Sub-ablative laser irradiation to prevent acid demineralisation of dental enamel. A systematic review of literature reporting *in vitro* studies



G. Lombardo, S. Pagano, S. Cianetti, B. Capobianco, M. Orso***, P. Negri, M. Paglia*, S. Friuli*, L. Paglia*, R. Gatto**, M. Severino**

Department of Surgical and Biomedical Sciences, Unit of Paediatric Dentistry, University of Perugia, Perugia, Italy
**Department of Pediatric Dentistry, Italian Stomatologic Institute, Milan, Italy*
***Department of Life, Health and Environmental Sciences, Division of Implantology and Prosthetic Dentistry, Dental Clinic, University of L'Aquila, L'Aquila, Italy*
****Health Planning Service, Department of Epidemiology, Regional Health Authority of Umbria, Italy*

e-mail: guido.lombardo@unipg.it

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Abstract

Aim Caries lesions begin with enamel acid demineralisation mediated by microorganisms. Lasers with sub-ablative energy might act as a prophylactic intervention to reinforce enamel against lesions caused by acid.

Materials and methods A systematic review of the literature was performed evaluating only *in vitro* studies published from 2010 to 2018. The research was performed using the following databases: Medline, Embase and the Web Of Science. A further search was performed consulting the list of references of the included studies as well as book chapters which dealt with this topic.

Results A total of 347 records were retrieved and, after their evaluation, 36 studies were included. CO_2 lasers were the most described and effective device in preventing acid demineralisation. This type of laser was unique in improving the already positive results obtained with fluoride-based interventions. Er,Cr:YSGG (with fluencies > 8.5J/cm^2), diode and argon lasers also improved enamel acid resistance (p-values ranging from 0.05 to 0.001) producing similar effects with fluoride-based interventions. Regarding the sealant retention outcome, the Er:YAG laser was able to perform an enamel etching which was as effective as the traditional acid etching with the advantage of being easier and usually well accepted by low-compliant patients (i.e. younger children). Nd:YAG presented the worst results. The most common structural changes after the laser irradiation were water and carbonate reduction in the enamel combined with a phosphate and calcium enamel content increase. Moreover, the calcium/phosphate ratio was found to reach the 1.67 ideal ratio.

Conclusion The *in vitro* studies that examined the prophylactic use of lasers for increasing enamel acid resistance presented interesting results that are enough to support a further in vivo experiment. This would entail the use of a clinical laser as an alternative or in combination with fluoride-based interventions.

KEYWORDS Caries prevention; Enamel demineralisation; Laser; Sub-ablative energy.

Introduction

Caries is a worldwide pandemic disease affecting all ages from early childhood to adulthood and the elderly. Although during the last decades the prevalence of caries in developed countries has been reduced, the disease still has significant relevance among socioeconomically disadvantaged individuals and during childhood. A recent review considering this disease among 5 year old children in 37 countries, described its prevalence as ranging from 23 to 90%, with a DMFT/dmft index that varied from 0.9 to 7.5. However, even more significantly, in 25 out of 37 studies the prevalence was higher than 50% [Chen et al., 2019]. The aetiology of caries is well understood (is caused by excessive sugar intake, reduced fluoride intake, and poor oral hygiene) and could be considered as a completely preventable disease. This supports the validity of prevention policies (educational and prophylactic) adopted by clinicians in addition to traditional dental care [Foley and Ruyter, 2019]. Several caries-preventive strategies can be adopted in the clinical practice. Among prophylactic interventions the most common are sealing of permanent molar pits and fissures as well as the use of enamel surface fluoride varnishes or gels [Ahovuo-Saloranta et al., 2017; Marinho et al., 2015 and 2013]. These prophylactic measures, although universally considered highly effective in preventing caries, present some questionable aspects. Fluoride varnish, although responsible for a DMFT reduction of 43% (95% CI 30% to 57%; P < 0.0001) [Marinho et al., 2013], could also be considered a potential cause of excessive fluoride intake in children due to its high F ion content (about 22.000 ppm). Similar observations could be made about the application of professional fluoride gel which showed a significant reduction in caries by 28% (95% CI 19% to 36%; P < 0.0001); yet, like the varnish, it has a high fluoride content (about 12.000 ppm). The pit and fissures sealing procedure also presented clinical advantages in reducing caries (OR 0.12; 95% CI 0.08 to 0.19, 7) [Ahovuo-Saloranta et al., 2017], however its effectiveness could be improved by limiting treatment failures, of which 5-10% are caused by detachments [Feigal, 1998]. These issues might justify new prophylactic approaches to the prevention of caries.

In the last few years, lasers have been introduced in the dental clinical practice. Until now lasers have been used essentially within ablative parameters for caries removal with

interesting results, particularly in paediatric dentistry, due to its high level of acceptance by patients [Birardi et al., 2004]. However, another applicative field for lasers could be their use at sub-ablative prophylactic parameters to reinforce dental enamel against caries acid demineralisation. The purpose of this review was to perform an *in vitro* analysis concerning the effects of laser irradiation on enamel surfaces in terms of acid resistance as well as morphological and structural tissue modifications.

Methods

Study design

Systematic review of the literature. Inclusion criteria: *In vitro* studies dealing with lasers used only at sub-ablative energies; studies describing prevention of enamel acid dissolution and/or improvement of traditional caries prophylactic interventions; studies reporting structural or morphological enamel variations after laser irradiation; studies referring to potential side effects related to laser irradiation. Exclusion criteria: *In vivo* studies; studies describing dental tissues and/or caries removal; studies performed on decayed teeth; studies without statistically significant differences among interventions and both negative (no treatment) or positive (other comparator intervention) control groups.

A specific literature research strategy for this review was produced with the following key and/or MeSH terms: (Laser OR Er:YAG OR Nd:YAG OR CO_2 OR Er,Cr,:YSGG OR argon OR diode) AND (enamel demineralisation OR mineral loss). This research strategy was developed for the Medline database and subsequently revised for the following further two databases: Embase and the web Of Science. The reference lists of the included studies and chapters from books related to this topic were also evaluated. Two researchers independently performed their research. In the case of disagreement between the two researchers on the records chosen, it was resolved by means of a dialogue. After the aforementioned studies had been chosen, their data extraction was performed by other two researchers.

Primary outcomes

Enamel acid demineralisation, sealant retention, fluoride uptake, side effects.

Secondary outcomes

Morphological enamel variations, structural enamel variations.

Statistical analysis

We narratively described the results as they were reported in the included studies. We analysed the results sorted by outcome and type of laser used. For each comparison, we reported the p-values of the statistical tests used in each included study. The most common statistical test used was the one-way analysis of variance with repeated measures (RM-ANOVA) with Tukey's multiple comparisons post-test. Due to the existing differences between the included studies in the type of interventions, comparators, and outcome measures, as well as the lack of reporting of means and standard deviations, we were able to perform a quantitative synthesis of the results only for the comparison of the CO_2 laser used alone versus no treatment for the outcome Integrated Reflectivity with Lesion Depth (ΔR). The pooled estimate was obtained by using means and standard deviations with a random-effect model, inverse variance method. Heterogeneity between studies included in the meta-analysis was also investigated. We considered as statistically significant p-values < 0.05. All statistical tests were performed using the RevMan 5.3 software.

Results

After searching through the electronic databases and the other bibliographic sources, 347 records were found. After eliminating the duplicates, 157 of the records were chosen. Following evaluation, 38 full texts were assessed and 36 studies were included. Three of the included studies did not derive directly from the electronic databases but from the reference list of studies already chosen. The overall research procedure was reported using a flow chart (Fig. 1). The records were analysed for single outcomes as reported in the following passages.

Enamel acid demineralisation

CO_2 laser used alone.

All nine studies dealing with this outcome showed a positive result from the use of the CO_2 laser in reducing enamel demineralisation with statistically significant differences in comparison with no treatment in terms of enamel softening (p-values from 0.0001 to 0.05) [Correa-Alfonso et al., 2012 b; Souza-Gabriel et al., 2010], mean depth of the acid lesions (p< 0.05) [Kim et al., 2017; Chang et al., 2017; Lee et al., 2017; Correa-Alfonso et al., 2012 b], percentage of mineral loss (p-values ranging 0.002 to 0.04) [Rechmann et al., 2011; Esteves-Oliveira et al., 2017) and hydroxyapatite calcium acid dissolution (p-values ranging from 0.0001 to 0.679) [Mathew

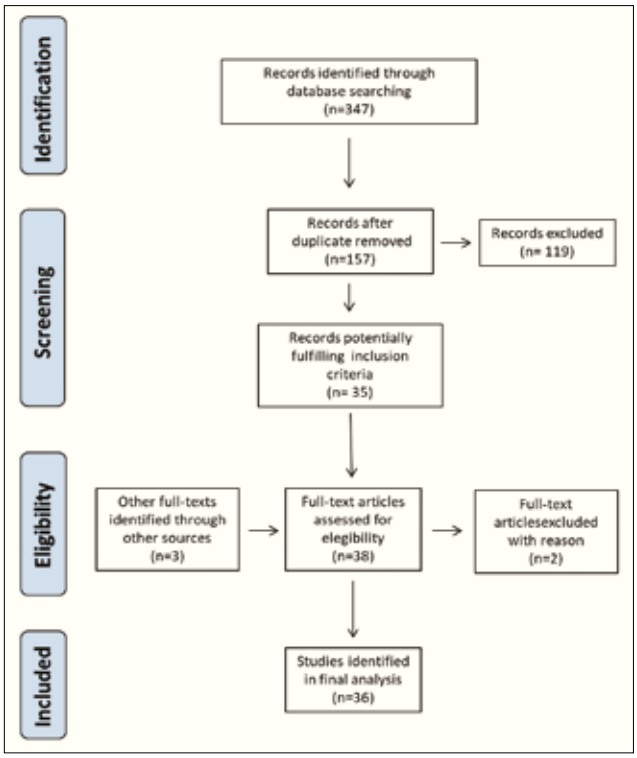


FIG. 1 Flow chart summarised the full search procedure of this literature review.

et al., 2014; Mirhashemi et al., 2016]. Particularly, when the parameter of Integrated Reflectivity with Lesion Depth (ΔR) was considered as a measure of enamel demineralisation, data was pooled with a meta-analysis procedure in three studies [Chang et al., 2017; Kim et al., 2017; Lee et al., 2017]. The pooled estimate (Fig. 2) showed a statistically significant reduction of ΔR (dB×μm) in the CO_2 laser group compared to the control group: the pooled mean difference of ΔR was -126.3, 95% CI -168.6 to -84.0 (p <0.001). The heterogeneity between studies was low (I2=33%; p =0.23).

CO_2 laser associated with other interventions.

When CO_2 lasers were used in association with 1.23% acidulated phosphate fluoride gel (APF) this improved enamel acid resistance considering parameters such as mean depth of acid induced lesions (p<0.05) [Chang et al., 2017; Kim et al., 2017; Lee et al., 2017] and calcium dissolution (p=0.0001) [Mathew et al., 2013]. Likewise, in another study, titanium tetra-fluoride (TiF4) gel in association with CO_2 laser reduced enamel demineralisation vs. no treatment (p-values ranging from 0.009 to 0.019) [Mirhashemi et al., 2016]. Moreover when a 5% fluoride varnish was combined with CO_2 laser there was a reduction in acid mineral loss in comparison with no treatment (p=0.025) [Esteves-Oliveira et al., 2017].

CO_2 laser compared with other fluoride interventions.

CO_2 lasers in comparison with 1.23% acidulated phosphate fluoride gel (APF) were more effective than the fluoride-based intervention with regards to parameters such as mean depth of the acid enamel lesions (p<0.05) [Kim et al., 2017; Lee et al., 2017], mineral loss (p = 0.0002) [Paulos et al., 2017], enamel softening (p <0.0001) [Souza-Gabriel et al., 2010], hydroxyapatite calcium dissolution [Mathew et al., 2013] and mean width of mineral loss areas [Seino et al., 2013]. Furthermore, where CO_2 lasers were compared with a 5% fluoride varnish, lasers were more effective than the fluoride-based intervention in reducing mineral loss when compared to no treatment (p<0.001) [Bahrololoomi et al., 2015; Noureldin et al., 2016]. In addition, when this combined intervention was repeated twice it was also more effective than the fluoride varnish used alone [Noureldin et al., 2016].

Er,Cr:YSGG laser used alone.

When the Er,Cr:YSGG laser was used at the highest sub-ablative fluencies (8.5-125 J/cm^2) it was effective in reducing the acid enamel softening (p-values ranging from 0.001 to 0.05) [de Oliveira et al., 2017; Geraldo-Martins et al., 2013; Ana et al., 2012; de Freitas et al., 2008] and the dissolution of hydroxyapatite ions such as calcium, phosphate (p< 0.01) [Baglar, 2014] and fluoride (p = 0.0119) [Ana et al., 2012]. Conversely, when the Er,Cr:YSGG laser was used at fluencies lower than 8.5 J/cm^2, negative or contrasting data were found regarding the effectiveness of the device in preventing acid demineralisation [Kumar et al., 2016; Ana et al., 2014; de Freitas et al., 2008]. Finally, one study described the Er,Cr:YSGG laser as ineffective in reducing the acid induced calcium dissolution. However, in this last study the laser beam fluency was not specified [Fekrazad and Ebrahimpour, 2014].

Er,Cr:YSGG laser associated with other interventions.

The Er,Cr:YSGG laser used in combination with other fluoride-based interventions (versus no treatment) was effective in preventing enamel demineralisation [Baglar, 2014; Ana et al., 2012]. Indeed, when lasers were combined with 1.23% acidulated phosphate fluoride gel or 5% fluoride varnish, the combined interventions were more effective than no treatment concerning parameters such as the loss of microhardness (p-values ranging from 0.01446 to 0.01) and calcium fluoride dissolution (p-values ranging from <0.01 to 0.0112) [Baglar, 2014; Ana et al., 2012].

Further considerations.

The Er,Cr:YSGG laser was more effective in preventing mineral loss when it was used without a water spray (p<0.05) [Geraldo-Martins et al., 2013]. Moreover, when the Er,Cr:YSGG laser was compared with the other fluoride-based interventions such as APF [Ana et al., 2012], 5% fluoride varnish [Baglar, 2014] and 2% NAF gel [de Freitas et al., 2008] no statistical difference in preventing enamel acid dissolution was found.

Three studies reported data on diode laser preventive treatment against acid-induced enamel lesions.

Diode laser used alone.

The diode laser used against enamel acid dissolution was more effective than no treatment [Chokhachi Zadeh Moghadam et al., 2018; Lacerda et al., 2014] in terms of loss of fluorescence as a parameter for a decrease in mineral loss (p<0.05) [Lacerda et al., 2014] and micro-hardness when the laser was used for at least four times consecutively (p = 0.008) [Chokhachi Zadeh Moghadam et al., 2018].

Diode laser associated with other interventions.

The diode laser was much more effective (versus no treatment) in preventing enamel demineralisation in terms of micro-hardness when combined with NaF light photo-adsorbing cream (p<0.05) [Lacerda et al., 2014] or with 5% fluoride varnish (P<0.001) [Bahrololoomi et al., 2015 b].

Diode laser compared with other interventions.

The diode laser compared with 5% fluoride varnish did not result in any difference in reducing the tendency of enamel to be softened after acid exposition (p-values ranging from 0.264 to 0.926) [Bahrololoomi et al., 2015b; Chokhachi Zadeh Moghadam et al., 2018].

Nd:YAG laser used alone.

Contrasting data on the effectiveness of the laser were found in the studies where the Nd:YAG laser was used. In fact in two studies, the Nd:YAG laser was described as completely incapable of improving enamel acid resistance when compared with no treatment (p>0.05) in terms of mean depth of acid-induced lesions [Chang et al., 2017] and percentage of mineral loss [Seino et al., 2013]. In two further studies, although the laser significantly reduced the mean depth of acid lesions (p< 0.05), it was not able to contain the enamel microhardness

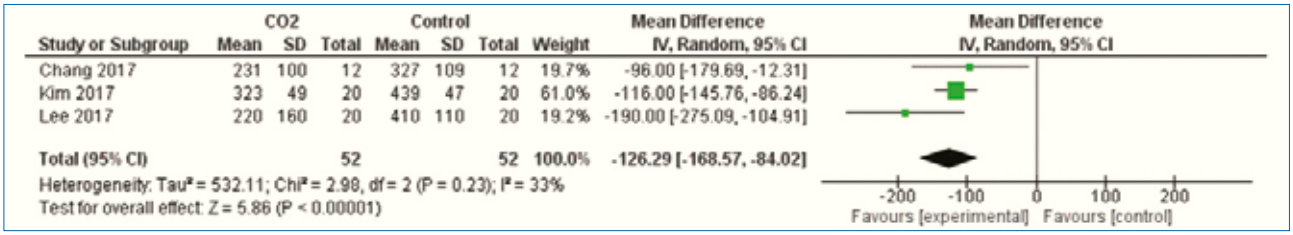


FIG. 2 Meta-analysis for the outcome enamel demineralisation measured by the Integrated Reflectivity with Lesion Depth (ΔR).

loss (p>0.05) [Azevedo et al., 2012; Correa-Alfonso et al., 2012 b]. In only one study, the Nd:YAG laser was found to be able to reduce the enamel softening parameter and mean depth of the mineral loss when the enamel was exposed to the acid stimuli (p<0.05) [Raucci Neto et al., 2014 a].

Nd:YAG laser associated with other interventions.

Similarly to the laser used alone, when this device was combined with other fluoride-based interventions, less than positive results were obtained. In two studies, the Nd:YAG laser was combined with a 5% fluoride varnish [Azevedo et al., 2012; Raucci Neto et al., 2014a]. In the first study this combined intervention did not hinder enamel softening and the mineral loss due to the tissue acid exposure (p>0.05) [Raucci Neto et al., 2014a]. In the second study, although the laser was able to reduce the mean depth of acid-induced lesions (p<0.05), it did not maintain this positive result in containing enamel softening after its acid exposure (p>0.05) [Azevedo et al., 2012]. Moreover, when the Nd:YAG laser was combined with a 1.23% acidulated fluoride phosphate gel (APF) no statistically significant difference with the controlled group (no treatment) was found (p>0.05) [Paulos et al., 2017].

Er:YAG laser used alone.

In the 6 studies which reported data about the Er:YAG laser preventive use against enamel acid demineralisation in comparison with no treatment contrasting data was found. In four studies, indeed, the Er:YAG laser was unable to improve the enamel acid resistance when parameters such as microhardness [Correa-Alfonso et al., 2012b; Fornaini el al, 2014], mean depth of acid lesions [Chang et al., 2017; Correa-Alfonso et al., 2012b] and hydroxyapatite calcium dissolution were considered (p > 0.05). Conversely, in the remaining two studies the Er:YAG laser was found to be able to prevent the enamel demineralisation. In one of these studies, in fact, the Er:YAG laser produced a 41% reduction in mineral loss (p< 0.001). In the other study the Er:YAG laser, used at 4 mm irradiation distance from the enamel surface with a 2mL/sec water cooling produced a significant reduction in the mean depth of enamel acid induced lesions (p<0.05) [Correa-Alfonso et al., 2010].

Er:YAG laser combined with other interventions.

The Er:YAG laser was effective in preventing enamel acid dissolution (versus no treatment) in terms of microhardness when combined with 5% fluoride varnish (p< 0.05) [Fornaini et al., 2014] as well as calcium dissolution when used with the 1.23% acidulated phosphate fluoride gel (APF) (p=0.0001) [Mathew et al., 2013]. Likewise, the laser when used with 2% NaF gel significantly reduced the acid-induced mineral loss versus no treatment (54%) and compared to this gel used alone (24%)(p< 0.001) [Liu et al., 2013]

Argon laser used alone.

Only one study dealt with the argon laser used alone. The laser irradiation significantly reduced the mean width of the acid enamel demineralisation when compared with no treatment or the Nd:YAG laser (p<0.05) [Tavares et al., 2012]. The argon laser produced a 61.2% reduction in demineralised areas compared to the control group [Tavares et al., 2012].

Fluoride uptake

There were two studies considering this outcome. Both studies indicated the laser as effective in improving the enamel fluoride uptake. In the first study the authors described both diode and CO_2 lasers (when combined with a 5% fluoride varnish) as more able than varnish used alone in inducing fluoride absorption into the enamel surfaces (P<0.001) [Bahrololoomi et al., 2015]. In the second study both diode at 7W and CO_2 at 1W lasers used in combination with an amine fluoride varnish (0.1 mg) improved the fluoride ion uptake than when this varnish was used alone (p<0.05) [González-Rodríguez et al., 2011].

Sealant retention

Three studies compared traditional acid etching with laser induced etching. In two of the studies (where the Er:YAG laser was used) the laser etching was comparable to the traditional acid etching in terms of sealant retention strength. These parameters were evaluated by measuring the sealant-enamel microleakage [Hossain et al., 2016] or the sealant penetration into the enamel pits and fissure [Markovic et al., 2010]. Moreover, where the Er:YAG laser was used in combination (before) with the traditional etching acids (such as 10% polyacrylic or 37% phosphoric acids) sealant retention was higher compared to that obtained with traditional acid etching only (p < 0.0001) [Markovic et al., 2010].

When a CO_2 laser etching was performed, also an increase in sealant retention was obtained in comparison with the traditional procedure ($p \leq 0.001$). This occurrence was particularly relevant when a very short laser pulse duration (3 μs) was adopted [Rechmann et al., 2017].

Side effects

Only one study reported information concerning this topic, describing the potential pulp damage due to excessive temperature increase during laser irradiation. In this study two types of lasers were described: The CO_2 laser used at 1 and 2 W and the diode laser at 5 and 7 W. The average temperature increase did not exceed 5.5°C which is considered harmful for pulpal vitality. Therefore, both lasers were considered as safe [González-Rodríguez et al., 2011].

Enamel structural changes

The most relevant structural changes involve the content of water inside the enamel and three hydroxyapatite components such as carbonate, phosphate and calcium ions. Both water and carbonate enamel content showed a significant decrease particularly when irradiated by the Nd:YAG laser (p<0.05) [Correa-Alfonso et al., 2015; Correa-Alfonso et al., 2012a]. A carbonate content reduction was found also when the Er:YAG laser was used (p<0.05) [Zamudio-Ortega, 2014]. Conversely, calcium and phosphate significantly increased their enamel content when irradiated by both the Er:YAG laser (p<0.05) [Zamudio-Ortega et al., 2014] and the Nd:YAG laser [Raucci Neto, 2014b] (p<0.05).In addition, the irradiated enamel presented a calcium phosphate ratio closer to the 1.67 ideal ratio than the untreated enamel [Zamudio-Ortega et al., 2014]. Also the hydroxyapatite stereoscopic structure was modified from the Er:YAG laser irradiation with a reduction in its transverse diameter (α-axis) varying from 0.004 to 0.011 Å [Liu et al., 2013b].

Enamel morphological changes

Regarding this outcome the CO_2 laser was the most described laser in literature with nine studies [Chang, 2017; Paulos, 2017; Mirhashemi, 2016; Correa-Alfonso, 2015; Seino, 2013; Correa-Alfonso, 2012a; González-Rodríguez, 2011; Correa-Alfonso, 2010; Souza-Gabriel, 2010]. With the exception of one study [Seino, 2013] in the remaining eight studies, although a laser was used at sub-ablative parameters, it was enough to melt enamel areas. Moreover, in two studies further lesions such as

cracks and/or craters were described in addition to the melted areas [Mirhashemi, 2016; González-Rodríguez, 2011]. In the only study dealing with the Er,Cr:YSGG, melted areas were also found [Kumar, 2016].

When the Nd:YAG laser was evaluated, conflicting data was found among five studies. Two studies indicated that the Nd:YAG laser irradiation resulted in melted areas [Correa-Alfonso et al., 2015; Wen et al., 2014; González-Rodríguez et al., 2011] while the other three studies described other enamel modifications such as an increased surface roughness and enamel areas showing prisms with a cone-like shape [Chang et al., 2017; Paulos et al., 2017]. Finally, in the four studies where the Er:YAG laser was adopted only increased enamel roughness or small abrasions were found without any evidence of melted areas [Chang et al., 2017; Zamudio-Ortega et al., 2014; Hossain et al., 2012; Correa-Alfonso et al., 2010] .

Discussion

In vitro laser irradiation on enamel dental surfaces, with the devices used at sub-ablative energy (lower than 100 J/cm^2), is a topic widely examined in literature. The first outcome evaluated in this review was the ability of lasers to reinforce enamel against acid-induced demineralisation. The most frequently described device was the CO_2 laser followed by the Er,Cr:YSGG, the Nd:YAG and Er:YAG lasers, while only a few papers dealt with diode and argon lasers. The best results against enamel acid dissolution were obtained using a CO_2 laser followed by Er,Cr:YSGG, diode and argon lasers. Infact, the CO_2 laser was the only laser which was able to improve the already positive prophylactic results obtained with the use of the fluoride-based interventions such as 5% fluoride varnish and 1.23% acidulated phosphate fluoride gel (p-values from <0.05 to <0.0001) [Kim et al., 2017; Lee et al., 2017; Paulos et al., 2017; Bahrololoomi et al., 2015; Mathew, 2013; Seino, 2013; Souza-Gabriel, 2010].

Positive results were also found when both Er,Cr:YSGG lasers (at fluencies higher than 8.5 J/cm^2) and diode lasers [Chokhachi Zadeh Moghadam et al., 2018; Geraldo-Martins et al., 2013; Ana et al., 2012; de Freitas, 2008] were used. These two types of laser, in fact, used alone or in combination with other fluoride-based interventions (i.e. 1.23% acidulated phosphate fluoride gel, 5% fluoride varnish or NaF photo-adsorbing cream) were significantly more effective than the control group (p-values from 0.001 to 0.05) [Chokhachi Zadeh Moghadam et al., 2018; Kumar et al., 2016; Baglar, 2014; Ana et al., 2012]. However, both lasers when used alone, in contrast with the CO_2 laser, did not improve the effectiveness shown by the fluoride interventions. The only study describing the argon laser shared similar positive prophylactic results with diode and Er,Cr:YSGG laser [Tavares et al., 2012]. Conversely, the Er:YAG laser when used alone was not effective in preventing enamel acid dissolution. However, this type of laser played an indirect role in preventing acid enamel damage as an alternative procedure to the traditional acid etching during teeth sealing treatments. Infact laser etching presented comparable results with the acid etching in terms of sealant retention but also more rapid and easier performances than traditional procedures. This feature could represent a relevant advantage when children with a high level of dental fear (10-20% of child population) and reduced compliance need to have sealing treatment [Cianetti et al., 2017; Paglia et al., 2017].

Another relevant outcome evaluated in this review was the fluoride intake on irradiated enamel surfaces. Both diode and CO_2 lasers were able to increase the absorption of the fluoride contained in the gels or varnishes (p-values from <0.001 to <0.05) [Bahrololoomi et al., 2015; González-Rodríguez et al., 2011]. This increased fluoride intake might represent an advantage in reducing caries prevalence considering the previously demonstrated prophylactic features of fluoride-based interventions [Marinh, 2013, 2015].

The last primary outcome analysed was the side effects resulting from low energy laser irradiation. The only study dealing with this outcome speculated that the potential pulp damage was due to the increase in enamel temperature after laser irradiation. In this study the use of CO_2 and diode lasers resulted as safe because they were unable to raise the dental pulp temperature over the critical value of 5.5°C from the basal temperature [González-Rodríguez et al., 2011].

This review also dealt with the morphological and structural enamel variation related to laser energy absorption into dental surfaces. Concerning the structural enamel modifications Er:YAG and Nd:YAG lasers were the most commonly used devices in the included studies. The modifications to enamel structure were due to the instantaneous and microstratified increase of enamel temperature from 100 to 1600°C [Fowler and Kuroda, 1986]. These parameters produced a reduction in water and carbonate enamel combined with an increase in calcium and phosphate tissue. In addition, the calcium phosphate ratio became closer to the 1.67 ideal ratio than non-irradiated enamel. Also the enamel prisms' tridimensional structure was modified after the Er:YAG laser irradiation, producing a reduction in their diameter. This data represents a further indirect support for a potential use of lasers against acid lesions. The lower enamel carbonate content, indeed, increased the hydroxyapatite crystallinity with a related improvement of the mechanical features [Xu et al., 2012] as well as an increased resistance to the caries which was characterised by the presence of an enamel rich in carbonate-substituted hydroxyapatite crystals [Seredin et al., 2015]. Furthermore, the laser-induced reduction of water molecules surrounding dental prisms (which occur in the enamel of elderly individuals) reduces the enamel permeability, and consequently the risk of caries [Kunin and Evdokimova, 2015]. Finally, the irradiated enamel has a calcium/phosphate ratio closer to the ideal ratio, a condition that increases the resistance of hydroxyapatite to acid demineralisation [Kunin and Evdokimova, 2015].

Moreover, when morphological enamel modifications due to laser irradiation were evaluated, evidence of enamel alterations were always described even though low energy levels were adopted in the studies. Lesions such as enamel melting areas were observed particularly when the CO_2 laser was used. Also Er,Cr:YSGG [Kumar et al., 2016] and Nd:YAG [Correa-Alfonso et al., 2015; Wen et al., 2014; González-Rodríguez et al., 2011] were described as being capable of generating enamel melting. Conversely, the Er:YAG was the only type of laser that was found not to produce melting areas [Chang, 2017; Zamudio-Ortega, 2014; Hossain, 2012]. Other morphological modifications such as an increased surface roughness and enamel prisms cone-like shape were also observed after laser use [Chang, 2017; Paulos, 2017; Zamudio-Ortega, 2014; Hossain, 2012].

The positive prophylactic action of a laser shown from the *in vitro* studies reported in this review was confirmed also by

data found in several clinical trials (*in vivo*) carried out over the past years. Lasers, therefore, also *in vivo* (tailored at sub-ablative energies) when used alone or combined with others fluoride-based interventions improved enamel acid resistance with a caries incidence reduction. Infact, in one study the laser used alone determined a significant caries decrease of 70% when compared with untreated teeth [RR= 0.30; (95% CI, 0.11-0.78), p=0.004] [Raucci Neto et al., 2015]. In another study the laser used in combination with a fluoride gel (1.23% acidulated phosphate fluoride gel) increased acid resistance than when only gel was used, with a caries decrease of 61% [RR=0.39 (95% CI, 0.22-0.71), p=0.001] [Zezell, 2019]. Likewise, also during pit and fissure sealing Er:YAG laser etching used in addition to traditional acid etching (35% phosphoric acid) was found to have a higher capability (56%) of reducing caries incidence than acid etching alone [RR=0.44 (95% CI, 0.20-0.97), p=0.03]. Moreover a significant reduction of sealant detachment was recorded when the combined etching procedure was adopted [RR=0.54 (95% CI, 0.34-0.87); p=0.01] [Durmus, 2017]. In addition the argon laser was found to be able to improve fluoride intake on enamel surfaces. In fact, this type of laser used in association with APF 1.25% gel determined a higher intake of fluoride than the gel used alone (ANOVA tests 95%, P<0.0001) [Nammour et al., 2013, 2015].

Laser treatment, as shown in the above mentioned *in vitro* studies, seems to be an interesting procedure which can share its clinical applications with other innovative materials (i.e modified glass ionomer cements) or devices (i.e. sonic-subsonic tips) for the treatment of specific patients with reduced compliance such as younger or highly fearful children [Pagano et al., 2019; Cianetti et al., 2018; Chieruzzi et al., 2018].

Conclusion

The CO_2 laser, among those described in the literature, was the most effective device in reducing acid enamel demineralisation *in vitro*. This type of laser was also able to improve fluoride-based interventions. The following further devices such as the Er,Cr:YSGG, diode and argon lasers presented significant positive abilities in preventing enamel acid lesions. During dental sealing procedures also the Er:YAG laser was considered to be an interesting device for its effectiveness in performing a valid enamel etching (alternative to the traditional acid etching) particularly well accepted by the younger patients for its clinical procedural ease. The only study dealing with safety did not report any pulpal damage when used at sub-ablative energies.

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