qualitative (color-coded projections of the maximum shear stress distribution in all models' components, Figures 3–8) and quantitative (Tables 2–7, in KPa).

All three forces (0.6 N, 1.2 N, and 2.4 N) displayed similar qualitative results (independently of bone loss level), while the quantitative results doubled for 1.2 N and quadrupled for 2.4 N when compared with 0.6 N.

Quantitatively, the highest amount of stress was displayed by rotation and translation, followed by tipping, while intrusion and extrusion were the least stressful.

3.1. Extrusion (Figure 3, Table 2)

Quantitatively, in intact periodontium, 0.6 N produced the highest amount of stress (i.e., 299.4 KPa, Figure 3A and Table 2) at bracket level, with a visible decreasing pattern in the other components. Qualitatively, the highest stress concentrated on and around the bracket (Figure 3B,C). However, when individually assessing each component, the most heavily stressed were dentin (Figure 3D), NVB (Figure 3E), and PDL (Figure 3F). In the dentine–cementum component, the vestibular cervical third displayed orange-yellow high stress areas, which are prone to external root resorption risks (i.e., 107.5 KPa, 6.7 times higher than the physiological MHP of 16 KPa). Qualitatively, despite the fact that PDL, pulp, and NVB displayed red-orange high stress areas, they did not quantitatively exceed MHP; thus, they can be safely applied in intact periodontium (Table 2). Moreover, despite the fact that bone and dentine–cementum (due to lesser vascularization) components exceeded MHP, they are not as sensitive to high pressures; thus, the resorptive and ischemic risks are smaller. The absorption–dissipation ability pattern of the above structures was clearly visible (Table 2): a progressive decrease in stress in radicular dentine (14.76–35.9%), alveolar bone socket (12.51–50.04%), PDL (1–2.24%), pulp (0.05%), and NVB (0.57%) when compared with the initial stress applied on the bracket (299.4 KPa).

In reduced periodontium (1–8 mm), the quantitative amount increases in correlation with bone loss. Nevertheless, the decreasing stress pattern (i.e., absorption–dissipation ability) in the model's components, which is visible in intact periodontium, was maintained. Qualitatively and quantitatively, coronal stress around and on the bracket increases (red-orange visible in Figure 3B,C), while radicular stress (Figure 3D) extends in the entire root (i.e., visible external resorptive risk areas are in the middle third). The progression of bone loss increases stresses in the entire alveolar socket (Figure 3G) and PDL (Figure 3F). Quantitatively, 0.6 N induces stresses in PDL cervical third exceeding MHP after 6 mm of loss, with higher ischemic and resorptive risks. From stresses manifested on the bracket level (305.5–392.5 KPa), only 1.24–5.75% was displayed in PDL, 0.05–0.09% in dental pulp, 0.63–1.11% in NVB, 14–50% in alveolar socket bone, and 24.51–63.47% in radicular dentine (Table 2). There was a visible increasing stress pattern in radicular dentine component from 24.51–42.77% in 1 mm loss to 63.47% in 8 mm loss, which is strictly correlated with periodontal breakdown.

The bracket absorption–dissipation ability (difference between tooth with bracket and without bracket coronal stress) was 15.48% in 0 mm, 16.43% in 1 mm, and 4.02% in 8 mm loss.

The enamel absorption–dissipation ability (difference between coronal tooth without bracket and coronal dentine stress) was 62.26% in 0 mm, 62.26% in 1 mm, and 65.44% in 8 mm loss.

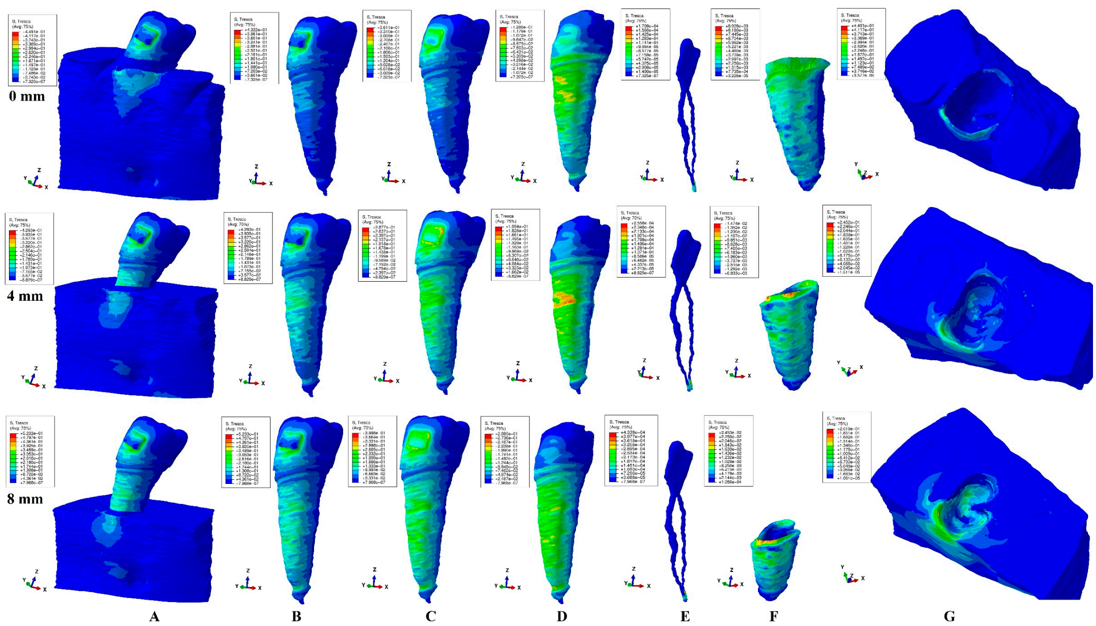

Figure 3. Extrusion—Tresca stress display in dental tissues subjected to 0.6 N for 0, 4, and 8 mm bone loss: (**A**)—dental tissues' structure, (**B**)—tooth with bracket, (**C**)—tooth without bracket, (**D**)—dentine component, (**E**)—dental pulp and NVB, (**F**)—PDL, (**G**)—bone with alveolar socket.

Table 2. Maximum stress average values (KPa) produced by orthodontic forces in tooth structure and dentine–cementum.

Resorption (mm)			**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Extrusion	Structure		**299.40**	**305.05**	**310.70**	**316.35**	**322.00**	**339.60**	**357.25**	**374.87**	**392.50**
0.6 N/60 gf	Tooth + bracket	a	72.03	107.67	143.31	178.98	214.60	237.27	259.94	282.61	305.30
Stress		m	108.00	134.65	161.30	187.95	214.60	237.27	259.94	282.61	305.30
Amount		c	216.10	233.63	251.15	268.67	286.20	290.97	295.75	300.52	305.30
In each		C	288.10	296.57	305.00	313.52	322.00	328.72	335.45	342.17	348.90
Component	Tooth	a	60.18	93.08	125.98	158.88	191.80	210.50	229.20	247.90	266.60
		m	90.28	115.66	141.04	166.42	191.80	210.50	229.20	247.90	266.60
		c	120.40	144.20	168.00	191.80	215.63	228.37	241.11	253.85	266.60
		C	240.70	246.45	252.20	257.95	263.70	281.05	298.40	315.75	333.10
	Dentine	a	44.18	74.76	105.34	135.92	166.50	187.15	207.81	228.46	249.11
		m	44.18	74.76	105.34	135.92	166.50	187.15	207.81	228.46	249.11
		c	107.50	130.48	153.45	176.43	199.40	211.83	224.26	236.68	249.11
		C	54.28	57.66	61.04	64.41	67.79	69.88	71.97	74.06	76.15
	Bone	a	37.46	43.43	49.40	55.36	61.33	62.82	64.31	65.80	67.30
		m	37.46	38.32	39.17	40.02	40.89	43.27	45.67	48.00	50.47
		c	149.83	153.25	156.67	160.09	163.51	164.71	165.92	167.12	168.34
	NVB	NVB	1.71	1.92	2.14	2.35	2.56	3.01	3.45	3.90	4.34
	Pulp	a	0.15	0.17	0.19	0.20	0.22	0.26	0.30	0.33	0.37
		c	0.15	0.17	0.19	0.20	0.22	0.26	0.30	0.33	0.37
	PDL	a	3.00	3.80	4.59	5.39	6.18	6.70	7.22	7.73	8.25
		m	3.00	3.80	4.59	5.39	6.18	6.70	7.22	7.73	8.25
		c	6.70	8.41	10.11	11.82	13.52	15.77	18.04	20.29	22.55
Extrusion	Structure %		**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
0.6 N/60 gf	Tooth + bracket %	a	**24.06**	**35.30**	**46.12**	**56.58**	**66.65**	**69.87**	**72.76**	**75.39**	**77.78**
Stress %		m	36.07	44.14	51.92	59.41	66.65	69.87	72.76	75.39	77.78
Reaching		c	72.18	76.59	80.83	84.93	88.88	85.68	82.79	80.17	77.78
Each		C	**96.23**	**97.22**	**98.17**	**99.11**	**100.00**	**96.80**	**93.90**	**91.28**	**88.89**
Component	Tooth %	a	**20.10**	**30.51**	**40.55**	**50.22**	**59.57**	**61.98**	**64.16**	**66.13**	**67.92**
		m	30.15	37.92	45.39	52.61	59.57	61.98	64.16	66.13	67.92
		c	40.21	47.27	54.07	60.63	66.96	67.25	67.49	67.72	67.92
		C	**80.39**	**80.79**	**81.17**	**81.54**	**81.89**	**82.76**	**83.53**	**84.23**	**84.87**
	Dentine %	a	**14.76**	**24.51**	**33.90**	**42.97**	**51.71**	**55.11**	**58.17**	**60.94**	**63.47**
		m	14.76	24.51	33.90	42.97	51.71	55.11	58.17	60.94	63.47
		c	35.91	42.77	49.39	55.77	61.93	62.38	62.77	63.14	63.47
		C	**18.13**	**18.90**	**19.64**	**20.36**	**21.05**	**20.58**	**20.15**	**19.76**	**19.40**
	Bone %	a	**12.51**	**14.24**	**15.90**	**17.50**	**19.05**	**18.50**	**18.00**	**17.55**	**17.15**
		m	12.51	12.56	12.61	12.65	12.70	12.74	12.78	12.80	12.86
		c	**50.04**	**50.24**	**50.43**	**50.61**	**50.78**	**48.50**	**46.44**	**44.58**	**42.89**
	NVB %	NVB	**0.57**	**0.63**	**0.69**	**0.74**	**0.79**	**0.89**	**0.97**	**1.04**	**1.11**
	Pulp %	a	**0.05**	**0.05**	**0.06**	**0.06**	**0.07**	**0.08**	**0.08**	**0.09**	**0.09**
		c	**0.05**	**0.05**	**0.06**	**0.06**	**0.07**	**0.08**	**0.08**	**0.09**	**0.09**
	PDL %	a	**1.00**	**1.24**	**1.48**	**1.70**	**1.92**	**1.97**	**2.02**	**2.06**	**2.10**
		m	1.00	1.24	1.48	1.70	1.92	1.97	2.02	2.06	2.10
		c	**2.24**	**2.76**	**3.25**	**3.73**	**4.20**	**4.64**	**5.05**	**5.41**	**5.75**

structure—stress displayed by the 3D model. Tooth + bracket, Tooth, Dentine, Bone, NVB, Pulp, PDL components—stress displayed by these components. a—apical third, m—middle third, c—cervical third, C—crown. Tooth + bracket %, Tooth %, Dentine %, Bone %, NVB %, Pulp %, PDL % components—% stress displayed by these components.

3.2. Intrusion (Figure 4, Table 3)

In intact and reduced periodontium, both qualitative and quantitative biomechanical behaviors are similar to extrusion. However, there are some visible differences (smaller amount of stress in cervical third) in PDL component, which are specific to the intrusion movement (Figure 4F).

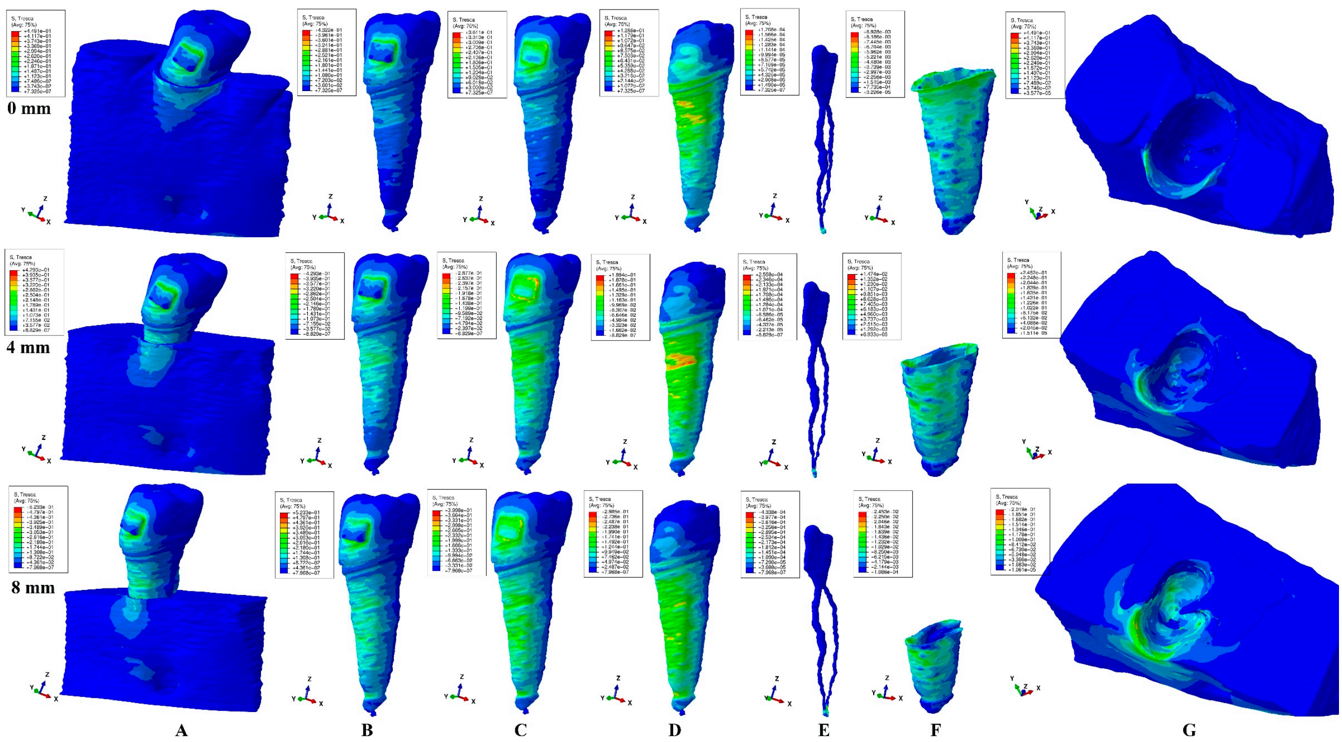

Figure 4. Intrusion—Tresca stress display in dental tissues subjected to 0.6 N for 0, 4, and 8 mm bone loss: (**A**)—dental tissues' structure, (**B**)—tooth with bracket, (**C**)—tooth without bracket, (**D**)—dentine component, (**E**)—dental pulp and NVB, (**F**)—PDL, (**G**)—bone with alveolar socket.

Table 3. Maximum stress average values (KPa) produced by orthodontic forces in tooth structure and dentine–cementum.

Resorption (mm)			**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Intrusion	Structure		**299.40**	**305.05**	**310.70**	**316.35**	**322.00**	**339.60**	**357.25**	**374.87**	**392.50**
0.6 N/60 gf	Tooth + bracket	a	72.03	107.67	143.31	178.98	214.60	237.27	259.94	282.61	305.30
Stress		m	108.00	134.65	161.30	187.95	214.60	237.27	259.94	282.61	305.30
Amount		c	216.10	233.63	251.15	268.67	286.20	290.97	295.75	300.52	305.30
In each		C	288.10	296.57	305.00	313.52	322.00	328.72	335.45	342.17	348.90
Component	Tooth	a	60.18	93.08	125.98	158.88	191.80	210.50	229.20	247.90	266.60
		m	90.28	115.66	141.04	166.42	191.80	210.50	229.20	247.90	266.60
		c	120.40	144.20	168.00	191.80	215.63	228.37	241.11	253.85	266.60
		C	240.70	246.45	252.20	257.95	263.70	281.05	298.40	315.75	333.10
	Dentine	a	44.18	74.76	105.34	135.92	166.50	187.15	207.81	228.46	249.11
		m	44.18	74.76	105.34	135.92	166.50	187.15	207.81	228.46	249.11
		c	107.50	130.48	153.45	176.43	199.40	211.83	224.26	236.68	249.11
		C	54.28	57.66	61.04	64.41	67.79	69.88	71.97	74.06	76.15
	Bone	a	37.46	43.43	49.40	55.36	61.33	62.82	64.31	65.80	67.30
		m	37.46	38.32	39.17	40.02	40.89	43.27	45.67	48.00	50.47
		c	149.83	153.25	156.67	160.09	163.51	164.71	165.92	167.12	168.34
	NVB	NVB	1.71	1.92	2.14	2.35	2.56	3.01	3.45	3.90	4.34
	Pulp	a	0.15	0.17	0.19	0.20	0.22	0.26	0.30	0.33	0.37
		c	0.15	0.17	0.19	0.20	0.22	0.26	0.30	0.33	0.37
	PDL	a	3.00	3.49	3.98	4.46	4.96	5.78	6.62	7.43	8.25
		m	3.00	5.21	5.51	6.31	7.41	7.62	7.83	8.04	8.25
		c	5.22	6.68	8.15	9.61	11.07	12.91	14.75	16.58	18.43
Intrusion	Structure %		**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
0.6 N/60 gf	Tooth + bracket %	a	**24.06**	**35.30**	**46.12**	**56.58**	**66.65**	**69.87**	**72.76**	**75.39**	**77.78**
Stress %		m	36.07	44.14	51.92	59.41	66.65	69.87	72.76	75.39	77.78
Reaching		c	72.18	76.59	80.83	84.93	88.88	85.68	82.79	80.17	77.78
Each		C	**96.23**	**97.22**	**98.17**	**99.11**	**100.00**	**96.80**	**93.90**	**91.28**	**88.89**

Table 3. *Cont.*

Resorption (mm)			0	1	2	3	4	5	6	7	8
Component	Tooth %	a	**20.10**	**30.51**	**40.55**	**50.22**	**59.57**	**61.98**	**64.16**	**66.13**	**67.92**
		m	30.15	37.92	45.39	52.61	59.57	61.98	64.16	66.13	67.92
		c	40.21	47.27	54.07	60.63	66.96	67.25	67.49	67.72	67.92
		C	**80.39**	**80.79**	**81.17**	**81.54**	**81.89**	**82.76**	**83.53**	**84.23**	**84.87**
	Dentine %	a	**14.76**	**24.51**	**33.90**	**42.97**	**51.71**	**55.11**	**58.17**	**60.94**	**63.47**
		m	14.76	24.51	33.90	42.97	51.71	55.11	58.17	60.94	63.47
		c	35.91	42.77	49.39	55.77	61.93	62.38	62.77	63.14	63.47
		C	**18.13**	**18.90**	**19.64**	**20.36**	**21.05**	**20.58**	**20.15**	**19.76**	**19.40**
	Bone %	a	**12.51**	**14.24**	**15.90**	**17.50**	**19.05**	**18.50**	**18.00**	**17.55**	**17.15**
		m	12.51	12.56	12.61	12.65	12.70	12.74	12.78	12.80	12.86
		c	**50.04**	**50.24**	**50.43**	**50.61**	**50.78**	**48.50**	**46.44**	**44.58**	**42.89**
	NVB %	NVB	**0.57**	**0.63**	**0.69**	**0.74**	**0.79**	**0.89**	**0.97**	**1.04**	**1.11**
	Pulp %	a	**0.05**	**0.05**	**0.06**	**0.06**	**0.07**	**0.08**	**0.08**	**0.09**	**0.09**
		c	**0.05**	**0.05**	**0.06**	**0.06**	**0.07**	**0.08**	**0.08**	**0.09**	**0.09**
	PDL %	a	**1.00**	**1.14**	**1.28**	**1.41**	**1.54**	**1.70**	**1.85**	**1.98**	**2.10**
		m	1.00	1.71	1.77	1.99	2.30	2.24	2.19	2.14	2.10
		c	**1.74**	**2.19**	**2.62**	**3.04**	**3.44**	**3.80**	**4.13**	**4.42**	**4.70**

structure—stress displayed by the 3D model. Tooth + bracket, Tooth, Dentine, Bone, NVB, Pulp, PDL components —stress displayed by these components. a—apical third, m—middle third, c—cervical third, C—crown. Tooth + bracket %, Tooth %, Dentine %, Bone %, NVB %, Pulp %, PDL % components—% stress displayed by these components.

3.3. Rotation (Figure 5, Table 4)

The highest quantitative stresses displayed among all five analyzed movements seem to be the most stressful movements. In intact periodontium, the stress around and on the bracket was 799.8 KPa, showing the same decreasing stress pattern (in tissular components) seen in extrusion and intrusion. In PDL cervical third, 0.6 N produced stresses under MHP. The dentine component displayed a high red-orange coronal stress under the bracket position. Stresses were concentrated in cervical third of radicular dentine, PDL, and alveolar socket bone. The quantitative amount of stress displayed by 1.2 N exceeded MHP only in cervical third of PDL, suggesting that it is safe to be clinically used (small neglectable areas of red-orange, Figure 5F). From a total of 799.8 KPa displayed at bracket level, only 8.98–21.19% are visible in radicular dentine, 9.6–38.37% in alveolar socket bone, 0.29–2.29% in PDL, 0.02–0.04% in pulp, and 0.22% in NVB.

In reduced periodontium, the displayed quantitative stresses at bracket level ranged between 803.35 and 890.4 KPa (for 0.6 N/approx. 60 gf), while only 16.96–77.69% reached radicular dentine, 10.54–45.19% alveolar bone socket, 0.38–7.67% PDL, 0.03–0.12% pulp, and 0.29–0.69% NVB. For radicular dentine component, a quantitative significant stress increase was visible during the periodontal breakdown (from 16–33.32% in 1 mm of loss to 58.35–77.69% in 8 mm of loss), while stress percentages for other components (alveolar bone socket, NVB, PDL, dental pulp) remained relatively constant. The radicular dentine displayed in the middle third qualitative red-orange areas of highly resorptive risks (especially in 8 mm of loss) correlated with bone loss. The coronal dentine stress decreases strictly correlated with bone loss. PDL cervical third stress (for 0.6 N) displayed qualitative red-orange areas which are more prone to further loss and quantitative values exceeding MHP (4 times for 8 mm of loss). Based on the above, 0.6 N can be safely applied in both intact and reduced periodontium, with the observation that PDL cervical third could suffer from ischemia and further loss. Rotational movement displayed increased stress in the entire alveolar bone socket (i.e., especially the cervical third, 40.18–45.19% of bracket stress) and correlated with periodontal breakdown. During the rotational movement in both intact and reduced periodontium, only the dentine component displayed significant red-orange high stress areas.

The 1.2 N force produced similar qualitative results as 0.6 N, and quantitative higher amounts of stress (doubling the numerical values when compared with 0.6 N), being prone to ischemic and resorptive risks in both middle and cervical third PDL and radicular dentine.

The bracket absorption–dissipation ability was 18.73% in 0 mm, 17.63% in 1 mm, and 17.48% in 8 mm loss.

The enamel absorption–dissipation ability was 60.09% in 0 mm, 60.09% in 1 mm, and 62.84% in 8 mm loss.

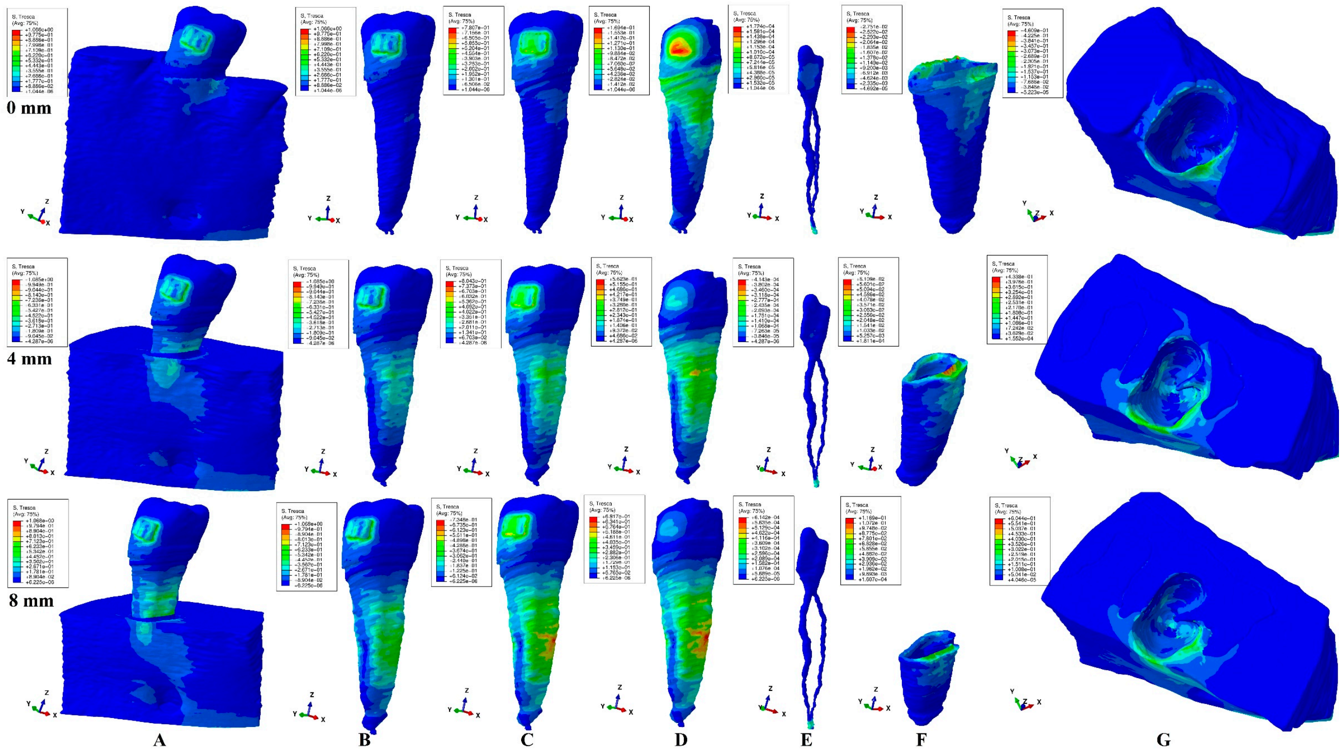

Figure 5. Rotation—Tresca stress display in dental tissues subjected to 0.6 N for 0, 4, and 8 mm bone loss: (**A**)—dental tissues' structure, (**B**)—tooth with bracket, (**C**)—tooth without bracket, (**D**)—dentine component, (**E**)—dental pulp and NVB, (**F**)—PDL, (**G**)—bone with alveolar socket.

Table 4. Maximum stress average values (KPa) produced by orthodontic forces in tooth structure and dentine–cementum.

Resorption (mm)			**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Rotation	Structure		**799.80**	**803.35**	**806.90**	**810.45**	**814.00**	**833.10**	**852.20**	**871.30**	**890.40**
0.6 N/60 gf	Tooth + bracket	a	88.60	156.90	225.20	293.50	361.80	427.17	492.54	557.91	623.30
Stress		m	88.60	224.70	360.84	496.96	633.10	675.15	717.20	759.25	801.30
Amount		c	177.70	291.55	405.40	519.25	633.10	652.90	672.70	692.50	712.30
In each		C	799.80	803.35	806.90	810.45	814.00	833.10	852.20	871.30	890.40
Component	Tooth	a	81.00	144.52	208.00	271.56	335.10	389.20	443.30	497.40	551.50
		m	81.00	178.00	275.10	372.15	469.20	535.60	602.00	668.40	734.80
		c	195.20	297.20	399.30	501.20	603.20	605.47	607.75	610.02	612.30
		C	650.00	657.32	664.65	671.97	679.30	693.17	707.05	720.92	734.80
	Dentine	a	71.84	136.25	200.66	265.07	329.48	376.99	424.50	472.01	519.52
		m	71.84	159.55	247.27	334.99	422.70	489.96	557.21	624.47	691.72
		c	169.46	267.67	365.89	464.10	562.31	565.96	569.62	573.27	576.92
		C	169.43	174.34	179.24	184.15	189.65	186.04	182.42	178.81	175.19
	Bone	a	76.74	84.65	92.57	100.48	108.39	119.02	129.64	140.26	150.88
		m	76.74	84.65	92.57	100.48	108.39	119.02	129.64	140.26	150.88
		c	306.88	322.78	338.66	354.55	370.45	378.43	386.42	394.42	402.41

Table 4. *Cont.*

Resorption (mm)			**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
	NVB	NVB	1.72	2.33	2.93	3.54	4.14	4.64	5.14	5.64	6.14
	Pulp	a	0.15	0.21	0.27	0.33	0.38	0.43	0.48	0.52	0.57
		c	0.29	0.43	0.51	0.62	0.73	0.82	0.91	0.99	1.08
	PDL	a	2.34	3.07	3.80	4.53	5.26	6.42	7.58	8.73	9.89
		m	4.62	6.05	7.48	8.90	10.33	12.65	14.98	17.30	19.62
		c	18.35	25.23	32.11	38.98	45.86	51.47	57.07	62.68	68.28
Rotation	Structure %		**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
0.6 N/60 gf	Tooth + bracket %	a	**11.08**	**19.53**	**27.91**	**36.21**	**44.45**	**51.27**	**57.80**	**64.03**	**70.00**
Stress %		m	11.08	27.97	44.72	61.32	77.78	81.04	84.16	87.14	89.99
Reaching		c	22.22	36.29	50.24	64.07	77.78	78.37	78.94	79.48	80.00
Each		C	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Component	Tooth %	a	**10.13**	**17.99**	**25.78**	**33.51**	**41.17**	**46.72**	**52.02**	**57.09**	**61.94**
		m	10.13	22.16	34.09	45.92	57.64	64.29	70.64	76.71	82.52
		c	24.41	37.00	49.49	61.84	74.10	72.68	71.32	70.01	68.77
		C	**81.27**	**81.82**	**82.37**	**82.91**	**83.45**	**83.20**	**82.97**	**82.74**	**82.52**
	Dentine %	a	**8.98**	**16.96**	**24.87**	**32.71**	**40.48**	**45.25**	**49.81**	**54.17**	**58.35**
		m	8.98	19.86	30.64	41.33	51.93	58.81	65.38	71.67	77.69
		c	21.19	33.32	45.34	57.26	69.08	67.93	66.84	65.79	64.79
		C	**21.18**	**21.70**	**22.21**	**22.72**	**23.30**	**22.33**	**21.41**	**20.52**	**19.68**
	Bone %	a	**9.60**	**10.54**	**11.47**	**12.40**	**13.32**	**14.29**	**15.21**	**16.10**	**16.95**
		m	9.60	10.54	11.47	12.40	13.32	14.29	15.21	16.10	16.95
		c	**38.37**	**40.18**	**41.97**	**43.75**	**45.51**	**45.42**	**45.34**	**45.27**	**45.19**
	NVB %	NVB	**0.22**	**0.29**	**0.36**	**0.44**	**0.51**	**0.56**	**0.60**	**0.65**	**0.69**
	Pulp %	a	**0.02**	**0.03**	**0.03**	**0.04**	**0.05**	**0.05**	**0.06**	**0.06**	**0.06**
		c	**0.04**	**0.05**	**0.06**	**0.08**	**0.09**	**0.10**	**0.11**	**0.11**	**0.12**
	PDL %	a	**0.29**	**0.38**	**0.47**	**0.56**	**0.65**	**0.77**	**0.89**	**1.00**	**1.11**
		m	0.58	0.75	0.93	1.10	1.27	1.52	1.76	1.99	2.20
		c	**2.29**	**3.14**	**3.98**	**4.81**	**5.63**	**6.18**	**6.70**	**7.19**	**7.67**

structure—stress displayed by the 3D model. Tooth + bracket, Tooth, Dentine, Bone, NVB, Pulp, PDL components—stress displayed by these components. a—apical third, m—middle third, c—cervical third, C—crown. Tooth + bracket %, Tooth %, Dentine %, Bone %, NVB %, Pulp %, PDL % components—% stress displayed by these components.

3.4. Translation (Figure 6, Table 5)

Based on quantitative and qualitative results, translation seems to be the second most stressful movement after rotation. In intact periodontium, translation displayed 504.4 KPa at bracket level, with visible stresses in cervical third of radicular dentine, PDL, and alveolar bone socket. Translation is the single movement that displayed a clearly visible stress in coronal pulp. From the 504.4 KPa displayed at bracket level, 13.47–39.67% reaches radicular dentine, 14–36.73% alveolar bone socket, 0.02–0.06% pulp, 0.4–3.51% PDL, and 0.22% NVB.

In reduced periodontium, the increase in quantitative stress results correlated with bone loss. Qualitatively, stress areas extended in the entire radicular dentine with red-orange areas displayed in middle and apical third, being prone to external resorptive and ischemic processes. Quantitively, a visible increase in stress amount during periodontal breakdown was clearly visible in the radicular dentine component ranging from 15.57–49.57% in 1 mm loss up to 62–92.9% in 8 mm loss (more pronounced in radicular middle third after 4 mm bone loss). The absorption–dissipation ability of the other tissular components remained constant, 16.98–41.57% in alveolar bone socket, 0.51–8.37% PDL, 0.02–0.08% pulp, and 0.25–0.32% in NVB during the bone loss process when compared with the other movements. The qualitative coronal pulp stress display remained visible during bone loss, but with a clearly decreasing pattern. Moreover, the qualitative alveolar bone socket stress display showed a decreasing stress pattern.

In both intact and reduced periodontium, 0.6 N seems to be safe for application (only PDL cervical third stress exceeded MHP). Both 0.6 N and 1.2 N showed similar qualitative stress display areas, with a doubling of quantitative results for 1.2 N. PDL displayed stresses exceeding physiological MHP during bone loss simulations, in both middle and cervical third (especially after 4 mm of loss, visible red-orange high stress areas), which seem to be prone to ischemic and resorptive processes.

The bracket absorption–dissipation ability was 29.04% in 0 mm, 28.79% in 1 mm, and 45.81% in 8 mm loss.

The enamel absorption–dissipation ability was 57.49% in 0 mm, 57.44% in 1 mm, and 38.53% in 8 mm loss.

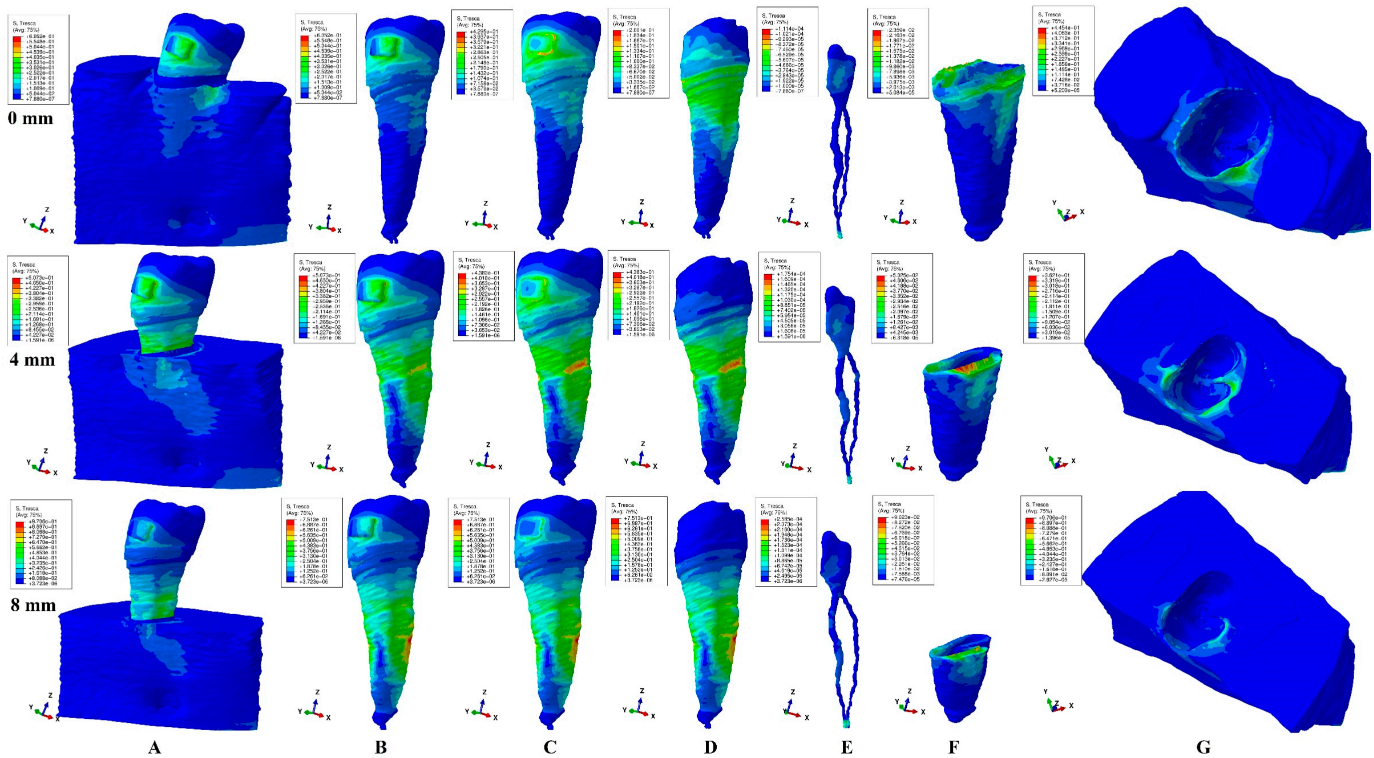

Figure 6. Translation—Tresca stress display in dental tissues subjected to 0.6 N for 0, 4, and 8 mm bone loss: (**A**)—dental tissues' structure, (**B**)—tooth with bracket, (**C**)—tooth without bracket, (**D**)—dentine component, (**E**)—dental pulp and NVB, (**F**)—PDL, (**G**)—bone with alveolar socket.

Table 5. Maximum stress average values (KPa) produced by orthodontic forces in tooth structure and dentine–cementum.

Resorption (mm)			**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Translation	Structure		**504.40**	**505.10**	**505.80**	**506.50**	**507.30**	**582.67**	**658.00**	**733.40**	**808.80**
0.6 N/60 gf	Tooth + bracket	a	100.90	117.95	135.00	152.05	169.10	267.70	366.30	464.90	563.61
Stress		m	100.90	191.92	282.90	373.90	465.00	536.58	608.10	679.70	751.35
Amount		c	202.41	268.00	333.69	399.30	465.00	489.60	415.30	538.90	563.61
In each		C	504.40	505.10	505.80	506.50	507.30	582.67	658.00	733.40	808.80
Component	Tooth	a	71.50	90.10	108.80	127.40	146.10	250.47	354.84	459.20	563.61
		m	71.50	163.20	254.90	346.60	438.31	516.50	594.80	673.00	751.35
		c	202.41	261.37	320.34	379.30	438.31	469.60	500.90	532.20	563.61
		C	357.90	359.70	361.60	363.40	365.30	383.55	401.80	420.00	438.30
	Dentine	a	67.94	78.67	89.40	100.12	110.85	208.50	306.16	403.81	501.46
		m	67.94	160.53	253.13	345.72	438.31	516.57	594.83	673.09	751.35
		c	200.11	250.38	300.65	350.91	401.18	441.88	482.57	523.27	563.96
		C	67.94	69.55	71.16	72.77	74.38	87.45	100.51	113.58	126.64
	Bone	a	74.17	85.75	97.33	108.92	120.51	130.76	141.04	151.30	161.57
		m	74.17	85.75	97.33	108.92	120.51	130.76	141.04	151.30	161.57
		c	185.28	191.68	198.08	204.49	210.90	220.91	230.93	240.95	250.97

Table 5. *Cont.*

Resorption (mm)			0	1	2	3	4	5	6	7	8
	NVB	NVB	1.11	1.27	1.43	1.59	1.75	1.97	2.18	2.38	2.59
	Pulp	a	0.11	0.12	0.13	0.15	0.16	0.18	0.21	0.23	0.25
		c	0.28	0.32	0.37	0.41	0.45	0.51	0.56	0.62	0.67
	PDL	a	2.01	2.57	3.13	3.69	4.25	5.06	5.88	6.69	7.51
		m	3.97	5.09	6.20	7.31	8.43	10.09	11.76	13.43	15.10
		c	17.71	23.76	29.80	35.84	41.89	48.29	54.69	61.10	67.70
Translation	Structure %		**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
0.6 N/60 gf	Tooth + bracket %	a	**20.00**	**23.35**	**26.69**	**30.02**	**33.33**	**45.94**	**55.67**	**63.39**	**69.68**
Stress %		m	20.00	38.00	55.93	73.82	91.66	92.09	92.42	92.68	92.90
Reaching		c	40.13	53.06	65.97	78.84	91.66	84.03	63.12	73.48	69.68
Each		C	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Component	Tooth %	a	**14.18**	**17.84**	**21.51**	**25.15**	**28.80**	**42.99**	**53.93**	**62.61**	**69.68**
		m	14.18	32.31	50.40	68.43	86.40	88.64	90.40	91.76	92.90
		c	40.13	51.75	63.33	74.89	86.40	80.59	76.12	72.57	69.68
		C	**70.96**	**71.21**	**71.49**	**71.75**	**72.01**	**65.83**	**61.06**	**57.27**	**54.19**
	Dentine %	a	**13.47**	**15.57**	**17.67**	**19.77**	**21.85**	**35.78**	**46.53**	**55.06**	**62.00**
		m	13.47	31.78	50.04	68.26	86.40	88.66	90.40	91.78	92.90
		c	39.67	49.57	59.44	69.28	79.08	75.84	73.34	71.35	69.73
		C	**13.47**	**13.77**	**14.07**	**14.37**	**14.66**	**15.01**	**15.28**	**15.49**	**15.66**
	Bone %	a	**14.70**	**16.98**	**19.24**	**21.50**	**23.75**	**22.44**	**21.43**	**20.63**	**19.98**
		m	14.70	16.98	19.24	21.50	23.75	22.44	21.43	20.63	19.98
		c	**36.73**	**37.95**	**39.16**	**40.37**	**41.57**	**37.91**	**35.10**	**32.85**	**31.03**
	NVB %	NVB	**0.22**	**0.25**	**0.28**	**0.31**	**0.35**	**0.34**	**0.33**	**0.32**	**0.32**
	Pulp %	a	**0.02**	**0.02**	**0.03**	**0.03**	**0.03**	**0.03**	**0.03**	**0.03**	**0.03**
		c	**0.06**	**0.06**	**0.07**	**0.08**	**0.09**	**0.09**	**0.09**	**0.08**	**0.08**
	PDL %	a	**0.40**	**0.51**	**0.62**	**0.73**	**0.84**	**0.87**	**0.89**	**0.91**	**0.93**
		m	0.79	1.01	1.23	1.44	1.66	1.73	1.79	1.83	1.87
		c	**3.51**	**4.70**	**5.89**	**7.08**	**8.26**	**8.29**	**8.31**	**8.33**	**8.37**

structure—stress displayed by the 3D model. Tooth + bracket, Tooth, Dentine, Bone, NVB, Pulp, PDL components—stress displayed by these components. a—apical third, m—middle third, c—cervical third, C—crown. Tooth + bracket %, Tooth %, Dentine %, Bone %, NVB %, Pulp %, PDL % components—% stress displayed by these components.

3.5. Tipping (Figure 7, Table 6)

In intact periodontium, the maximum amount of stress displayed at bracket level was 366.1 KPa, which is closer to the intrusion and extrusion movements, whereas the qualitative stress was concentrated in cervical third of radicular dentine and PDL. Of the total amount of stress, only 16.39–32.4% reached radicular dentine, 20–40% alveolar bone socket, 0.42–3.31% PDL, 0.03–0.04% pulp, and 0.4% NVB. All quantitative stresses displayed in PDL, dental pulp, and NVB were lower than MHP.

Reduced periodontium displayed an extension of high stress areas (i.e., red-orange) in the entire radicular dentine and PDL cervical third, signaling potential areas of ischemic and resorptive risks. The stress increases in bone alveolar socket correlated with bone loss. Quantitatively, the circulatory sensitive tissues (PDL, NVB, and dental pulp) for both 0.6 and 1.2 N amounts of stress were lower than MHP (except in cervical third after 2–5 mm of loss), which seem to be safe for application up to 8 mm of bone loss (Figure 8 and Table 7). The radicular dentine component showed a quantitatively increasing stress pattern ranging from 23.4–40.68% in 1 mm loss to 70.94–85% in 8 mm loss, which is correlated with the bone loss process and qualitatively visible (red-orange areas are prone to resorptive processes). Of the total amount of stress of 380.4–544.1 KPa, only 20.63–44.46% reached alveolar bone socket, 0.62–8.25% PDL, 0.04–0.07% pulp, and 0.48–0.82% NVB.

The bracket absorption–dissipation ability was 16.69% in 0 mm, 18.16% in 1 mm, and 16.35% in 8 mm loss.

The enamel absorption–dissipation ability was 50.29% in 0 mm, 49.09% in 1 mm, and 42.31% in 8 mm loss.

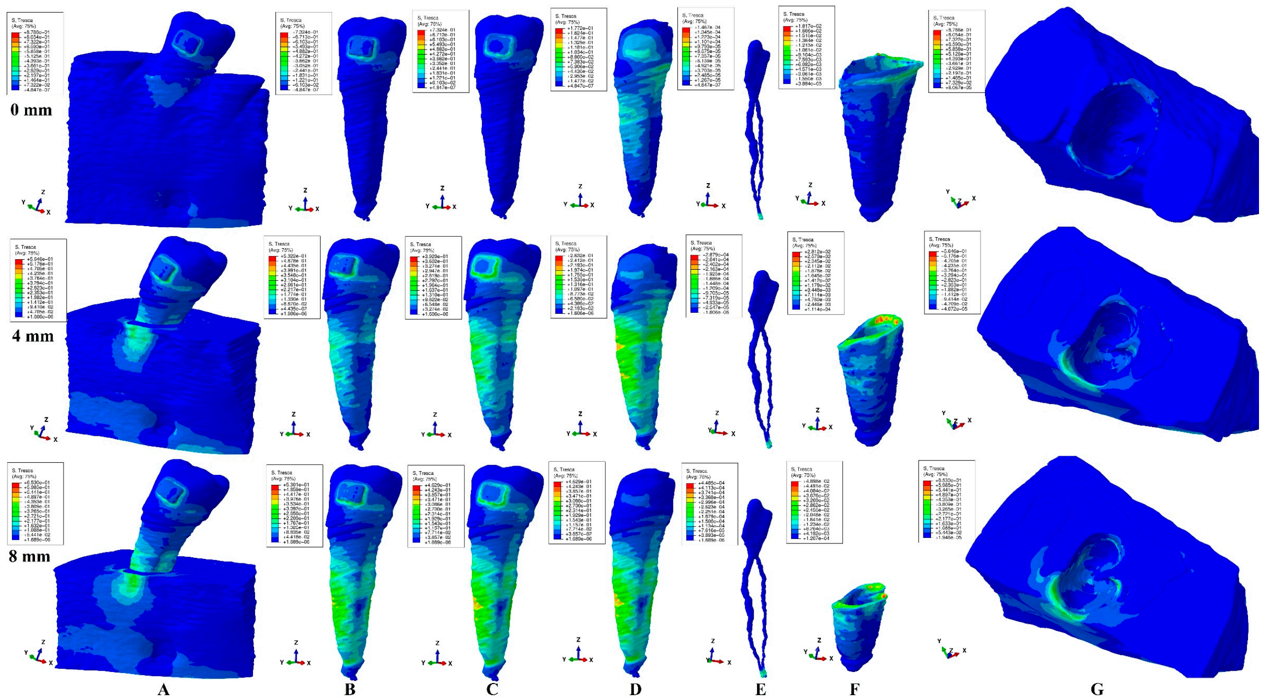

Figure 7. Tipping—Tresca stress display in dental tissues subjected to 0.6 N for 0, 4, and 8 mm bone loss: (**A**)—dental tissues' structure, (**B**)—tooth with bracket, (**C**)—tooth without bracket, (**D**)—dentine component, (**E**)—dental pulp and NVB, (**F**)—PDL, (**G**)—bone with alveolar socket.

Table 6. Maximum stress average values (KPa) produced by orthodontic forces in tooth structure and dentine–cementum.

Resorption (mm)			**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Tipping	Structure		**366.10**	**380.40**	**394.80**	**409.15**	**423.50**	**453.60**	**483.80**	**513.90**	**544.10**
0.6 N/60 gf	Tooth + bracket	a	61.67	112.85	164.03	215.20	266.41	299.20	331.90	364.70	397.60
Stress		m	61.67	134.95	208.20	281.50	354.80	387.50	420.30	453.10	485.90
Amount		c	122.61	180.60	238.70	296.70	354.80	365.50	376.20	386.90	397.60
In each		C	305.20	317.60	330.00	342.50	354.80	365.50	376.20	386.20	397.60
Component	Tooth	a	61.67	96.35	128.00	162.70	196.40	243.70	291.00	338.36	385.70
		m	61.67	119.90	178.10	236.40	294.70	336.75	378.80	420.80	462.92
		c	122.61	165.60	208.60	251.60	294.70	317.40	340.20	362.90	385.70
		C	244.10	248.50	253.00	257.40	261.90	273.50	285.20	296.90	308.60
	Dentine	a	60.01	89.00	118.00	147.00	176.00	228.50	281.00	333.50	386.00
		m	60.01	99.92	139.84	179.75	219.66	280.48	341.29	402.11	462.92
		c	118.61	154.76	190.91	227.06	263.21	293.91	324.61	355.30	386.00
		C	60.01	61.76	63.51	65.26	67.01	69.85	72.70	75.54	78.38
	Bone	a	73.27	78.48	83.70	88.92	94.14	97.81	101.50	105.17	108.85
		m	73.27	78.48	83.70	88.92	94.14	97.81	101.50	105.17	108.85
		c	146.53	156.97	167.41	177.85	188.29	195.64	202.99	210.34	217.69
	NVB	NVB	1.47	1.82	2.18	2.53	2.88	3.28	3.69	4.09	4.49
	Pulp	a	0.13	0.17	0.19	0.23	0.25	0.29	0.33	0.36	0.39
		c	0.15	0.18	0.21	0.23	0.25	0.29	0.33	0.36	0.39
	PDL	a	1.55	2.36	3.16	3.96	4.78	5.65	6.52	7.39	8.26
		m	3.06	4.07	5.09	6.10	7.11	8.42	9.73	11.03	12.34
		c	12.13	15.55	18.96	22.38	25.79	30.57	35.35	40.13	44.91
Tipping	Structure %		**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
0.6 N/60 gf	Tooth + bracket %	a	**16.84**	**29.67**	**41.55**	**52.60**	**62.91**	**65.96**	**68.60**	**70.97**	**73.07**
Stress %		m	16.84	35.48	52.74	68.80	83.78	85.43	86.87	88.17	89.30
Reaching		c	33.49	47.48	60.46	72.52	83.78	80.58	77.76	75.29	73.07
Each		C	**83.37**	**83.49**	**83.59**	**83.71**	**83.78**	**80.58**	**77.76**	**75.15**	**73.07**
Component	Tooth %	a	**16.84**	**25.33**	**32.42**	**39.77**	**46.38**	**53.73**	**60.15**	**65.84**	**70.89**
		m	16.84	31.52	45.11	57.78	69.59	74.24	78.30	81.88	85.08
		c	33.49	43.53	52.84	61.49	69.59	69.97	70.32	70.62	70.89

Table 6. *Cont.*

Resorption (mm)		**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
	C	**66.68**	**65.33**	**64.08**	**62.91**	**61.84**	**60.30**	**58.95**	**57.77**	**56.72**
Dentine %	a	**16.39**	**23.40**	**29.89**	**35.93**	**41.56**	**50.37**	**58.08**	**64.90**	**70.94**
	m	16.39	26.27	35.42	43.93	51.87	61.83	70.54	78.25	85.08
	c	32.40	40.68	48.36	55.50	62.15	64.79	67.09	69.14	70.94
	C	**16.39**	**16.24**	**16.09**	**15.95**	**15.82**	**15.40**	**15.03**	**14.70**	**14.41**
Bone %	a	**20.01**	**20.63**	**21.20**	**21.73**	**22.23**	**21.56**	**20.98**	**20.46**	**20.01**
	m	20.01	20.63	21.20	21.73	22.23	21.56	20.98	20.46	20.01
	c	**40.02**	**41.26**	**42.40**	**43.47**	**44.46**	**43.13**	**41.96**	**40.93**	**40.01**
NVB %	NVB	**0.40**	**0.48**	**0.55**	**0.62**	**0.68**	**0.72**	**0.76**	**0.80**	**0.82**
Pulp %	a	**0.03**	**0.04**	**0.05**	**0.06**	**0.06**	**0.06**	**0.07**	**0.07**	**0.07**
	c	**0.04**	**0.05**	**0.05**	**0.06**	**0.06**	**0.06**	**0.07**	**0.07**	**0.07**
PDL %	a	**0.42**	**0.62**	**0.80**	**0.97**	**1.13**	**1.25**	**1.35**	**1.44**	**1.52**
	m	0.84	1.07	1.29	1.49	1.68	1.86	2.01	2.15	2.27
	c	**3.31**	**4.09**	**4.80**	**5.47**	**6.09**	**6.74**	**7.31**	**7.81**	**8.25**

structure—stress displayed by the 3D model. Tooth + bracket, Tooth, Dentine, Bone, NVB, Pulp, PDL components —stress displayed by these components. a—apical third, m—middle third, c—cervical third, C—crown. Tooth + bracket %, Tooth %, Dentine %, Bone %, NVB %, Pulp %, PDL % components—% stress displayed by these components.

In intact periodontium, both forces (0.6 and 1.2 N) seem to be safe for application (lower than MHP). Nevertheless, in reduced periodontium, 0.6 N of the applied force produced a cervical third PDL stress that is higher than physiological MHP (i.e., after 2–5 mm of loss, with higher resorptive risks). A force of 2.4 N exceeded the physiological MHP for all movements and bone levels, thus ischemic and resorptive risks are the highest (Figure 8 and Table 7). Nevertheless, the stress absorption–dissipation pattern displayed by the other two lower loads remained constant.

As biomechanically expected in all movements and bone loss levels, the radicular dentine component showed variable quantitative and qualitative stress displays (% of total stress reaching the dentine component, Figures 3–7, Tables 2–6), while all other tissular components showed a relatively constant %, emphasizing dentine and enamel components' primary role in the absorption–dissipation ability of dental structures.

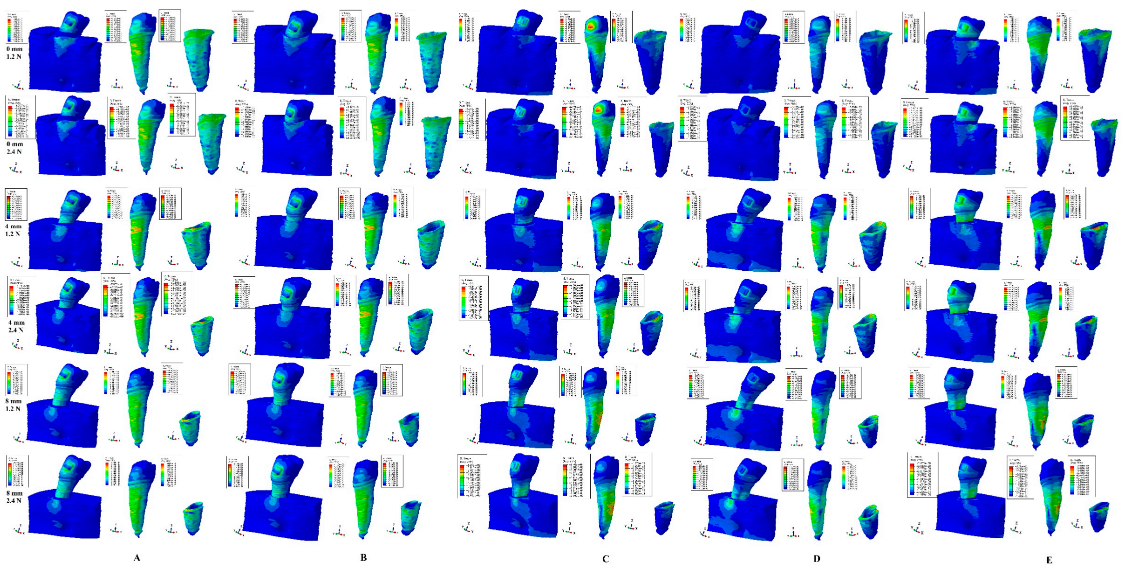

Figure 8. Comparative qualitative and quantitative results for 1.2 and 2.4 N forces for 0, 4, and 8 mm bone loss for dental tissues' structure, dentine, and PDL components: (**A**)—extrusion, (**B**)—intrusion, (**C**)—rotation, (**D**)—tipping, (**E**)—translation.

Table 7. Maximum stress average values (KPa) produced by orthodontic forces in tooth structure, dentine–cementum, and PDL.

Resorption (mm)			0	1	2	3	4	5	6	7	8
Extrusion 1.2 N/ 120 gf	Structure		598.8	610.1	621.4	632.7	644	679.2	714.5	749.74	785
	Dentine	a	88.35	149.52	210.68	271.84	333.00	374.31	415.61	456.92	498.22
		m	88.35	149.52	210.68	271.84	333.00	374.31	415.61	456.92	498.22
		c	215.00	260.95	306.90	352.85	398.80	423.66	448.51	473.37	498.22
		C	108.56	115.32	122.07	128.83	135.57	139.76	143.94	148.12	152.30
PDL Stress Exceeding MHP	**PDL**	a	5.99	7.59	9.18	10.77	12.37	13.40	14.43	15.47	16.50
		m	5.99	7.59	9.18	10.77	12.37	13.40	14.43	15.47	16.50
		c	**13.41**	**16.81**	**20.22**	**23.63**	**27.04**	**31.54**	**36.07**	**40.59**	**45.10**
2.4 N/ 240 gf	Structure		1197.6	1220.2	1242.8	1265.4	1288	1358.4	1429	1499.48	1570
	Dentine	a	176.71	299.04	421.36	543.68	666.00	748.61	831.22	913.83	996.43
		m	176.71	299.04	421.36	543.68	666.00	748.61	831.22	913.83	996.43
		c	430.00	521.90	613.80	705.70	797.60	847.31	897.02	946.73	996.43
		C	217.12	230.63	244.14	257.65	271.14	279.52	287.88	296.24	304.60
PDL Stress Exceeding MHP	**PDL**	**a**	11.99	15.18	18.36	**21.54**	**24.73**	**26.79**	**28.86**	**30.93**	**33.00**
		m	11.99	15.18	18.36	**21.54**	**24.73**	**26.79**	**28.86**	**30.93**	**33.00**
		c	**26.82**	**33.62**	**40.44**	**47.26**	**54.08**	**63.08**	**72.14**	**81.17**	**90.20**
Intrusion 1.2 N/ 120 gf	Structure		598.8	610.1	621.4	632.7	644	679.2	714.5	749.74	785
	Dentine	a	88.35	149.52	210.68	271.84	333.00	374.31	415.61	456.92	498.22
		m	88.35	149.52	210.68	271.84	333.00	374.31	415.61	456.92	498.22
		c	215.00	260.95	306.90	352.85	398.80	423.66	448.51	473.37	498.22
		C	108.56	115.32	122.07	128.83	135.57	139.76	143.94	148.12	152.30
PDL Stress Exceeding MHP	**PDL**	a	5.99	6.98	7.95	8.93	9.92	11.55	13.23	14.85	16.50
		m	5.99	10.41	11.01	12.62	14.81	15.24	15.66	16.08	16.50
		c	10.44	13.37	16.30	**19.22**	**22.15**	**25.82**	**29.49**	**33.17**	**36.86**
2.4 N/ 240 gf	Structure		1197.6	1220.2	1242.8	1265.4	1288	1358.4	1429	1499.48	1570
	Dentine	a	176.71	299.04	421.36	543.68	666.00	748.61	831.22	913.83	996.43
		m	176.71	299.04	421.36	543.68	666.00	748.61	831.22	913.83	996.43
		c	430.00	521.90	613.80	705.70	797.60	847.31	897.02	946.73	996.43
		C	217.12	230.63	244.14	257.65	271.14	279.52	287.88	296.24	304.60
PDL Stress Exceeding MHP	**PDL**	**a**	11.99	13.95	15.90	**17.85**	**19.84**	**23.10**	**26.46**	**29.70**	**33.00**
		m	11.99	**20.83**	**22.02**	**25.23**	**29.62**	**30.48**	**31.32**	**32.16**	**33.00**
		c	**20.88**	**26.73**	**32.59**	**38.43**	**44.29**	**51.63**	**58.99**	**66.33**	**73.71**
Rotation 1.2 N/ 120 gf	Structure		1599.60	1606.70	1613.80	1620.90	1628.00	1666.20	1704.40	1742.60	1780.80
	Dentine	a	143.67	272.50	401.32	530.14	658.95	753.98	849.00	944.02	1039.04
		m	143.67	319.10	494.54	669.97	845.40	979.91	1114.42	1248.93	1383.44
		c	338.91	535.35	731.77	928.20	1124.63	1131.93	1139.23	1146.54	1153.84
		C	338.86	348.67	358.48	368.29	379.30	372.07	364.84	357.61	350.37
PDL Stress Exceeding MHP	PDL	**a**	4.67	6.14	7.60	9.06	10.51	12.83	15.15	**17.47**	**19.79**
		m	9.25	12.10	14.95	**17.81**	**20.67**	**25.31**	**29.95**	**34.60**	**39.24**
		c	**36.70**	**50.46**	**64.21**	**77.97**	**91.72**	**102.93**	**114.14**	**125.35**	**136.56**
2.4 N/ 240 gf	Structure		3199.20	3213.40	3227.60	3241.80	3256.00	3332.40	3408.80	3485.20	3561.60
	Dentine	a	287.34	545.00	802.64	1060.28	1317.90	1507.96	1698.00	1888.04	2078.07
		m	287.34	638.20	989.08	1339.94	1690.79	1959.82	2228.84	2497.86	2766.87
		c	677.82	1070.69	1463.54	1856.39	2249.26	2263.85	2278.46	2293.07	2307.67
		C	677.72	697.34	716.96	736.58	758.60	744.14	729.68	715.22	700.75
PDL Stress Exceeding MHP	**PDL**	**a**	9.34	12.29	15.21	**18.13**	**21.03**	**25.67**	**30.30**	**34.93**	**39.57**
		m	**18.50**	**24.19**	**29.90**	**35.61**	**41.34**	**50.61**	**59.90**	**69.19**	**78.48**
		c	**73.41**	**100.91**	**128.42**	**155.93**	**183.44**	**205.86**	**228.29**	**250.70**	**273.12**
Translation 1.2 N/ 120 gf	Structure		1008.80	1010.20	1011.60	1013.00	1014.60	1165.34	1316.00	1466.80	1617.60
	Dentine	a	135.88	157.34	178.79	200.25	221.69	417.01	612.31	807.62	1002.92
		m	135.88	321.07	506.25	691.44	876.62	1033.14	1189.66	1346.18	1502.69
		c	400.21	500.76	601.29	701.83	802.36	883.75	965.14	1046.53	1127.93
		C	135.88	139.10	142.32	145.54	148.76	174.89	201.02	227.15	253.28

Table 7. *Cont.*

Resorption (mm)			0	1	2	3	4	5	6	7	8
PDL Stress	**PDL**	a	4.03	5.15	6.26	7.38	8.49	10.13	11.75	13.39	15.02
Exceeding		**m**	7.95	10.18	12.40	14.63	16.85	**20.19**	**23.53**	**26.86**	**30.20**
MHP		**c**	**35.43**	**47.52**	**59.60**	**71.69**	**83.77**	**96.58**	**109.39**	**122.19**	**135.40**
2.4 N/ 240 gf	Structure		2017.60	2020.40	2023.20	2026.00	2029.20	2330.68	2632.00	2933.60	3235.20
	Dentine	a	271.76	314.67	357.58	400.49	443.39	834.01	1224.62	1615.23	2005.84
		m	271.76	642.13	1012.50	1382.87	1753.25	2066.28	2379.32	2692.36	3005.39
		c	800.43	1001.51	1202.58	1403.65	1604.72	1767.50	1930.28	2093.06	2255.86
		C	271.76	278.20	284.64	291.08	297.52	349.78	402.04	454.30	506.56
PDL Stress	**PDL**	**a**	8.05	10.29	12.52	14.75	16.98	**20.26**	**23.50**	**26.78**	**30.05**
Exceeding		**m**	15.90	**20.35**	**24.80**	**29.25**	**33.70**	**40.38**	**47.05**	**53.73**	**60.40**
MHP		**c**	**70.86**	**95.03**	**119.20**	**143.37**	**167.54**	**193.16**	**218.77**	**244.39**	**270.80**
Tipping	Structure		732.20	760.80	789.60	818.30	847.00	907.20	967.60	1027.80	1088.20
1.2 N/ 120 gf	Dentine	a	120.03	178.00	236.00	294.00	352.00	457.00	562.00	667.00	772.00
		m	120.03	199.85	279.67	359.50	439.33	560.95	682.58	804.21	925.83
		c	237.21	309.52	381.82	454.12	526.42	587.82	649.21	710.61	772.00
		C	120.03	123.52	127.02	130.52	134.02	139.71	145.39	151.08	156.77
PDL Stress	**PDL**	a	3.10	4.72	6.32	7.92	9.56	11.30	13.04	14.78	**16.53**
Exceeding		**m**	6.12	8.15	10.17	12.20	14.22	16.84	**19.45**	**22.07**	**24.68**
MHP		**c**	**24.26**	**31.09**	**37.92**	**44.75**	**51.58**	**61.14**	**70.70**	**80.26**	**89.82**
2.4 N/ 240 gf	Structure		1464.40	1521.60	1579.20	1636.60	1694.00	1814.40	1935.20	2055.60	2176.40
	Dentine	a	240.06	356.00	472.00	588.00	704.01	914.00	1124.00	1334.00	1544.00
		m	240.06	399.69	559.34	718.99	878.66	1121.90	1365.16	1608.42	1851.66
		c	474.43	619.04	763.64	908.24	1052.84	1175.63	1298.42	1421.21	1544.00
		C	240.06	247.04	254.04	261.04	268.05	279.41	290.78	302.15	313.54
PDL Stress	**PDL**	**a**	6.20	9.43	12.63	15.83	**19.12**	**22.60**	**26.08**	**29.56**	**33.06**
Exceeding		**m**	12.24	16.29	**20.34**	**24.39**	**28.44**	**33.67**	**38.90**	**44.13**	**49.36**
MHP		**c**	**48.52**	**62.18**	**75.84**	**89.50**	**103.16**	**122.28**	**141.40**	**160.52**	**179.64**

structure—stress displayed by the entire tooth structure including the applied bracket. dentine—stress displayed by the dentine–cementum component of the tooth structure. a—root apical third, m—root middle third, c—root cervical third, C—crown.

Herein, the quantitative results were lower (i.e., 890.4 KPa, rotation, 8 mm loss, 0.6 N; 1.78 MPa rotation, 8 mm loss, 1.2 N) than the acknowledged dental components' physical properties.

Since all three applied forces showed similar qualitative stress displays and increasing quantitative results, the assumed applied boundary conditions (isotropy, linear elasticity, and homogeneity) are correct up to 2.4 N, if the Tresca failure criterion (for ductile non-homogenous materials) is employed.

4. Discussion

The present numerical analysis (eighty-one 3D models and 810 FEA simulations) assessed the absorption–dissipation ability of dental tissues under three forces (0.6, 1.2, and 2.4 N), and five most used orthodontic movements during 0–8 mm periodontal breakdown. Here, the simulation is the first study of its kind to bring an original approach in the dental studies field and new data with impact over clinical perspective. The absorption–dissipation ability of tooth as a single-stand structure was biomechanically recognized [1–3,11,14,48], and not yet investigated except for our previous research [2,3,9,10,48].

Additionally, by applying a third force (of 2.4 N), the correctness of using the assumptions of isotropy, linear elasticity, and homogeneity in FEA studies of dental tissues was assessed.

The biomechanical behavioral assessment following the progressive reduction in stress is individually displayed in each model's component. There was a visible progressive quantitative stress increase in all five movements strictly correlated with bone loss.

In both intact and reduced periodontium from the total amount of stress applied on and around the stainless-steel bracket base, only a constant percentage reached the alveolar bone socket (9.6–20% in 0 mm loss up to 36.73–50.04% in 8 mm loss), PDL (0.29–1.2% in 0 mm loss up to 2.24–8.37% in 8 mm loss), NVB (0.22–0.57% in 0 mm loss up to 0.32–0.69% in 8 mm loss), and dental pulp (0.02–0.05% in 0 mm up to 0.02–0.12% in 8 mm loss). However, due to the internal micro-architecture [1,11,13], the dentine component displayed an increase in variable percentage values which is correlated with the progression of periodontal breakdown (8.98–39.67% in 0 mm loss, 15.57–49.57% in 1 mm loss up to 58.35–92.9% in 8 mm loss).

The comparison between stresses displayed in the tooth with bracket and tooth without bracket showed a limited absorption–dissipation ability of stainless-steel stress bracket (15.48–18.73% in 0 mm, 16.43–18.16% in 1 mm loss up to 4.02–16.35% in 8 mm loss) except for translation which displayed doubled values (probably due to the movement's biomechanical specificity). The enamel component (comparison between tooth without bracket and coronal dentine) displayed a higher absorption–dissipation ability (50.29–62.26% in 0 mm, 49.09–62.26% in 1 mm loss up to 38.53–65.44% in 8 mm loss) than bracket. There is little difference (i.e., % of stress) at root level between the three structures (tooth with bracket, tooth without bracket, and dentine), the main differences being visible only in coronal part (where the main absorption–dissipation seems to take place).

Thus, the largest absorption–dissipation is performed by dentine (i.e., approx. 40–93%) and enamel (i.e., approx. 40–65%) components, while the stainless-steel bracket base has a limited ability (i.e., approx. 16%). Nevertheless, biomechanically, all three above components act similar to a single-stand structure that absorbs–dissipates most of the stresses produced by orthodontic loads, allowing for only a fraction of these stresses to be manifested in the circulatory sensitive tissues (i.e., approx. 0.3–8.4% in PDL, 0.2–0.7% in NVB, 0.02–0.12% in dental pulp, which are quantitatively under a physiological amount of 16 KPa of MHP). These agree with our previous reports about the absorption–dissipation ability found in PDL, dental pulp, NVB, tooth, and bone [5,6,9,10,16,48]. Regarding the bone alveolar socket, only approx. 10–20% in intact periodontium and 35–50% in 8 mm reduced periodontium reached the bone cervical third (with reduced circulatory vessels) and exceeded MHP, but with smaller percentages for apical and middle third where the circulatory component is better represented. The above biomechanical behavioral data were found for all three orthodontic loads since they displayed similar qualitative results and increased quantitative values (doubling for 1.2 N and quadrupling for 2.4 N).

A previous research [2,3,9,10,48] of our team (0.5–1.2 N of force, five movements, Tresca criteria, intact and reduced periodontium) reported a tooth structure absorption–dissipation ability of approx. 85% of stresses before reaching circulatory sensitive tissues (i.e., 86.66–97.5% dissipation before reaching PDL, 98% before reaching NVB, and 99.6–99.94% before reaching pulp) similar to the research herein.

Thus, the herein numerical simulation confirmed that 0.6–1.2 N are safe for application in intact periodontium, producing only minor limited ischemic and resorptive risks especially in the cervical third of PDL and bone alveolar socket, which is also in agreement with previous reports [5,6,9,10,16,48]. Nevertheless, in reduced periodontium, the same amounts of force increased ischemic and resorptive risks for radicular dentine and PDL, which are strictly correlated with the periodontal breakdown process, as previously reported [5,6,9,10,16,48]. This FEA simulation confirmed the importance of support tissues in biomechanical orthodontic behavior and the need to reduce orthodontic loads applied after 4 mm bone loss (to 0.2–0.6 N) to eliminate ischemic and resorptive risks, as previously recommended [5,6,9,10,16,48]. Therefore, the maximum force safely applied in these tissues should be the one that is safer for the weakest component (i.e., PDL and NVB). The 2.4 N force displayed in intact periodontium, for intrusion and extrusion, PDL cervical third stresses of 20.88–26.82 KPa exceeded the 16 KPa of MHP (prone to ischemic and resorptive risks), while in the other three movements the stresses were 3–4.5 times higher.

The above biomechanical behavior is due to the physical properties and internal micro-architecture of each tissular component. In both our previous [5,6,9,10,16,48] and other studies [1,2,7,8,11–15,17,18,51], dental tissues were reported to resemble ductile materials (i.e., elastic deformation with recovering of original form). Besides the main ductile nature, each one of these materials possesses a certain amount of brittle mode flow [5,6,9,10,16,48] (i.e., small deformation with cracking and destructions).

Enamel, due to its internal micro-architecture that is made of hydroxyapatite, was seen as brittle-like [1,11]. Nevertheless, this behavior is not clinically sustained (no cracking or destruction). Here, simulations confirmed very good absorption–dissipation deformation ability (i.e., 40–65%) and dentine resemblance (40–93%), thus being a ductile-like material, in agreement with a previous report [9]. Herein, the simulation is the first of its kind that scientifically proves that the enamel resembles a ductile material (confirming clinical knowledge) and is of extreme importance for the numerical studies (i.e., selection of failure criteria and boundary conditions). Moreover, it is the first FEA study to scientifically confirm the clinical knowledge about the tooth absorption–dissipation ability as a single-stand structure.

The other aim of the simulation herein was to assess the correctness of the boundary conditions' assumptions of isotropy, linear elasticity, and homogeneity for dental tissues when loads higher than 1 N loads were investigated. The results showed that qualitatively displayed stress was similar for all three loads, while quantitatively displayed stress increased (doubling for 1.2 N and quadrupling for 2.4 N). Thus, it seems that up to 2.4 N these assumptions can be used in FEA studies and obtain correct results. We must emphasize that these assumptions were investigated only under Tresca failure criteria (designed for non-homogenous ductile materials with a certain brittle flow mode). Moreover, both qualitative and quantitative results agreed with clinical and numerical data [2–6,9,10,16,48]. The explanation for this biomechanical behavior is that the displacements and deformations of tooth and surrounding tissues are extremely small, and thus the mechanical principles are compiled (i.e., under 1 N and small displacements all materials show linear elasticity and isotropy).

Since the simulation herein is the first of this type, the only possible correlation besides our previous research [5,6,9,10,16,48] was to indirectly compare the results with similar numerical studies [19–47] that assessed various components of dental tissues. The employed failure criterion was Von Mises (overall stress, for homogenous ductile-like materials), since no Tresca (shear stress, for non-homogenous ductile-like materials having a brittle flow mode) studies were found. Both criteria are mathematically similar, with Tresca quantitative results being 15–30% higher when compared with Von Mises (thus, correlations are acceptable). Most of the studies had similar boundary conditions with those in [19–47]. These comparisons assessed both qualitative behavioral stress display and quantitative results, which are correlated with physiological MHP (to confirm the amounts).

Merdji et al. [44] (lower third molar, intact periodontium, single model, sample size of one, Von Mises criteria, intrusion: 10 N, tipping/translation: 3 N, bone: 142,305 elements, global element size: 0.25–1 mm), reported similar qualitative results (cervical third alveolar bone socket stress), but with an extension on both vestibular and lingual sides (due to the three rooted anatomical reconstructions that are closer to bone–implant models [19–27] than tooth models). By following anatomical correctness, we addressed this issue in the herein models. Merdji et al. [44] quantitatively reported 10.5 MPa for 10 N of intrusion, 11.5 MPa for 3 N of tipping, and 16.83 MPa for 3 N of translation for alveolar bone socket cervical third and higher than herein for cervical radicular dentine for translation 20.36 MPa, intrusion 18.36 MPa, tipping 19.62 MPa, while in our study 0.6 N produced 149.83 KPa/0.149 MPa (intrusion), 146.53 KPa/0.146 MPa (tipping), and 185.28 KPa/0.185 MPa (translation), and 2.4 N displayed 599.32 KPa/0.599 MPa (intrusion), 586.12 KPa/0.586 MPa (tipping), and 741.14 KPa/0.741 MPa (translation). It was assumed that these differences were due to boundary conditions (global element size 0.25–1 mm and 142,305 elements [44] vs. global element size 0.08–0.116 mm and 5,117,355 elements herein) and models' anatomy (idealized third molar [44] vs. anatomically correct second premolar).

Field et al. [43] (intact periodontium, two models, sample size of two, Von Mises criteria, tipping: 0.35/0.5 N, canine model: 23,565 elements, incisor-canine-first premolar model: 32,812 element, global element size: 1.2 mm) reported qualitative resembling results (as extension and topography—in bone, PDL, and radicular dentine). Nevertheless, it must be emphasized that their color-coded results were red—orange in the entire stress areas (high ischemic and resorptive risks) for light forces of 0.35 N, that clinically is not true. They also reported PDL quantitative stresses of 32–324.5 KPa, exceeding MHP signaling high resorptive risks, in total disagreement with herein and clinical data.

In intact periodontium, both Maravic et al. [49] (single simplified model of second upper premolar, intrusion) and Huang et al. [50] (single simplified model of first lower premolar, intrusion) reported comparable qualitative but higher quantitative results.

Hohmann et al. [37,38] (intact periodontium, one model, sample size of one, hydrostatic pressure criteria, maxillary first molar, intrusion: 0.5–1 N, PDL: 195,881–215,887 elements, tooth: 71,114–74,777 elements) reported maximum stresses of 9.95e-00TPa in the entire radicular dentine apical third, suggesting extended resorptive risks, in total disagreement with herein and contradicting clinical data. Moreover, the hydrostatic pressure criterion was specially designed for liquids (with no shear stress), thus its employment in dental tissues is not correct, as proven in other reports [3,9,48].

Shaw et al. [29] (upper incisor, intact periodontium, one model, sample size of one, Von Mises criteria, intrusion, extrusion, tipping, translation, and rotation, model: 11,924 elements and 20,852 nodes) reported lower amounts of alveolar bone socket and radicular dentine cervical stress as well as intrusion and extrusion to be more stressful than rotation and tipping, in total disagreement with herein (most likely due to boundary condition differences).

Shetty et al. [42] (upper first molar, intact periodontium, one model, sample size of one, Von Mises criteria, intrusion and tipping: 150 N, model: 30,838 nodes and 167,089 elements) quantitatively reported 1.33–1.95 MPa for intrusion and 2.16–8.15 MPa for tipping, and tipping to be more stressful than intrusion (in agreement with herein), but qualitatively displaying extended stress areas in the entire alveolar socket (in disagreement with herein).

The common issues found in these numerical studies [19–47] were the lack of correlation quantitative results of MHP, absence of scientifical motivation for employing a certain failure criterion (most studies), and biomechanical and physical mechanical explanations of boundary conditions when using higher loads.

Perrez et al. [30] partially approached these issues in endodontic root canal filling (concentrating on the brittleness aspect of root filling) but without any mention of Tresca or homogeneity/non-homogeneity, and linear- non-linear issues.

Comparative studies [2,3,9,16,48] have proven that only Von Mises and Tresca criteria supply correct results close to clinical data. Both criteria are specially designed for ductile materials, with Von Mises (overall stress) for homogenous materials and Tresca (shear stress) for non-homogenous materials with a brittle flow mode. By employing Tresca (maximum shear stress) criteria, the non-homogeneity nature of dental tissues was approached.

Multiple numerical studies by employing the hydrostatic pressure criteria (specially designed for liquids) and Ogdeon hyper-elastic model (specially designed for hyper-elastic rubbers) investigated an optimal PDL force in intact periodontium but with various and contradictive reports. Thus, Wu et al. [39–41] reported various optimal forces (in the range of 0.28–3.31 N) for canine, premolar, and lateral incisive, with significant differences for the same tooth (e.g., canine: rotation 1.7–2.1 N [41] and 3.31 N [39]; extrusion 0.38–0.4 N [41] and 2.3–2.6 N [40]; premolar: rotation 2.8–2.9 N [39]), much higher than 0.6–1.2 N reported by Proffit et al. [4] (0.1–1 N), and Hemanth et al. [32,33] (0.3–1 N).

Almost all FEA studies [19–47] employed the following boundary conditions: linear elasticity, isotropy, and homogeneity. Biomechanically, this is acceptable when subjected to small amounts of loads of up to 1 N. However, higher loads imply larger movements and displacements, and the use of linear elasticity and the isotropy approach may not be correct [2,3,9,16,48]. A study of linearity vs. non-linearity was conducted by Hemanth et al. [32,33] in PDL of upper incisor subjected to 0.2–1 N of intrusion and tipping, which employed the S1 and S3 (brittle failure) criteria. The authors reported that up to 20–50% less quantitative applied force is needed for non-linearity vs. linearity. However, the employed failure criterion was of brittle-like material, while PDL is a ductile-like material; thus, their reports have accuracy issues. The use of failure criteria, which are specially designed for non-homogenous materials (as Tresca), deals with the homogeneity/non-homogeneity issue.

The main limit of an FEA numerical analysis is related to the fact that it cannot accurately reproduce clinical conditions. Clinically, there are no pure movements, but rather an association and combination; thus, the amount of stress displayed at tissular level could be smaller than the herein results. We foresee this limit and compensate through data interpretation (especially those close to the physiological limit). Nevertheless, the main advantage of numerical analysis is related to the fact that it can produce individual analyses of each tissular component (the only available method), while by changing the boundary conditions, it requires a small sample size. This is why most FEA studies [19–44] employed an acceptable sample size of one (one patient, one model, few simulations). To obtain correct and valid results, we approached this issue by using a larger sample size of nine (nine patients, eighty-one models, and 810 simulations), which was found to be superior to other numerical studies.

A limit could also be seen to the sample size of nine (i.e., nine patients). However, it must be emphasized that all of the previous numerical studies used a sample size of one (one patient with one FEA 3D model and only few simulations), while this study used 81 3D models and 810 numerical simulations. The FEA method allows for multiple changes in the physical properties and boundary conditions of the models, allowing for many simulations over the same models; thereby supplying reliable results even if a low number of models is used.

A good example of these changes is represented in our study by the tissular reconstruction of missing tissues (bone and PDL) and by the reduction of 1 mm to simulate various levels of bone loss (small enough to be numerically quantifiable and clinically relevant, since 1 mm is considered to be clinically relevant in both periodontics and orthodontics). It must be emphasized that if a large amount of bone loss occurs, the chances of keeping the tooth's functionality reduces, and thus the viability of keeping it in the oral cavity.

Most FEA analyses fasten the process by employing anatomical simplified models. Our approach was the manual reconstruction segmentation process (automated detection software missed some areas). Thus, our intact periodontium models had 5.06–6.05 million C3D4 tetrahedral elements, 0.97–1.07 million nodes, and a global element size of 0.08–0.116 mm, no error element, and only a limited number of elements. When compared with other FEA analyses, a lower number of elements and nodes and higher global element size were used, with influence over results accuracy: 142,305 elements [44], 23,565–32,812 elements [43], 30,838 nodes and 167,089 elements [42], 148,097 elements and 239,666 nodes [32,33], 11,924 elements and 20,852 nodes [29], and higher global element size of 1.2 mm [43] and 0.25–1 mm [44].

To confirm numerical simulations, correct results must be compared and correlated with both physiological constants, with clinical data, and other studies. Herein, the analysis approached the above-mentioned issues by correlating the results with MHP found in periodontal and dental pulp circulatory vessels and known clinical data.

5. Conclusions

1. The largest absorption–dissipation is performed by dentine (i.e., approx. 40–93%) and enamel (i.e., approx. 40–65%) components, while the stainless-steel bracket base has a limited ability (i.e., approx. 16%).
2. The main absorption–dissipation of stresses takes place in coronal part since there is little difference (i.e., % of stress) at root level between the three structures (i.e., tooth

with bracket, tooth without bracket, and dentine), the main differences being visible only in coronal part.
3. Enamel, dentine, and stainless-steel bracket biomechanically behave as a single-stand structure, allowing for only a limited fraction of stresses to reach the circulatory sensitive tissues (i.e., approx. 0.3–8.4% in PDL, 0.2–0.7% in NVB, 0.02–0.12% in dental pulp, and quantitatively under the physiological amount of 16 KPa of MHP).
4. Enamel component displayed dentine resemblance of absorption–dissipation ability, thus being proven to resemble more ductile materials (with a certain brittle flow mode).
5. Tooth behaves as a single-stand structure showing the highest absorption–dissipation ability among dental tissues.
6. These numerical simulations confirmed clinical biomechanical knowledge, showing that 0.6–1.2 N of force are safe to be applied in intact periodontium, while for reduced periodontium, forces higher than 0.6 N are prone to resorptive and ischemic risks.
7. For reducing ischemic and resorptive risks, after 4 mm of bone loss, 0.2–0.6 N of force are recommended, to keep stresses under the 16 KPa physiological limit.
8. The rotational and translational movements were the most stressful, followed by tipping.
9. Both intrusion and extrusion supplied similar quantitative and quantitative results.
10. Forces of 0.6 N, 1.2 N, and 2.4 N displayed similar qualitative results for all movements and bone levels, while quantitative results doubled for 1.2 N and quadrupled for 2.4 N when compared with 0.6 N.
11. The employment of isotropy, linear elasticity, and homogeneity as assumed boundary conditions for the study of dental tissues seems to be correct when Tresca criterion (for non-homogenous materials) is used, for loads up to 2.4 N.

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Levels of IL-23/IL-17 Axis in Plasma and Gingival Tissue of Periodontitis Patients According to the New Classification

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Abstract: Background: Periodontitis (P) is a chronic inflammatory disease characterized by the destruction of periodontium support tissue generated by different immuno-inflammatory mechanisms, including the RANK/RANKL/OPG and the IL-23/IL-17 axis. Methods: The study was performed with healthy subjects (HS) and patients with periodontitis. Plasma samples were obtained from peripheral blood and the gingival tissue (GT) during periodontal surgery. The ELISA technique was used to evaluate the levels of IL-23, IL-17A, IL-23R, and IL-17RA. Results: In the plasma, a significant decrease in IL-17A was observed in patients with periodontitis than HS. In the GT, IL-23, IL-17A, and IL-17RA levels were increased in periodontitis patients; on the contrary, IL-23R levels were decreased in periodontitis patients when compared with HS. Finally, several positive correlations were found: soluble IL-17RA (sIL-17RA) levels in plasma between the percentage of radiographic bone loss (RBL%), and IL-23 with IL-17A in gingival tissue. Conclusions: The detection of the IL-23/IL-17A axis in gingival tissue and plasma provides us with more information on the behavior of this axis in a localized way in the periodontal microenvironment, in contrast to the systemic levels evaluated according to the new classification of periodontitis.

Keywords: Interleukin-23; Interleukin-17; Interleukin-23 receptor; Interleukin-17 receptor; periodontitis

1. Introduction

Periodontitis is caused by multiple risk factors such as bacterial dysbiosis, destructive host immune responses, and environmental factors such as smoking [1]. It is distinguished by an immuno-inflammatory solid response characterized by gingival inflammation, periodontal pockets (sites with deep probing depths), attachment loss, and radiographic bone loss [1,2]. However, dysbiosis is not sufficient to induce loss of attachment or erosion of the alveolar bone [3]. In this context, dendritic cells and macrophages stimulated by periodontal pathogenic bacteria produce IL-23 which plays an important role in the innate immune responses to bacterial and fungal infections diseases [4,5]. IL-23 binds to IL-23R which is expressed in several cells such as macrophages, dendritic cells, and natural killer (NK) cells, but principally on Th17 cells [6]. After binding of IL-23 to IL-23R, Th17 cells become activated and release IL-17A, IL-17F, IL-6, IL-22, and tumor necrosis factor α (TNF-α) [6,7], and the receptor activator of the NF-κB ligand (RANKL) [8]. IL-17 activates cells such as fibroblasts through the IL-17A receptor (IL-17RA/RC) and induces the release of RANKL [9]. RANKL released by fibroblasts and Th17 cells binds to RANK expressed on osteoclast precursor and induces their maturation to initiate bone destruction in periodontitis [9].

Significantly, IL-23R and IL-17RA can be in soluble form (sIL-23R and sIL-17RA) [10]. IL-23 binds to IL-23R and forms a complex (IL-23-IL-23R), acting as an inhibitor or activator of Th17 cell signaling [10,11] while sIL-17RA can function as a blocker of IL-17A signaling [12].

IL-23/IL-17 axis in periodontitis has been studied in different biological samples. Regarding IL-23 in gingival crevicular fluid (CGF), high concentrations have been reported in patients with periodontitis [13,14], as well as in saliva [15]. Contrary to Sadeghi R et al., who found decreased IL-23 when compared to healthy subjects (HS) [16]. On the other hand, some study groups did not observe significant differences in IL-23 between HS and periodontitis in GCF samples [17,18]. Other study groups demonstrated that patients with periodontitis have higher levels of IL-23 compared in serum and plasma [19,20]. Regarding IL-17 concentrations, elevated concentrations have been reported in CGF samples from patients with periodontitis compared to HS [17,21–23]. Elevated concentrations of IL-17A were reported in serum, plasma, and saliva samples in patients with periodontitis compared to HS [13–15,24,25]. Contrarily, Ozçaka O et al., found higher IL-17 in the saliva of HS compared to patients with periodontitis [26], but in other study however, in another study, there were no significant differences [27].

In gingival tissue samples, few working groups have evaluated the IL-23/IL-17 axis, of which IL-17 is elevated in patients with periodontitis compared to healthy subjects [21,28]. However, Takahashi K et al. only detected IL-17A in 10 of 16 gingival tissue samples from patients with periodontitis [29]. Ohyama H et al., reported higher IL-23 and IL-23R mRNA expression in early and advanced periodontal lesions compared to biopsies from healthy sites of periodontitis [30].

Regarding the receptors of IL-23R and IL-17RA, our study group have described that IL-23R is diminished and IL-17RA increased in gingival tissue from chronic and aggressive periodontitis patients [31]. Based on studies conducted on gingival tissue of the IL-23/IL-17 axis in periodontitis patients diagnosed according to the 1999 classification, we hypothesized that the molecules of the IL-23/IL-17A axis are increased in the gingival tissue in periodontitis coupled with the progression and severity of periodontitis based on the classification of periodontal disease (2018).

2. Materials and Methods

2.1. Ethical Approval and Informed Consent

This study was submitted and approved by the Ethics and Research Committees of Guadalajara University, and the Regulations of the General Health Law with the approval number (CI-08020). Additionally, the study was conducted according to the regulations of the World Medical Association Declaration of Helsinki 2013.

2.2. Study Subjects

Sixty-two subjects were enrolled to participate in the Periodontics Clinic of the University of Guadalajara from 2019 to 2020.

Inclusion criteria: Patients who showed typical characteristics of periodontitis and periodontally healthy. All the included subjects had not received periodontal therapy, medicaments such as antibiotics, anti-inflammatory drugs, or immunomodulators in the six months prior to the study.

Exclusion criteria: Smoking subjects, pregnant women, subjects who were under prophylactic antibiotic or dental treatment, those who took drugs that affect the gingiva, and subjects who presented a systemic disease were excluded from the study.

2.3. Periodontal Clinical Parameters

A clinical examination was performed on six sites per tooth of all existing dental organs of all participants with a Hu Friedy periodontal probe of 15 mm long and 0.5 mm in diameter (University of North Carolina UNC-15 Hu Friedy, Chicago, IL, USA), and the results were averaged [32,33]. The probing depth (PD), clinical attachment loss (CAL), percentage of bleeding on probing (BoP%), and percentage of radiographic bone loss RBL% were measured. The percentage of radiographic bone loss RBL% was assessed in relation to the root size of the most affected tooth. Likewise, data was collected from the specific area from which the gingival tissue was obtained.

All the HS did not present gingival inflammation and radiographic bone erosion. All patients with periodontitis were diagnosed according to the classification of periodontal diseases (2018) [34]. The diagnosis and classification of periodontitis were carried out by two calibrated specialists from the Periodontics Clinic of the University of Guadalajara.

2.4. Study Groups

Group of healthy subjects: This group consisted of 28 HS, (6 male, and 22 female), with a mean age of 37.26 $\pm$ 1.82 who were treated at the Periodontics Clinic for cosmetic surgery or crown lengthening. The group of healthy subjects showed a PD $\leq$ 2, CAL $\leq$ 1, BoP% < 10%, and no evidence of RBL%.

Group of periodontitis patients: This group consisted of 34 patients, (8 male, and 26 female); with a mean age of 40.5 $\pm$ 2.06, who presented periodontal pockets (CAL $\geq$ 5 mm and PD $\geq$ 4 mm), BoP% $\geq$ 10%, and RBL%. Patients with periodontal stage III and IV were included because, according to periodontal treatment, these patients did require surgery.

2.5. Plasma Sample Collection

Peripheral blood was obtained from all study subjects by venous puncture in a tube with EDTA and then centrifuged for 10 min at 700$\times$ g at room temperature. Next, the plasma was collected and immediately stored at -80 °C until ELISA assay.

2.6. Gingival Tissue Collection and Protein Extraction

Gingival tissue collection from HS and periodontitis patients were performed during aesthetic surgery or crown lengthening, and during the surgical phase of treatment, respectively. Sections from each tissue were collected, weighed, and placed in a microtube with 300 µl of PBS buffer. The microtubes were then placed in ice (4 °C), transported to the laboratory, and stored in an ultrafreezer at -80 °C until analysis.

To obtain total proteins, the samples were thawed at room temperature, subjected to the vapors of liquid nitrogen to crystallize them, and then crushed by compression. The triturated gingival tissue was immersed in 300 µL of RIPA buffer (Sigma, River Edge, NJ, USA) plus protease inhibitor (Complete, Roche Diagnostic GmbH, Risch, Switzerland) for 20 min at 4 °C. The homogenate was centrifuged at 12,300$\times$ g for 10 min and the supernatants with the total proteins were collected and stored at -80 °C until the quantification of IL-23, IL-17A, IL-23R, and IL-17RA by the ELISA method.

The quantification of total proteins was performed by the Bradford Coomassie Protein Assay Kit method (Thermo Fisher Scientific, Waltham, MA, USA). It was read on a spectrophotometer at 590 nm. The total protein concentration was obtained in mg/mL.

2.7. Enzyme-Linked Immunosorbent Assay (ELISA)

The plasma and gingival tissue samples were added in triplicate to the wells of microtiter plates to determine the concentrations of human IL-23, IL-17A, IL-23R, and IL-17RA using DuoSet® ELISA Kits (R&D Systems, Minneapolis, MN, USA). The absorbance of each well was read at 450 nm in a microplate spectrophotometer (Poweam Medical Systems, Co., Nanjing, China). The IL-23, IL-17A, IL-23R, and IL-17RA levels were calculated from the standard curves included in each assay. The levels in the plasma were expressed as pg/m, and in gingival tissue expressed as pg/mg of gingival tissue. When performing the ELISA technique, the concentrations are shown in pg/mL; thus, the picograms of each molecule were adjusted according to the tissue weight using the following formula:

$$\frac{\boldsymbol{pg/mL\ of\ citokyne\ or\ receptor\ by\ ELISA}}{\boldsymbol{mg/mL\ of\ gingival\ tissue}} = \boldsymbol{pg/m} \tag{1}$$

2.8. Statistical Analysis

For a small sample size, the data distribution was evaluated using the Shapiro–Wilk test. The data were abnormally distributed therefore, a nonparametric Mann–Whitney U test was used to compare the median differences between the study groups. A Chi-squared test was used to compare the sex. The correlation of the clinical findings and the levels of IL-23, IL-23R, IL-17A, and IL-17RA in the plasma and gingival tissues were evaluated by Spearman rank correlation coefficient. A value of $p \leq 0.05$ was considered significant. These results were analyzed using SPSS software version 25.0 (Chicago, IL, USA). Finally, the effect size and power of the study were analyzed with the G * P3.1 software.

3. Results

3.1. Demographic Characteristics and Clinical Parameters

The female gender dominated in both study groups; in terms of age, patients with periodontitis were older than healthy subjects. Regarding the periodontal characteristics, these were measured in all the areas of the teeth present and averaged, likewise was measured the exact value of the area where the gingival tissue was obtained. Patients with periodontitis presented a significant increase in PD, CAL, and BoP% in general measurements, as well as in the area of gingival tissue collected. It is worth mentioning that HS do not present RBL%, however, patients with periodontitis showed bone loss above 50% (Table 1).

Table 1. Demographic characteristics and clinical parameters.

	HS	P
Gender M/F	6/22	8/26
Age (years)	37.26 ± 1.82	40.85 ± 2.06 *
PD (mm)	2.14 ± 0.13	4.87 ± 0.4 *
CAL (mm)	1.02 ± 0.2	5.24 ± 0.49 *
BoP%	0.52 ± 0.52	22.65 ± 6.77 *
RBL%	-	60.55 ± 5.18
PD GT (mm)	2.31 ± 0.11	5.21 ± 0.44 *
CAL GT (mm)	1.07 ± 0.25	5.47 ± 0.46 *
BoP GT%	1.38 ± 1.38	24.47 ± 8.56
Stage III	-	26
Stage IV	-	8
Grade A	-	17
Grade B	-	11
Grade C	-	6

The results are expressed as mean and standard error or percentage. HS: Healthy Subjects, P: Periodontitis, M: Male, F = Female, PD: Probing depth, CAL: Clinical Attachment loss, BoP%: Bleeding of Probing percentage, RBL%: Percentage of Radiographic Bone Loss. GT: site of gingival tissue was obtained. * Significant difference between HS and P. A $p \leq 0.05$ was considered as significant.

3.2. Levels of IL-23/IL-17A Axis and Soluble Receptors in Plasma

No significant differences were observed in IL-23 and sIL-23R between the group of patients with periodontitis and HS (Figure 1a,b). A significant increase in IL-17A was observed in HS than periodontitis group (Figure 1c). Additionally, sIL-17RA was similar between healthy subjects and patients with periodontitis (Figure 1d).

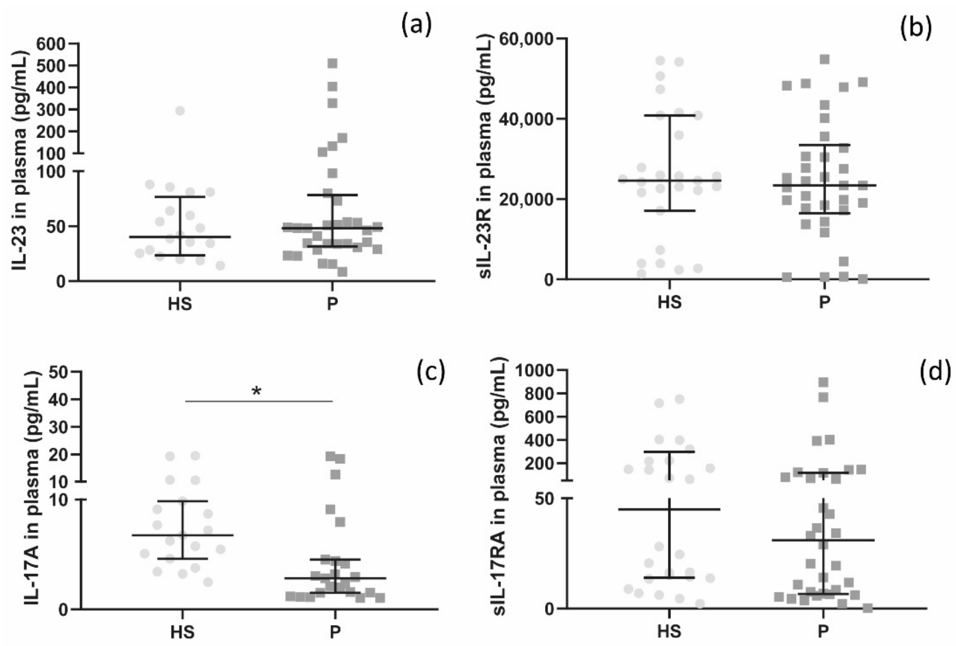

Figure 1. IL-23, IL-17A, sIL-23R, and sIL-17RA levels in plasma. plasma levels of IL-23 (**a**), sIL-23R (**b**), IL-17A (**c**), and sIL-17RA (**d**) from healthy subjects (HS) and periodontitis patients (P) were detected by ELISA and are expressed as pg/mL. The results are shown as median and interquartile ranges. * A *p*-value $\leq$ 0.05 was considered significant.

3.3. Levels of IL-23/IL-17A Axis and Receptors in the Gingival Tissue

In this study, we found an increase in IL-23 in the gingival tissue of patients with periodontitis compared with HS (Figure 2a). On the other hand, IL-23R levels in the gingival tissue were found to be higher in healthy subjects compared with patients with periodontitis (Figure 2b).

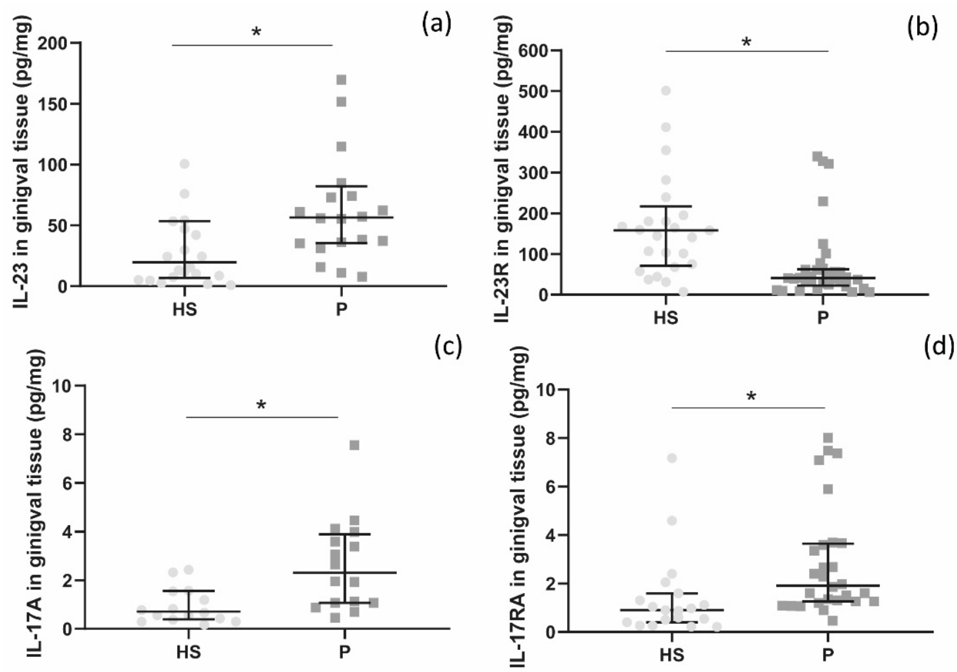

Figure 2. Levels of IL-23, IL-17A, IL-23R, and IL-17RA in gingival tissue. gingival tissue levels of IL-23 (**a**), IL-23R (**b**), IL-17A (**c**), and IL-17RA (**d**) from healthy subjects (HS) and periodontitis patients (P) which were detected by ELISA and are expressed as pg for each mg of gingival tissue. The results are shown as median and interquartile ranges. * A *p*-value $\leq$ 0.05 was considered significant.

Regarding IL-17A in gingival tissue, it was found to be elevated in patients with periodontitis compared with HS (Figure 2c). Similarly, IL-17RA in gingival tissue was found elevated in periodontitis patients compared with HS (Figure 2d).

3.4. Correlation between IL-23, IL-17A, IL-23R, and IL-17RA with Clinical Parameters in Plasma and Gingival Tissue

A Spearman correlation test was implemented in IL-23, IL-17A, IL-23R, and IL-17RA levels between plasma and gingival tissue and the clinical parameters. Only a positive correlation was observed in plasma molecules: IL-17RA vs. IL-23R r = 0.381 p = 0.003. Regarding the clinical characteristics, two positive correlations were found between IL-17RA vs. RBL % r = 0.575 p = 0.010 and PS vs. CAL r = 0.855 p = 0.000.

Regarding the gingival tissue, several positive correlations were observed: IL-17A-GT vs. IL-23-GT r = 0.592 p = 0.000; IL-23-GT vs. IL-17RA-GT r = 0.683 p = 0.000; IL-17A-GT vs. IL-17RA-GT r = 0.448 p = 0.013. As well as negative correlation: IL-23-GT vs. IL-23R-GT r = −0.329 p = 0.033. In the same way, correlations were found with the specific clinical parameters of the gingival tissue obtaining areas: IL-23R-GT vs. PS-GT r = −0.514 p = 0.000; IL-23R-GT vs. CAL-GT r = −0.508 p = 0.000; IL-17A-GT vs. PS-GT r = 0.462 p = 0.030; IL-17A-GT vs. CAL-GT r = 0.469 p = 0.037; IL-17RA-GT vs. CAL-GT r = 0.371 p = 0.024, and PS-GT vs. CAL-GT r = 0.877 p = 0.000.

Finally, we made a correlation of the IL-23/IL-17 axes molecules between plasma and GT as well as with the clinical characteristics obtained. We found both positive and negative correlations: IL-17A vs. IL-17A-GT r = −0.592 p = 0.001; IL-23R-GT vs. PS r vs. = −0.477 p = 0.000; IL-23R-GT vs. CAL r = −0.480 p = 0.001; IL-17RA-GT vs. CAL r = 0.320 p = 0.047; PS vs. PS-GT r = 0.895 p = 0.000; PS CAL-GT r = 0.808 p = 0.000; CAL vs. PS-GT r = 0.821 p = 0.000; CAL vs. CAL-TG r = 0.944 p = 0.000; BoP % vs. PS-GT r = 0.501 p = 0.004, and BoP % vs. BoP %-GT r = 0.891 p = 0.000 (Figure 3).

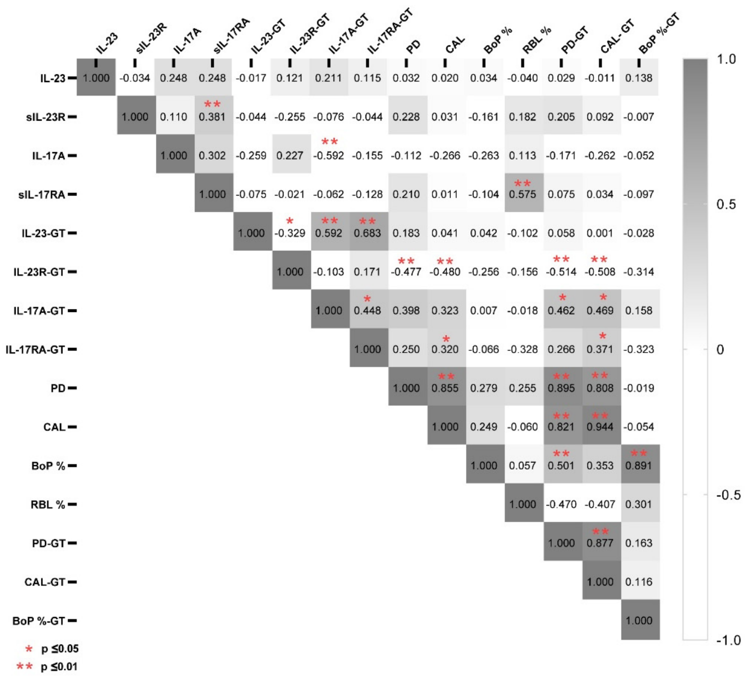

Figure 3. Correlation between the IL-23/IL-17A axis molecules and IL-23R IL-17RA receptors with clinical parameters in plasma and gingival tissue were analyzed using the Spearman correlation test. PD: probing depth, CAL: clinical attachment loss, BoP%: bleeding on probing percentage, RBL%: percentage of radiographic bone loss in plasma and (GT): gingival tissue values. * $p \leq 0.05$, ** $p \leq 0.01$.

3.5. Power and Effect Size

A post hoc exploration of the effect size and power of the study were analyzed with the G * P3.1 software, considering a unilateral hypothesis with α = 0.05. Only the sIL-23R and IL-23-GT showed a poor effect size and power (Table 2).

Table 2. Power and effect size of IL-23/IL-17 axes in periodontitis.

	IL-23	sIL-23R	IL-17A	sIL-17RA	IL-23-GT	IL-23R-GT	IL-17A-GT	IL-17RA-GT
Power	0.99 *	0.098	1 *	0.99 *	0.43	1 *	0.99 *	1 *
Effect size	1.04 *	0.075	2.02 *	0.89 *	0.37	2.61 *	1.46 *	2.55 *

GT: site of gingival tissue was obtained. * power ≥ 80 or effect size ≥ 0.5.

4. Discussion

Interactions between various cells and molecules in the immune system of the host's periodontal tissue are necessary, such as the IL-23/IL-17A axis, which can mediate the destruction of the periodontium. In this study, we expected to find an increase in the IL-23/IL-17A axis in the plasma and gingival tissue of patients with periodontitis. However, this behaviour was not observed in all the molecules of the IL-23/IL-17A axis, according to the classification of periodontal disease (2018).

Regarding IL-23 and sIL-23R in plasma, a significant difference was not observed between healthy subjects and patients with periodontitis. In this sense, the power and size of the effect for sIL-23R were not sufficient to establish an absolute result, so it is pertinent to increase the size of the samples. Regarding IL-23 in plasma, it is possible that in periodontitis patients this citokyne are diminished in the chornic fase (stage III and IV) of this disease, getting similar levels of IL-23 as healthy subjects. This phenom can be explained by the hyphotesis "Inflammation-Mediated Polymicrobial-Emergenceand Dysbiotic-Exacerbation"(IMPEDE), this hypothesis states that in the periodontitis cours a cycle where the inflammation resolution can restore the periodontal health afther a treatment [35]. It is important to say that the periodontitis patients from this study have non-surgical treatment before participating. Likewise, other studies have evaluated somes citokynes before and after treatment, and have shown that some citokyne levels decreased after non-surgical treatment (mechanical removal of dental plaque) and some periodontitis patients can regulate the production of proimflamatory citokynes managing to maintain similar levels similarly to healthy subjects [19,22,36].

Contrarily, an increase in IL-23 was observed only in the gingival tissue of patients with periodontitis compared to healthy subjects, according to Ohyama H. et al., that evaluated this cytokine in gingival tissue by immunohistochemistry and mRNA expression via PCR [30]. It is possible that the evaluation of cytokynes is better or more exactly in the specific tissue that by systemic way, because is the place where the inflammation is occurring and these molecules (IL-23/IL-17 axis) can be expressed according to the stimulus on the periodontum or by the periodontal stage and grade as proposing van Dick et al. [35].

In the present study, significant differences were not observed in sIL-23R in plasma samples. However, a decrease in IL-23R in the gingival tissue of periodontitis patients was demonstrated. In this sense, it is known that IL-23 can regulate the expression of IL-23R [37]. Considering that IL-23 in gingival tissue is increased in the periodontitis patients in this study, we expected to find an increase in IL-23R. In addition to this finding, the IL-23 increase and IL-23R decrease in gingival tissue are reflected in a negative correlation. In this regard, it has been observed that ADAM17 is elevated in the periodontal tissue of periodontitis patients [38,39], and ADAM17 has been shown to cleave IL-23R expressed on the cell membrane [11]. This mechanism likely partly explains the decreased expression of IL-23R that was found in the gingival tissue of periodontitis patients, which could be since that there are multiple protein isoforms of IL-23R, including soluble isoforms [11] that could not be detected by the ELISA method. It would be interesting to analyze IL-23R by western blot to identify possible isoforms of IL-23R and then to characterize these isoforms by mass spectrometric and grades of glycosylation of IL-23R [40], due to the fact that some IL-23R isoforms have been described in Bowel's disease [41].

On the other hand, in plasma samples, there is a decrease in IL-17A in patients with periodontitis, similar to that reported by some authors regarding GCF [42] and plasma [22]. In contrast, other authors have described an increment in the concentration of IL-17A in different biological fluid samples from periodontitis patients [17,18,26], while others still failed to detect this cytokine in patients with periodontitis [12,13]. However, IL-17A is a cytokine highly studied in inflammatory and autoimmune diseases such as Sjögren's syndrome and rheumatoid arthritis, among others [43–46]. In this sense, Ridgley LA. et al. [47] propose that several cytokines, such as IL-17A, are inversely proportional to the course and chronicity of rheumatoid arthritis. In addition, an increase in IL-17A was observed in the preclinical phase, and this cytokine decreases as the chronicity of RA enhances [47]. Although, periodontal disease affects the bone and periodontal tissue and is known to have a similarity to RA [48]. IL-17A in periodontitis may exhibit behavior similar to what Ridgley LA et al. propose in rheumatoid arthritis.

Contrary to plasma, in gingival tissue samples an increase in IL-17A was observed in periodontitis patients than HS, which coincides with that reported by Johnson RB et al., and Ruiz AC et al., in gingival tissue [28,31] as well as by Vernal R et al., in gingival culture supernatant [21]. This increase in IL-17A can be attributed to the gingival microenvironment generated by the severity of periodontitis, since in this study all patients were between periodontal stages III and IV, being the most advanced of periodontitis [1]. This finding can apply the Ridgley observation in some molecules, such as IL-17A that are overexpressed according to the severity and chronicity of rheumatoid arthritis [47].

Regarding sIL-17RA in plasma, differences between HS and periodontitis patients were not found. An increase in sIL-17RA was expected in periodontitis because in a previous study, this receptor was observed increased in the gingival tissue of patients with periodontitis [31], sowe considered that sIL-17RA could be elevated in plasma because several soluble isoforms of sIL-17RA can be secreted as a result of alternative splicing [12]. In addition, an increase in IL-17RA has been observed in other pathologies [49–51].

On the other hand, sIL-17RA positively correlated with the RBL%, which is a parameter that indicates the periodontal grade (severity); in this regard, the inhibitory effect of some IL-17A soluble receptors on IL-17A in vitro has been demonstrated [52,53]. The study of the sIL-17RA expression isoforms in periodontitis could elucidate the role that these molecules play in bone resorption in this disease.

Regarding IL-17RA in gingival tissue, an increase was observed in patients with periodontitis as reported by previous studies in patients with chronic and aggressive periodontitis [31]. It has been shown that there is an increase in RANKL produced by fibroblasts under the stimulus of IL-17A when binding to its receptor [54]. Therefore, the enhanced levels of IL-17A [21,28,55] and IL-17RA [31] in the gingival tissue of patients with periodontitis reported by other authors agree with the increase in RANKL reported by Bi CS et al. [54].

In addition to this, the positive correlation between IL-17A with PD and CAL in gingival tissue agrees with that reported by another study group [28]; the higher the IL-17A concentration, the greater the probing depth, and insertion loss. Therefore, IL-17A and IL-17RA positively correlate in the gingival tissue and appear to be crucial to the loss of insertion and alveolar resorption. This could be because when IL-17A binds to IL-17RA it triggers the expression of chemokines, pro-inflammatory cytokines, and metalloproteinases (MMPs), promoting a crucial pro-inflammatory environment for osteoclastogenesis and bone erosion in periodontitis [56].

When correlating the levels of IL-17A between the plasma samples and gingival tissues, an inverse behavior was observed. While in plasma, there is a decrease in IL-17A levels, in gingival tissue the levels of this cytokine increase. This is probably because plasma can only detect IL-17A released by cells. In contrast, in gingival tissue, it can determine whether IL-17A is bound to the receptor on the cell membrane or intracellularly.

Similarly, this is probably because that IL-17A in plasma is blocked by the sIL-17RA, as other authors have proposed [12]. This could explain the discrepancy of IL-17A levels in different biological fluids.

Furthermore to these findings, a positive correlation was found between IL-23 and IL-17A in the gingival tissue that coincides with the path of the IL-23/IL-17 axis since the production of IL-17A depends on the stimulus of IL-23 [6].

This study has some limitations, principally the effect size and power of IL-23R in plasma and IL-23 in gingival tissue that was inadequate to evaluate the complete behavior of the IL-23/IL-17 axis. Another limitation was the sample size; it is important to evaluate these molecules in more patients with periodontitis to have better study groups classified by the periodontal grades and stages, this can help to determine if there is a significant difference in the severity and progresion of periodontal disease.

In the future, the IL-23/IL-17A axis and their receptors in the gingival tissue of periodontitis patients will be analyzed by Western blot and mass spectrometry, which will allow us to study the functionality of the isoforms from the IL-23/IL-17 axis and their association with periodontitis progression in detail. In this sense, different biological drugs (monoclonal antibodies) have been used in different pathologies such as rheumatoid arthritis, ankylosing spondylitis, and psoriasis to counteract the proinflammatory effect of various cytokines and receptors, including IL-17, IL-23, and IL-17RA, and extensive improvements have been observed in the clinical and histological features of these diseases [57–60]. For example, brodalumab, approved for the treatment of psoriasis, which consists of an IgG2a monoclonal antibody that specifically binds to IL-17RA and blocks the signaling of various IL-17 isoforms (IL-17A and IL-17F) [61,62]. It would be interesting to carry out the corresponding experimental phases to test this kind of brodalumab, because in this study, we found elevated IL-17RA in the gingival tissue of patients with periodontitis. In this way, it may be possible to reduce RANKL production by fibroblasts [9], and thus block bone resorption generated by the IL-17/IL-17RA and RANK/RANKL systems in patients with periodontitis.

5. Conclusions

The detection of the IL-23/IL-17 axis and its receptors in the gingival tissue provides more information on the behavior of the IL-23/IL-17 axis in a localized way, since it is found in the periodontal microenvironment, unlike the systemic evaluation as in plasma. In addition, elevated levels of IL-23, IL-17A, and IL-17RA were found in gingival tissues, and these findings correlate with clinical periodontal characteristics.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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