Laser and remineralising agents in dental erosion: a systematic review and meta-analysis

A. Pardo[1], A. Butera[2], A. Signoriello[1,*], M. Marchiori[1], V. Fiorini[1], N. Branz[1], A. Scribante[2], G. Lombardo[1]

[1]*Dentistry and Maxillofacial Surgery Section, Department of Surgery, Dentistry, Pediatrics and Gy-necology, University of Verona, Verona, Italy*
[2]*Unit of Dental Hygiene, Section of Dentistry, Department of Clinical, Surgical, Diagnostic and Pe-diatric Sciences, University of Pavia, 27100, Pavia, Italy*

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email: annarita.signoriello@univr.it

Abstract

Aim This systematic review aimed to evaluate, by means of statistical comparison between selected studies, the effectiveness of laser irradiation on dental erosion applied alone or in combination with anti-erosive agents (fluoride, Acidulated phosphate fluoride APF and CPP-amorphous calcium fluoride phosphate CPP-ACFP), through optical profilometry and microhardness measurement.

Methods The searching strategy was conducted according to the International PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines, to answer research questions regarding the effectiveness of lasers used in the context of dental erosion (such as Nd:YAG, CO2, Er:YAG, Er,Cr:YSGG, Diode, and Argon Ion lasers). A comprehensive and unrestricted sys-tematic searching was performed using the electronic databases of PubMed and Cochrane Library, for all articles published up to the end of January 2023. The review record was registered by the International prospective register of systematic reviews (PROSPERO) under the identification number CRD408639.

Results Of the 16 studies included, only 7 were selected for data of interest, enabling a comparison between the experimental and control groups for metanalysis. A comparison between laser and the control group (no agent or fluoride agent) showed improved enamel microhardness (standardised mean deviation, SMD 1.47, p=0.05, I^2 =88%) and tissue loss assessed for optical profilometry (SMD 2.53, p=0.0002, I^2 =87%). Regarding active ingredients compared to the control group, the overall SMD was 1.44 (p=0.0003, I^2=81%). For the other groups, outcomes concerning microhardness were as follows: SMD -0.26 (p=0.5, I^2=0%) for APF gel + Laser versus control group; SMD -0.85 (p=0.005, I^2=81%) for APF gel + Laser versus APF gel; SMD -0.65 (p=0.09, I^2=64%) for APF gel + laser versus Laser; SMD -1.13 (p=0.00001, I^2=91%) for Laser + APF gel versus APF gel.

Conclusions Change in microhardness for the lasers group, irrespective of the type of laser used in the sample, was significantly greater compared with the one in the control group. Regards microhardness, the combination of Laser and APF gel showed promising results compared to APF gel agent.

KEYWORDS dental erosion, tooth erosion and laser, fluoride and laser, dental enamel, dental erosion, fluoride agent and prevention.

Introduction

The repair of dental tissues constitutes a widely discussed topic in terms of clinically relevant needs, as prevalence of tooth erosion represents an ever increasing phenomenon among all age groups, especially considering the progressive aging of the population.

In this regard, prevalence of dental erosion was geographically explored, finding extremely varied results in different areas of the world. Further data reveal that approximately 30% of the population aged between 18 and 35 years has at least one tooth affected by erosive processes [Carvalho et al., 2015]. Even if erosion can be a consequence of wear and tear following physiological aging of tooth structures, it can occur during childhood, negatively compromising the development of a healthy and correct dentition. In adults, the damage caused by this phenomenon may thus require extensive treatment in terms of cost and clinical complexity [McCarthy et al., 2021]. Moreover, it is relevant to say that nowadays the prevalence of dental erosion has a similar prevalence than 30 years ago, but the condition seems to be more severe [Stenhagen et al., 2017]. Among hard dental tissues, enamel is the most exposed to demineralisation and subsequent tissue loss, leading the underlying dentin to degenerative processes. The outermost enamel region and eventually exposed dentine are both in direct contact with saliva and oral fluids, and the crystalline structures in hard tissues are in equilibrium with the adjacent aqueous phases. The quantity of these mineral components eventually dissolved in the aqueous phase is restored and compensated by those reformed through the action of saliva components [Li et al., 2014]. Dental hydroxyapatite generally dissolves in a process known as carious demineralisation at a pH of 5.5, which can be easily achieved through the action of cariogenic bacteria on food introduced with the diet, with consequent production of acidic substances. Differently, erosion [Cheng et al., 2009; Litonjua et al., 2003] tends to occur at a pH value around 4, also lower than one characterising in vitro condition, for which ions leach out of the tooth structure even at a milder pH of 6 [Milosevic and O'Sullivan, 2008]. Over the years, research has intensified in the direction of new approaches able to reestablish dental functions by repairing lost tooth tissues (eroded hydroxyapatite crystals). Primary and secondary prevention

protocols were implemented to reduce tooth erosion, including the use of topical fluoride in the form of solutions, gels, or varnishes [Magalhães et al., 2009]. However, other devices with different fields of applications, such as lasers, are not only used in periodontal therapy [Pardo et al., 2023], but also in enamel treatment. Several studies [González-Rodríguez et al., 2011; Lombardo et al., 2019] have shown that certain types of lasers, such as Nd:YAG, CO_2, Er:YAG, Er,Cr:YSGG, Diode, and Argon Ion lasers, can reduce the demineralisation rate of the enamel surface, both in permanent and in deciduous teeth with similar results [Kornblit et al., 2009]. Regarding these outcomes, several explanations were given for the increased acid resistance of laser-treated enamel, in terms of decreased enamel permeability, altered chemical composition, or combination of both. Despite some limitations, these data revealed an increased absorption of fluoride by laser-treated enamel, thus increasing resistance and decreasing susceptibility to acids in the context of dental erosion.

Objective

The primary aim of this systematic review of the literature was to evaluate, by means of statistical comparison between selected studies, the effectiveness of laser irradiation applied alone or in association with anti-erosive fluoride agents, and the effects of these combinations on dental erosion. The study also aimed to detect eventual potential protective effects of lasers, in adjunction with fluoride agents, on enamel erosion.

Methods

Search strategy and study selection

This systematic review was prepared according to the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement [Moher et al., 2009].

The focused question was proposed using the "PICO" format:

- Problem: Dental erosion at the level of permanent teeth;
- Intervention: Use of laser in combination with anti-erosive agents;
- Comparison: Application of anti-erosive agents, laser application, no treatment;
- Outcome: Reducing demineralisation and increasing resistance of dental hard tissue to acid attacks.

The review record was registered by the International prospective register of systematic reviews (PROSPERO) under the identification number CRD408639.

A comprehensive unrestricted systematic searching was performed using the electronic databases of PubMed and Cochrane Library. The searching strategy was conducted between January 2011 and January 2023, using the following key terms and their combinations:

- tooth erosion and laser;
- dental erosion and laser;
- fluoride and laser;
- dental enamel;
- dentine;
- dental erosion and prevention.

Data collection process

The articles were manually selected according to the criteria defined by P.I.C.O by two independent operator and were viewed and selected reading title and abstract, and by applying the inclusion and exclusion criteria mentioned below. All disagreements between individual judgements were resolved with another reviewer of team research. Full-text articles were read and analysed to be confirmed for selection in the systematic review, which included comparative in vitro studies performed on human enamel and dentine samples.

Data items

Data extracted for this study were: study year, number of teeth (size sample), erosive cycle, case and control interventions, type of vehicle, laser group parameters (frequency and length of consumption).

Eligibility criteria

The inclusion criteria for the studies were as follows:

- Type of study: comparative in vitro studies; clinical studies were thus excluded;
- Type of sample: sample of human enamel and dentine, permanent dental elements;
- Language: English language studies;
- Time interval of research: eleven years (2011-2023).

The exclusion criteria were as follows:

- Deciduous dental elements;
- Sample of enamel and dentine on non-human tissue;
- Studies involving the use of lasers for other purposes, such as tooth whitening, treatment of dental hypersensitivity, and/or demineralisation, in the context of caries prevention.

Statistical analysis

Of the studies included in the systematic review, only those which included data on sample size, mean and standard deviation (SD) of mineral loss, and microhardness (SMH) of dental tissues were extracted for analysis and reported the mean change in the final microhardness without considering the starting values. The analysis included the evaluation of outcomes using: all laser types included in the studies versus control group (no agent or fluoride agent); active ingredients versus control group; APF (acidulated phosphate fluoride) gel + Laser versus control group; APF gel + Laser versus Laser; APF gel + Laser versus APF gel; Laser + APF gel versus APF gel (compared to previous options, the order of application was reversed: first laser and then APF gel). The analysis was performed using the Review Manager (RevMan, software version 5.4, The Cochrane Collaboration, Copenhagen, Denmark, 2020). The results were presented graphically using a forest plot. Considering the clear presence of heterogeneity between the methodologies used by authors, the analysis was conducted applying a random effects model, based on standardised mean difference in mineral loss (degradation of the surface and subsurface structures of teeth), and dental hard tissue microhardness (surface softening) data, to estimate the cumulative effect of each intervention. Statistical significance (p) was set at 0.05. Heterogeneity (I^2) values were established as follows not important if it was between 0 and 40%; moderate if between 30 and 60%; substantial if between 50 and 90%; considerable if between 75 and 100%. To evaluate it, subgroups were therefore created for separate analysis.

Results

Study selection

The initial literature searching identified sixty-six potentially relevant publications up to January 2023; specifically, fifty-five articles were found in PubMed database, while eleven in the Cochrane Library. Following the evaluation of titles and abstracts,

duplicate publications and studies not considering treatments with a combination of laser and anti-erosive agents in the context of dental erosions were excluded, resulting in twenty-two articles. Of the latter, articles performed on non-human enamel and dentine samples were discarded. At the end of the selection process, twenty-eight studies were removed based on the exclusion criteria; the remaining final sixteen studies met the eligibility criteria and were included in this systematic review (Fig. 1).

Study Characteristics

Studies finally included in the systematic review, published between 2007 and 2023, were comparative in vitro studies performed on human enamel and dentine samples; in particular, the treatments were performed on extracted premolars and molars. Different types of lasers were used: Diode lasers [Vlacic et al., 2007; Sayed et al., 2012], CO_2 laser [Sayed et al., 2012; Esteves-Oliveira et al., 2011; Ramos-Oliveira et al., 2014; Esteves-Oliveira et al., 2015; Wegehaupt et al., 2011; João-Souza et al., 2015; Belcheva et al., 2018; Kasraei et al., 2021; Silva et al., 2020; AlShamrani et al., 2021],Er,Cr:YSGG (an erbium, chromium-doped yttrium, scandium, gallium) [Sobral et al., 2009; Moslemi et al., 2009], Er:YAG (erbium-doped yttrium, aluminum) [Eltinok et al., 2011; Molla Asadollah et al., 2019], Nd:YAG (Neodymium:YAG laser) [Vlacic et al., 2007; Sayed et al., 2012; João-Souza et al., 2015; Sobral et al., 2009; Braga et al., 2017], KTP laser (potassium titanyl phosphate) [Vlacic et al., 2007; Sayed et al., 2012], and an Argon Ion laser [Vlacic et al., 2007], with different parameters (power, energy, energy density, wavelength, application time, frequency, and emission mode (see Table 1). In most studies, the experimental groups consisted in the use of laser before or after the application of anti-erosive agents and samples in which only anti-erosive agents were used in comparison with no agents. Other studies evaluated lasers performed individually with one or more applications. On the other hand, two publications included a control group in which no treatment was performed [Sayed et al., 2012] and subjected only to the erosive cycle [Vlacic et al., 2007], and experimental groups on which different types of laser (Nd:YAG, KTP, Diode, CO_2, Argon Ion) and NaF gel (1.23%) were used; this is called laser-activated fluoride therapy (LAF) therapy, involving the activation of fluoride by laser irradiation. In these two studies, therefore, the efficacy of adding laser in combination with anti-erosive agents was not compared with the single use of the latter, but the potential of LAF therapy in reducing demineralisation process and increasing the resistance of dental hard tissue to acid attacks in the context of dental erosion was evaluated. It can be evidenced that most of the publications used acidulated fluoride phosphate gel as a remineralising product (APF gel,1.23%) [Ramos-Oliveira et al., 2014; Belcheva et al., 2018; AlShamrani et al., 2021; Sobral et al., 2009; Moslemi et al., 2009; Altinok et al., 2011; Molla Asadollah et al., 2019; Braga et al., 2017]. Moreover, in one study [Kasraei et al., 2021] a paste containing CPP-ACFP was applied, while the others evaluated the effect of sodium fluoride (NaF) [Vlacic et al., 2007; Sayed et al., 2012; João-Souza et al., 2015], titanium tetrafluoride (TiF_4) [Silva et al., 2020], the combination of amine fluoride and sodium fluoride (AmF/Na/F) [Esteves-Oliveira et al., 2011; Ramos-Oliveira et al., 2014], and amine fluoride, sodium fluoride and stannous chloride ($SnCl_2$) [Ramos-Oliveira et al., 2014; Silva et al., 2020], in gel or solution form. One study [Wegehaupt et al., 2011] tested the use of a lanthanide solution in combination with a fluoride solution, which is a solution of cerium chloride ($CeCl_3$) combined with a solution of amine fluoride (AmF): this solution was already evaluated in the

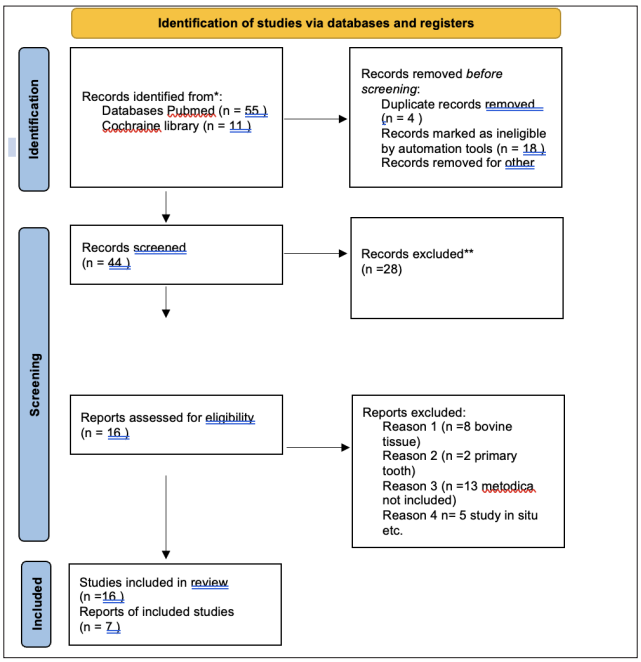

FIG. 1 Preferred reporting items for systematic review and meta-analysis (PRISMA) flow diagram gram for studies retrieved through the searching and selection process.

context of acid resistance of the dentine samples that was evaluated by measuring the calcium released during an erosive attack (EA).To investigate the surface loss of dental tissue minerals, surface microhardness (SMH) was measured in some papers using the Vickers [Vlacic et al., 2007; Sayed et al., 2012; Belcheva et al., 2018; AlShamrani et al., 2021; Sobral et al., 2009; Altinok et al., 2011; Molla Asadollah et al., 2019] and Knoop [Esteves-Oliveira et al., 2011; Ramos-Oliveira et al., 2014; Kasraei et al., 2021] methods; in other studies, surface loss and roughness (in µm) were examined using optical profilometry [Esteves-Oliveira et al., 2015; João-Souza et al., 2015; Silva et al., 2020]; others studies shown resistance to acid attack by measuring the calcium released during the erosive cycle using atomic absorption spectroscopy, an instrumental analytical technique used for both quantitative and qualitative determination of metal ions in solution [Wegehaupt et al., 2011; Moslemi et al., 2009], or by means of atomic emission spectroscopy [Braga et al., 2017]. Finally, in a few studies included in this review, in addition to mineral loss assessment, scanning electron microscopy (SEM) investigations were performed to assess the morphology of tooth surfaces after treatment and after the erosive cycle [João-Souza et al., 2015; Silva et al., 2020; Braga et al., 2017](see Table 2).

Statistical comparisons

Of the 16 studies included in the systematic review, only 7 were selected because of data of interest, enabling a comparison between the experimental and control groups. Among these seven, those necessary for the research purposes were selected, depending on the objective of the statistical analysis. Regard microhardness, the comparison of the effect of laser individually (L) and the control group (C) on dental tissue microhardness, due to high heterogeneity ($I^2 = 88\%$) among studies, was conducted as a global analysis and a subgroup analysis comparing CO_2 laser vs. control in the first subgroup, and Er/ErCr laser vs. control in the second subgroup. However, the heterogeneity was not reduced: for the subgroup CO_2 laser vs. control, I^2 was 89%, while for the subgroup Er/ErCr laser vs. control I^2 was 60%. As shown

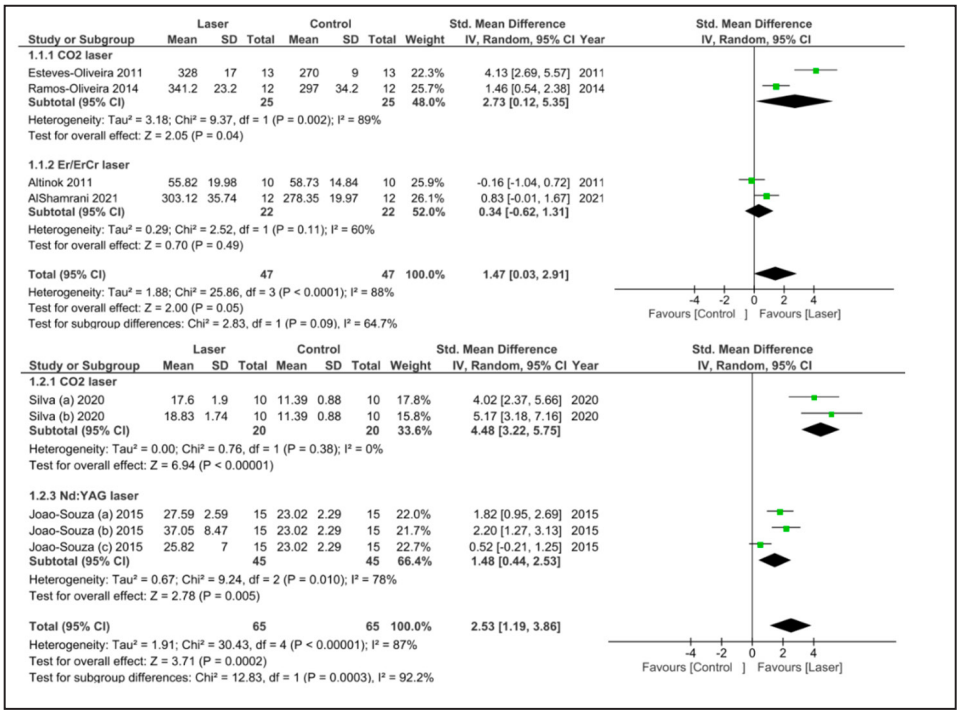

FIG. 2. Comparison of the effect of laser treatment versus the control group (erosive cycle only) on the microhard-ness (SMH) of dental tissue and on dental tissue loss assessed by optical profilometry.

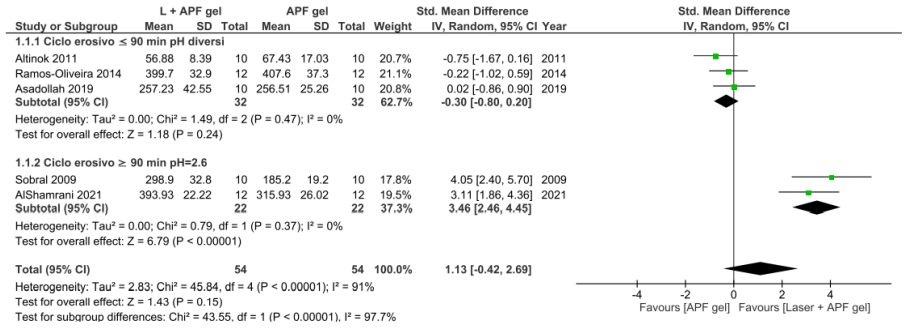

FIG.3 Comparison of the effect of the combined treatment (laser + APF gel) versus the APF gel group on dental tissue microhardness.

in Figure 2, the standardised mean difference of 2.73 (95% CI:0.12, 5.35) was statistically significant (p=0.04), in favor of laser for the first group (CO_2 laser); the standardised mean difference of 0.34 (95% CI: -0.62, 1.31) was not statistically significant (p=0.49) for the second group (Er/ErCr laser); the overall standardised mean difference was 1.47 (95% CI:0.03, 2.91, I=88%), indicating that mean change in microhardness following laser, irrespective of the type of laser used in the sample, was significantly greater compared with the one in the control group.

A low heterogeneity (I^2 = 0%) was observed in the first subgroup (CO_2 laser vs. control) where the standardised mean difference of 4.48 (95% CI:3.22, 5.75) was statistically significant (p<0.00001), and a higher mineral loss was found in the laser group than in the control group. Considerable heterogeneity (I^2 = 78%) was found in the second subgroup (Nd:YAG laser vs. control), in which the standardised mean difference of 1.48 (95% CI:0.44, 2.53, I = 87%) was statistically significant (p=0.005), to the disadvantage of the laser group (in which greater mineral loss was found). As the same comparison as above, yet assessing the mineral loss of dental tissue, showed high heterogeneity (I^2 = 87%) of the studies, a global analysis and a subgroup analysis (CO_2 laser vs. control; Nd:YAG laser vs. control) were conducted. The global standardised mean difference was 2.53 (95% CI:1.19, 3.86), indicating that the mineral loss of the control group was significantly lower compared to the laser (Fig. 2).

A further analysis comparing the effect of treatment with laser + APF gel versus the APF gel group on the microhardness of dental tissue was conducted (Fig. 3). Due to high heterogeneity (I^2 = 91%) of the studies, global and subgroup analyses were conducted. By performing a subgroup analysis, separating the studies with an erosive cycle ≥ 90 min at a pH of 2.6, from the studies with a shorter erosive cycle and varying pH (pH< 2.6 and pH>2.6), the heterogeneity was significantly reduced (I^2 = 0%). In the first subgroup (erosive cycle ≤ 90 minutes and different pH) the overall standardised mean difference was -0.30 (95% CI: -0.80, 0.20). The microhardness of dental tissue following APF gel agent was greater than that following the combined treatment (L+APF gel), but this difference was not statistically significant (p = 0.24). In the second subgroup (erosive cycle ≥90 minutes and pH = 2.6), the overall standardised mean difference was 3.46 (95% CI:2.46, 4.45). The microhardness of the dental tissue following laser + APF gel treatment was significantly higher (p<0.00001) than that following APF gel agent. The overall standardised mean difference was 1.13 (95% CI: -0.42, 2.69), indicating a greater microhardness (mean increase) of the laser + APF gel group compared to that of the APF gel agent; however, this difference was not statistically significant (p = 0.15, see Fig. 3). In the overall analysis on the comparison between the effects

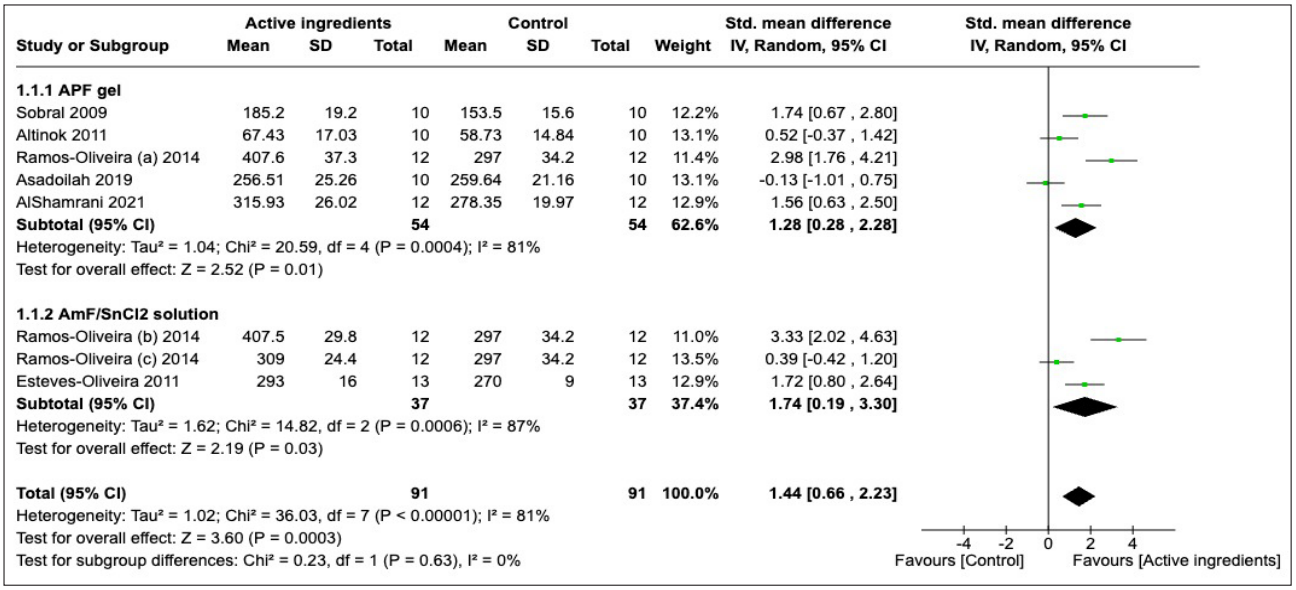

FIG. 4 Comparison of the effect of the combined treatment Active Ingredients versus control group on dental tissue microhardness.

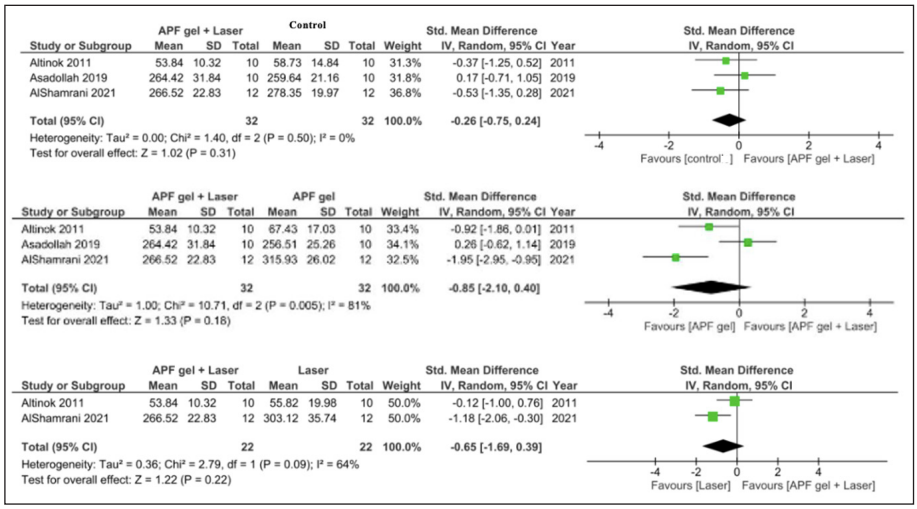

FIG. 5 Comparison of the effect of the combined treatments APF gel with Laser versus control group, APF with Laser versus APF gel, APF gel with Laser versus Laser on dental tissue microhardness.

of active ingredients and the control group, a high heterogeneity I^2 = 81% was found; by carrying out a subgroup analysis (first subgroup APF gel vs. control; second subgroup AmF/NaF gel, AmF/$SnCl_2$ solution vs. control), this could not be reduced: for the first subgroup I^2 was 81%, for the second subgroup I^2 was 87%. The overall standardised mean difference was 1.44 (95% CI:0.66, 2.23), indicating that a mean change in microhardness after treatment with anti-erosive agents was significantly greater (p = 0.0003) compared to the control group. Due to considerable heterogeneity, both subgroups showed a statistically significant standardised mean difference (p = 0.0003), in favor of the active ingredients. The mineral loss of dental tissue was depicted by comparing the use of the anti-erosive agents and the control group. The anti-erosive agents used were NaF gel [Esteves-Oliveira et al., 2011], TiF_4 gel [Wegehaupt et al., 2011], and AmF/NaF/$SnCl_2$ [Wegehaupt et al., 2011]. Heterogeneity was considerable (I^2 = 94%) and the standardised mean difference was -2.64 (95% CI: -5.53, 0.25), indicating that loss of minerals following treatment with anti-erosive agents was not significantly different from that in the control group (p=0.07, see Fig. 4).

Only three studies presented data (mean and standard deviation) for the analysis of the effect of the combined treatment APF gel + laser compared to the control group (erosive cycle only) on dental tissue microhardness. Low heterogeneity was observed (I^2 = 0%), which allowed comparison. The standardised mean difference was -0.26 (95% CI: -0.75, 0.24), indicating that the mean change in microhardness following the combined treatment was smaller compared to the control group; however, this difference was not statistically significant (p=0.5). The comparison of the combined APF gel + laser treatment versus the APF gel group shows an overall standardised mean difference of -0.85 (95% CI: -2.10, 0.40). This result indicated that the mean change in tooth tissue microhardness following APF gel agent was greater compared to that following combined treatment, but this difference was not statistically significant (p=0.005). Heterogeneity of the studies was considerable (I^2 = 81%). The comparison between the effect following the combined treatment (APF gel + laser) and the laser group showed an overall standardised mean difference of -0.65 (95% CI: -1.69, 0.39). The mean increase in the microhardness value of dental tissue following laser was greater compared to that following the combined treatment, but this difference was not statistically significant (p=0.09). Heterogeneity of the studies was substantial (I^2 = 64%, see Fig. 5).

Discussion

This systematic review and meta-analysis on the erosion of

Author Year Place	Type of study and sample	N. per group	Experimental groups	Erosion cycle	Results
Vlacic et al. 2007; Brisbane	In vitro study; human enamel; molars and premolars.	10	Group 0: control, erosive cycle without application of NaF and laser; Groups 1-7: NaF gel + laser.	Hydrochloric acid (1.0 M) for 5 mins.	Laser activation of fluoride (LAF therapy) offers protection to enamel in the presence of acid attacks.
Moslemi et al. 2009; Iran	In vitro study; human enamel; premolars.	17	Group 1(C): control; Group 2(L): Er,Cr:YSGG laser; Group 3(F): APF gel; Group 4(LF): laser + APF gel; Group 5(FL): APF gel + laser.	Acetate buffer solution (0.2M, pH 4.8) for 10 days.	Er,Cr:YSGG laser + APF gel showed an increase in enamel resistance to acid attack compared to the use of APF gel alone. There is no significant difference in resistance to acid attack if Er,Cr:YSGG laser irradiation is carried out before or after the application of APF.
Sobral et al. 2009; Brazil	In vitro study; human enamel; molars.	10	Group 1(C): control; Group 2(F): APF gel; Group 3(L): laser light absorber + Nd:YAG laser; Group 4(LF): Nd:YAG laser + APF gel.	2% citric acid solution (pH 2.6) for 90 mins.	Nd:YAG laser + APF gel increased the strength of the enamel.
Altinok et al. 2011; Turkey	In vitro study; human enamel; permanent molars.	10	Group 1(C): control; Group 2(F): APF gel; Group 3(LF): Er: YAG laser + APF gel; Group 4(FL): APF + Er: YAG; Group 5(L): Er:YAG laser.	Immersion of the samples in a drink (not specified which one), for 10 mins.	Er: YAG laser alone or combined with APF gel decreased enamel solubility. The combined treatment showed no significant additional effect.
Esteves-Oliveira et al. 2011; Brazil	In vitro study; human enamel; permanent molars.	13	Group 1(C): surface rinse with distilled water, negative control; Group 2(L): laser irradiation; Group 3(LF) laser irradiation followed by fluoride gel treatment; Group 4(FL): fluoride gel + laser irradiation; Group 5(F) fluoride treatment.	1% citric acid, pH 4.0, for 3 mins.	CO2 laser significantly decreased the mineral loss due to erosive attack (97 per cent) and increased the microhardness of previously demineralised enamel.
Wegehaupt et al. 2011; Switzerland	In vitro study; human dentine; premolars.	12	1) placebo (G1 /G2); 2) amine fluoride (AmF) (G3 / G4); G3 /G4); 3) Cerium chloride (CeCl3) (G5 /G6); 4) amine fluoride/Cerium chloride (AmF/CeCl3:G7 / G8). The groups G2, G4, G6, G8 were irradiated with C02 lasers.	Lactic acid (pH 3.0) for 5 minutes 6 times.	CeCl3 + AmF showed the highest anti-erosive potential. CO2 laser irradiation had no additional effect.
Sayed, et al. 2012; India	In vitro study; human enamel; premolars.	10	Group 1(C): control; Group 2: KTP; Group 3: Nd: YAG; Group 4: Diodes; Group 5: CO2.	Hydrochloric acid (1.0M) for 5 mins.	After acid attack, the least reduction in enamel hardness was found: 1) in the Nd: YAG group, 2) KTP laser, 3) CO2 laser, 4) Diode laser.
Ramos-Oliveira et al. 2014; Brazil	In vitro study; human enamel; permanent molars.	12	Group 1: eroded enamel (no treatment); Group 2: APF gel; Group 3: AmF/NaF gel; Group 4: AmF/SnF solution2 ; Group 5: CO2 laser; Group 6: CO2 laser + APF gel; Group 7: CO2 laser + AmF/NaF gel; Group 8: CO2 laser + AmF/SnF solution2 ; Group 9: intact enamel.	1% citric acid (pH 4.0) for 3 min. Immersion in a pH 7.0 solution for 24 h.	AmF/NaF and APF have the potential to control and reduce the progression of erosion in dental enamel; CO2 laser did not affect their effectiveness. CO2 laser alone can significantly decrease enamel mineral loss, albeit at lower levels.
Braga et al. 2015; Brazil	In vitro study; human enamel; permanent molars.	10	Group 1(C): control; Group 2(F): APF gel; Group 3(L): Nd: YAG laser; Group 4(FL): APF + laser; Group 5(LF): Laser + APF.	Hydrochloric acid (pH 2.2) for 5 mins, immersion in artificial saliva for 3h. Cycle repetition: 4 times/day x 14 days.	APF gel + Nd:YAG laser significantly reduced the dissolution of the tooth enamel surface.
Esteves-Oliveira et al. 2015; Brazil	In vitro study; human enamel; permanent molars.	16	Group 1(C): control; Group 2(F): daily application; Group 3(Lx1): laser irradiation once before the erosive cycle; Group 4(Lx2): laser irradiation twice (once before the erosive cycle + once before the sixth day of the cycle); Group 5(L1 /F): daily application of fluoride solution + laser irradiation once before the erosive cycle. Group 6(L2 /F): daily application of fluoride solution + laser irradiation twice (before the first erosive cycle and before the sixth cycle day).	pH 2.3 solution for 2 mins; immersion in pH 6.5 solution. Repeat cycle: 6 times/day for 10 days.	Fluoride solution causes reduction (88%) of enamel surface loss; solution + CO2 laser almost completely hinders loss due to erosive attacks.

João-Souza et al. 2015; Brazil	In vitro study; human dentine.	15	Group 1(C): control; Group 2(F): NaF gel; Group 3(L1): Nd:YAG laser, P1 ; Group 4(L2): Nd:YAG, P2 ; Group 5(L3): Nd:YAG, P3 ; Group 6(FL1):NaF gel + Nd:YAG1; Group 7(FL2): NaF gel + Nd:YAG2; Group 8(FL3): NaF gel + Nd:YAG3	1% citric acid (pH 2.3) for 3 mins. Immersion in artificial saliva pH 7.0 for 60 mins Cycle repetition: 6 times/day x 5 days.	Nd: YAG lasers: P1, P2, P3 caused ablation with removal of dentine; NaF gel (2%) was the only treatment able to reduce further demineralisation on eroded dentine.
Belcheva et al. 2018; Bulgaria	In vitro study; human enamel; permanent molars.	10	Group 1(C): control; Group 2(F): APF gel; Group 3(L): CO2 laser; Group 4(FL): APF gel + laser.	Soft drink (pH = 2.75) for 10 mins; immersion in artificial saliva for 1 h.	CO2 laser + APF gel is more effective in protecting the enamel surface and reducing demineralisation after an acid attack than applying the two treatments separately.
Asadollah et al. 2019; Iran	In vitro study; human enamel; permanent molars.	10	Group 1(C): control; Group 2(F): APF gel; Group 3(FL): APF gel + Er: YAG laser; Group 4(LF): Er: YAG laser + APF gel.	Coca Cola + phosphoric acid, pH 2.4 for 2 min; immersion in artificial saliva (pH 6.5) for 2 h; Repeat cycle: 4 times/ day x 5 days.	Er: YAG laser + APF gel does not prevent erosion; however, less reduction in enamel microhardness after acid attack was observed compared to group C and F.
Silva CV et al. 2020; United States	In vitro study (RCT); human enamel; permanent molars.	10	Group 1(C): control; Group 2: 4% TiF4 gel; Group 3(E) AmF/NaF/SnCl solution2 ; Group 4(L1): CO2 laser (P1); Group 5(L1/TiF4): CO2 laser (P1) + 4% TiF4 gel; Group 6(L1/E): CO2 laser (P1) + AmF/NaF/SnCl solution2 ; Group 7(L2): CO2 laser (P2); Group 8(L2 / TiF4): CO2 laser (P2) + 4% TiF4 gel; Group 9 (L2 /E): CO2 laser (P2) + AmF/NaF/SnCl solution.	Citric acid solution, pH 2.3, for 2 min; immersion in pH 6.7 solution. Repeat cycle: 6 times/day for 10 days.	CO2 laser (P1 and P2) + AmF/NaF/ SnCl solution2 significantly reduced the progression of dental enamel erosion.
AlShamrani et al. 2021; Saudi Arabia	In vitro study; human enamel; premolars.	12	Group 1(C): control group; Group 2(F): APF gel; Group 3(L): Er, Cr:YSGG laser; Group 4(FL): APF gel + laser; Group 5(LF): laser + APF gel. After treatment, all samples were stored in artificial saliva (pH 6.8) for 24h.	0.3% citric acid solution (pH 2.6) for 5 min; immersion in artificial saliva. Repeat cycle: 4 times/day, for 5 days.	Compared to the control group, Er,Cr:YSGG laser + APF gel increased the microhardness of the enamel surface preventing the progression of erosion.
Kasraei et al. 2021; Iran (18)	In vitro study; human enamel; premolars.	14	Group 0: positive control, no erosive cycle or treatment; Group 1: negative control, erosive cycle only; Group 2: CO2 laser; Group 3: CPP-ACFP pastes; Group 4: CO2 laser + CPP-ACFP pastes; Group 5: CPP-ACFP paste + CO2 laser. Afterwards, the samples were immersed in artificial saliva (pH = 7.0) for 48h.	Carbonated cola drink (pH 2.5), 2 minutes 3 times (tot. 8 minutes).	CO2 laser or CPP-ACFP individually increase the surface hardness of eroded enamel; however, their simultaneous application is more effective. CPP-ACFP + CO2 laser with appropriate parameters can significantly increase the surface hardness of enamel.

TABLE 1. Characteristics of the studies included in the systematic review.

dental hard tissues statistically evaluated data from in vitro studies, as the searching did not yield any results regarding clinical or in vivo studies. The application of anti-erosive agents appeared to be a valuable treatment for increasing the resistance of dental enamel to erosion progression and improving its microhardness. The use of laser, able to interact with dental hard tissue by producing chemical and morphological changes on its surface, presents contradictory results, as it was found to induce an increase in enamel microhardness; however, a loss of minerals could be observed, due to the ablative effect [Ramos-Oliveira et al., 2014]. It was found that the use of lasers at the dentine level causes a loss of minerals, inferior to the one at the enamel level: this is precisely due to the composition of dentine tissue, which is less mineralised than enamel. In this proposal, only two articles investigating the effect of laser irradiation on erosion at the dentine level were selected and included according to the inclusion criteria [Wegehaupt et al., 2011; João-Souza et al., 2015]. Outcomes of statistical analysis derived from the comparison of data on dentine minerals loss in the laser-treated group versus the control group showed that loss of hard tissue following laser was significantly greater ($p = 0.005$), indicating that laser had an ablative effect on dentine by causing a loss of substance. However, these considerations must be considered with caution, because of the heterogeneity of the studies and insufficient current scientific evidence on the effectiveness of laser for dentine erosion. Some authors who examined dentine tissue rejected the hypothesis that irradiation would improve dentine resistance in presence of acid attacks. Wegehaupt et al. [2011] found that release of calcium from acids was higher after laser irradiation; this effect could be attributed to the roughening of surface of irradiated samples after the erosive cycle. João-Souza et al. [2015] showed that the use of the Nd:YAG laser at 1064 nm with 3 protocols, for 40 seconds, caused carbonisation and tissue loss with all protocols at different levels. In this proposal, progression of dental erosion at dentine level is different from enamel level: as a consequence, the mechanism which causes changes in chemical composition and morphology of the substrates, responsible for the protective effect of laser against demineralisation, does not have the same effect on both tissues, due to a higher water and protein contents at the dentine level [Hara et al., 2005]. On the contrary, irradiation of human dental enamel using lasers can cause increased resistance to acid attacks and a decrease of the critical pH. Even in combination with topical applications of fluoride or non-fluoridated anti-erosive agents, an increase in resistance of tooth structure to mineral loss was observed. This effect could be linked to deposition of calcium fluoride, formation of microspaces in dental hard tissue, formation of tricalcium phosphate, and transformation of hydroxyapatite

Author, Year, Place	Laser group parameters	Application of remineralising agents
Vlacic et al. 2007; Brisbane (10)	Argon ion laser: λ=488nm; power 0.3W, exposure time 10sec, power density 1530 mW/cm2; Argon ion laser λ=514.5 nm, 0.3W, 10sec, 1530 mW/cm2; KTP λ=532nm, 0.1W, 60sec (30 sec before NaF + 30 sec after NaF application), 510 mW/cm2; Diode laser λ=633nm, 0.05W, 60sec, 255 mW/cm2; Diode laser λ=670nm, 0.01W, 300sec, 51 mW/cm2; Diode laser λ=830nm, 0.06W, 50sec, 36mW/cm2; Nd:YAG λ=1064nm, 0.03W, 10sec, 153mW/cm2 . *Continuous mode for all lasers; Nd:YAG pulsed mode.	1.23% NaF (12 300ppm) + laser activation.
Moslemi et al. 2009; Iran (22)	Er,Cr:YSGG laser λ=2780 nm; power 0.25 W; pulse duration 140 ms; pulse energy 12.5 mJ; No water cooling; 11 % air cooling; irradiation time 10 s. Frequency 20 Hz; distance 1-2 mm from tooth surface.	1.23 % APF gel, 4 min.
Sobral et al. 2009; Brazil (21)	Nd:YAG laser λ= 1064 nm; 1 W, 100 mJ, 10 Hz, 125 J/cm2 , no water cooling, irradiation time 3 min.	APF (1.23% NaF; pH 5.3), repeated 3 times, for 4 min.
Altinok et al. 2011; Turkey (23)	Er:YAG laser λ=2940 nm; energy density 1.2 J/cm2 , frequency 10Hz, water cooling, non-contact mode.	APF gel (1.23% NaF, pH 3.5), 1 min.
Esteves-Oliveira et al. 2011; Brazil (12)	CO2 laser λ=10.600 nm; energy density 0.30 J/cm2; pulse duration 5 µs; irradiation time 9 s; frequency 226 Hz; pulse energy 15 mJ; beam diameter 2.5 mm; irradiation distance 19.8 cm.	AmF/NaF Gel (1.25% F, pH 4.8-6.0) for 4 min.
Wegehaupt et al. 2011; Switzerland (15)	CO2 laser λ= 10,600 nm; power 0.5 W; frequency 20 Hz; pulse duration 100 ms; beam diameter 1.1 mm; irradiation time 30 s.	G1, G3, G5 and G7: solutions were applied for 30s. *AmF: 9250 ppm Olaflur and 750 ppm Dectaflur; Elmex fluid; Ph 3.9. *CeCl3): solution consisting of 10.00 g Cerium chloride, 0.10 g sodium benzoate and 89.90 g distilled water (pH 4.94).
Sayed, et al. 2012; India (11)	CO2 laser λ=10,600 nm; power 0.52 W; exposure time 60s. Diode laser λ=810-940 nm; 2.0 W; 60s. KTP laser λ=532 nm; 0.40 W; 60s. Nd YAG laser λ=1064 nm; 1.50 W; 60s.	NaF gel (1.23%) before laser irradiation.
Ramos-Oliveira et al. 2014; Brazil (13)	CO2 laser λ=10.600 nm; energy density 0.45 J/cm2; pulse duration 15 µs; frequency 128 Hz; pulse energy 22 mJ; irradiation time 9 s; no air/water cooling; irradiation distance 19.8 cm.	G2 and G5: APF gel (1.23% NaF, pH 3.6-3.9) for 4 min; G3 and G6: AmF/NaF gel (1.25%F, pH 4.8-6.0) for 4 min; G4 and G7: SnF solution2 (0.16%AmF+0.05%SnF2, pH 4.2) for 3 min. G6, G7, G8: application of fluorinated agents immediately after laser exposure.
Braga et al. 2015; Brazil (25)	Nd:YAG laser λ=1064 nm, E.max 100 mJ, power 1 W, frequency 10 Hz, energy density 141.5 J/cm2 , irradiation time: 30s.	1.23% APF gel, for 4 min.
Esteves-Oliveira et al. 2015; Brazil (15)	CO2 laser λ=10.600 nm; pulse duration:5 µs, frequency 226 Hz, energy density of 0.3 J/cm2 , No water cooling; irradiation distance 19.8 cm, beam diameter 2.5 mm; irradiation time: 9s	Solution (pH = 4.5) containing amine fluoride (AmF 125 ppm F) + sodium fluoride (NaF 375 ppm F) + stannous chloride (SnCl2 800 ppm). Immersion of the samples in the solution for 30 s per day. * L1F and L2F: The first application of fluoride was completed before laser irradiation.
João-Souza et al. 2015; Brazil (16)	Nd:YAG laser λ=1064 nm; Protocol 1: power 0.5 W; E max50 mJ; energy density 41.66 J/cm2 ; frequency 10 Hz; Protocol 2: 0.7 W; 70 mJ; 62.50 J/cm2; 10 Hz. P1 and P2: contact mode, irradiation time: 40 s. Protocol 3:1 W; 100 mJ; 54.16 J/cm2 ; 10 Hz, at a distance of 1 mm, for 40 s.	2% NaF gel according to the manufacturer's instructions.

Belcheva et al. 2018; Bulgaria (17)	CO2 laser λ= 10,600 nm. Time on: 100 μs; time off: 40 ms; Power 0.73 W; maximum power 292.73 W; Movement speed 2 mm/s; Energy density 5 J/cm2; Distance from tooth surface 20 mm; Irradiation time 30 s.	1.23% APF gel for 4 min, according to manufacturer's instructions.
Asadollah et al. 2019; Iran (24)	Er:YAG laser λ=2940 nm, E.max 100 mJ , energy density 1.59 J/cm2 , power 1 W, frequency 10Hz, pulse duration 250 μs, irradiation time: 10s, beam diameter 1 mm, water cooling 50%, distance 2 mm	1.23% APF gel; 4 min.
Silva et al. 2020; Brazil (19)	CO2 laser λ= 9300 nm; Protocol 1: beam diameter at irradiation point 0.63 mm; pulse duration 14.6 μs; energy density 1.9 J/cm2; frequency 100 Hz; power 0.58 W; air cooling; irradiation time 10 s. Protocol 2: beam diameter at irradiation point 0.63 mm; pulse duration 18 μs; energy density 2.2 J/cm2; frequency 100 Hz; power 0.69 W; air cooling; irradiation time 10 s.	Groups TiF4, L1/TiF4, and L2/TiF4: one application of 4% TiF4 gel (pH 1.5; 24,533 ppm F-), for 4 minutes, before the start of the erosion cycle (simulation of a fluoride varnish application in the study occurring every 6 months). Groups E, L1/E, and L2/E: immersion in the AmF/NaF/SnCl solution2 (pH 4.5; 500 ppm F-; 800 ppm Sn2) for 2 minutes, every day, before the erosive cycle (simulation daily use of a fluoride mouthwash).
AlShamrani et al. 2021; Saudi Arabia (20)	Er, Cr:YSGG laser λ= 2780 nm; power 0.5 W, frequency 20 Hz, pulse duration 60 μs, exposure time 10 s, air cooling 11%, energy density 8.8 J/ cm2 , irradiation distance 1 mm.	APF gel (1.23%, pH: 3.6- 3.9) for 4 min.
Kasraei et al. 2021; Iran (18)	CO2 laser λ=10.600 nm; power 0.7 W, pulse duration 0.4 μs, beam diameter 0.4 mm, frequency 50 Hz, distance 10 mm, energy density 10.66 J/cm2, air cooling 60%, irradiation time 10 s.	CPP-ACFP (MI Paste Plus, Recaldent™, GC Co., USA) applied to 3mm tooth surface diameter, 1mm thickness, for 3 min.

TABLE 2 Parameters of the lasers used and application of anti-erosion agents.

into fluorapatite (more resistant to acids compared to carbonated hydroxyapatite) [Shellis et al., 2010; Field et al., 2010]. Laser irradiation generally causes chemical and morphological changes on surfaces. More specifically, chemical changes usually occur after removal of carbonate apatite (carbonate-hydroxyapatite crystals are more soluble than pure hydroxyapatite crystals), while morphological changes result from the increase in surface temperature, which causes protein denaturation, melting, and subsequent resolidification of hard tissues [Schmidlin et al., 2017]. The effects consist in an altered mineral composition, a decreased enamel solubility, and an increased absorption of remineralising agents on tooth surface. Furthermore, air/water cooling is important for controlling temperature and avoiding formation of inhomogeneous surfaces (SEM), being less resistant to acid attacks. This result was mostly found for studies using lasers on dentine after the erosive cycle [Vlacic et al., 2007 ; West et al., 2020]. Regarding studies carried out on human enamel, from the evaluations of statistical comparisons, data on the effects on laser-treated dental elements (CO_2 laser) compared to the untreated ones (control group) reported an average increase in enamel microhardness of 2.73 (Knoop hardness test), with a statistical significance of p=0.04; however, this result cannot be considered reliable, due to the high heterogeneity of the studies. It can be only hypothesised that enamel microhardness is increased, confirming that the laser can be a valid mean to counteract or reduce demineralisation following acid attacks. This concept was also confirmed by Esteves-Oliveira [2011] and Ramos-Oliveira [2014]. However, these studies evaluated the efficacy of this treatment based on a single day, simulating incipient erosive lesions created by a single exposure to citric acid for 3 min, and using a CO_2 laser and fluorinated agents to simulate a single professional application: a limitation of this experimental model is that long-term effects of therapy cannot be predicted.

The potential of anti-erosive agents (first subgroup APF gel vs. control; second subgroup AmF/NaF gel, AmF/$SnCl_2$ solution vs. control) to prevent or reduce mineral loss during the initial stages of enamel erosion was also analysed. An increase in enamel microhardness of 1.44 was observed with a statistical significance; however, the efficacy may depend on the nature of fluoride compound, type of product (solution, gel, varnish), concentration of the active ingredient, application time, and duration of treatment. Indeed, in the studies evaluated, application times were short and probably insufficient to allow proper formation of a protective layer; different products, demineralisation cycles, and formulations (gel, solution, or varnish) were used, making it difficult to interpret results about the role of anti-erosive agents.

Concerning studies about the evaluation of the effects of combined agents (APF gel + laser), it was found that topical application of this agent can be a preventive method in improving the resistance of dental hard tissue in presence of acid attacks; a calcium fluoride deposit (CaF_2) is obtained on surface crystals, as a 'reservoir' releasing fluoride during the demineralisation process.

Furthermore, topical application of APF gel before or after laser irradiation leads to an increase in fluoride absorption and a decrease in rate of dissolution in an acid solution [González-Rodríguez et al., 2011; Moher et al., 2009]. Statistical analysis, taking into account the considerable heterogeneity of the compared studies, yielded an overall standardised mean difference of 1.13, indicating that microhardness of laser + APF gel group was greater compared to APF gel group; however, as this difference was not statistically significant (p=0.15), this result appears to be not reliable for the use of this combination. In a

recent systematic review, it has emerged that the CO_2 lasers with 1.23% APF gel, improve the enamel acid resistance in the parameter of mean depth of acid induced lesions ($p<0.05$) (11).

The subgroup analysis showed that use of laser and subsequent associated APF gel, in short erosive cycles (≤90 min), did not produce a significant effect, compared with APF gel alone. Despite potential limitations, it could be thus stated that clinical dental elements subjected to prolonged erosive cycles (e.g., in presence of eating disorders such as anorexia and bulimia) could benefit from the application of laser followed by APF gel, compared with the use of APF gel alone. In addition, as mentioned before, only one study included [Esteves-Oliveira et al., 2015] applied a paste containing CPP-ACFP; a prospective study compared CPP-ACP and CPP-ACFP, concluding that the second one is superior to fluoride varnish at remineralising smooth-surface white spot lesions [Llena et al., 2015].

Comparison of data from studies regarding the combined APF gel + laser treatment, that is, compared with previous observations, by reversing the order of application and first using the APF gel and then the laser, showed a decrease in mean value of enamel microhardness compared with APF gel alone, and a decrease in mean value of microhardness: again, as results obtained were not statistically significant, this combination cannot be considered as valid.

Results of studies which considered microhardness of dental tissue after laser application compared to the control group, and those of the studies which assessed the microhardness of dental tissue after application of anti-erosive agents compared to the control group, were statistically significant; however, owing to the considerable heterogeneity, they cannot be finally considered as scientifically relevant.

It must be considered that in vitro conditions differ from in vivo conditions. Other less invasive techniques, like fluoride-based varnishes, casein calcium phosphate pastes and biomimetic hydroxyapatite were evaluated in literature for their anti-erosive effects on both enamel and dentine [Butera et al., 2023; Colombo et al., 2017].

However, there is evidence in literature that some varnishes are less biocompatible than others and can induce some changes in the number of mitochondria [Escobar-García et al., 2021]. Therefore, the results of this review must be interpreted with caution, because of inherent limitations, regarding difficulty in simulating oral conditions with the same cycles of demineralisation and remineralisation, absence of salivary flow, oral bacteria and other protective factors, such as salivary proteins. Saliva plays an important role in preventing erosion of dental hard tissue as it acts as a diffusion barrier for acids: in studies performed in vitro, a higher rate of enamel surface loss is likely to be obtained than in a real clinical situation.

Because of the variety of parameters and methodologies used in literature, it is difficult to make comparisons between studies or to identify appropriate and standardised protocols, as the studies involved the use of different types of lasers and parameters (energy density, average power, frequency, irradiation time, and irradiation mode); plus, polishing of enamel increases the accuracy of microhardness measurements, but it exposes, at the same time, a less acid-resistant inner enamel layer. This means that the erosive demineralisation occurring in polished enamel is somewhat higher than in natural enamel; therefore, the observed mineral dissolution rates are likely to be lower when evaluated in vivo. In addition, age variations of donors may give rise to variations in enamel mineral density, which may influence the results of previous studies. In the light of these consideration, outcomes were standardised using a standardised mean difference rather than mean difference when carrying out statistical analysis, and subgroup analysis were also performed. Despite this, no entirely reliable data were obtained; it was therefore not possible to effectively compare all studies with each other and to develop a complete meta-analysis. Finally, it must be noted that the studies reported values of mean change in the final microhardness without considering the starting values, introducing a bias. To obtain more reliable results regarding the effects on post-treatment dental hard tissue and to reduce the risk of bias, it would be advisable to make a com-parison between studies which start from equal microhardness data at the starting time.

Conclusions

Change in microhardness for the lasers group, irrespective of the type of laser used in the sample, was significantly greater compared with the one in the control group. While use of lasers associated with fluoride did not give any significant outcomes, the use of laser alone was found to induce an increase in enamel microhardness. Regards microhardness, the combination of Laser and APF gel showed promising results compared to APF gel agent. However, whether used before or after laser, the effectiveness of laser in combination with anti-erosive agents remains still unclear: no statistically significant values were obtained from the analysis of the present review.

Supplementary Materials
Table 1 and Table 2.

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