



In vitro study of high-power Er: YAG laser parameters for the management of dentin hypersensitivity in hypomineralized permanent teeth

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Abstract

Dentin hypersensitivity (DH) is characterized by intense, short-lasting pain, triggered by dentin exposure to different stimuli. It is often associated with hypomineralized teeth due to increased enamel porosity and exposed dentinal tubules. The high-power Er: YAG laser has emerged as a promising therapeutic strategy for the treatment of this condition. The aim of this in vitro study was to test different protocols for managing dentin hypersensitivity in hypomineralized teeth using a high-power Er: YAG laser. Six protocols were evaluated, varying in power, frequency, application mode, and the use of cooling. Qualitative analysis of morphological changes were evaluated by scanning electron microscopy (SEM). Further, the most promising protocol – 20 mJ, 10 Hz, 0.20 watts, applied in focused mode, without cooling – was selected for assessing intrapulpal temperature variation using thermocouples connected to a monitoring system. The SEM images showed that this protocol promoted homogeneous surface melting of dentin, effectively sealing the dentinal tubules without inducing a thermal increase greater than 2 °C. These findings support the feasibility of using the Er: YAG laser with conservative parameters and no cooling as a safe and effective alternative for the management of DH in hypomineralized teeth. However, clinical trials are recommended to validate its applicability in pediatric dentistry.

Keywords Er:YAG laser · Dentin hypersensitivity · Hypomineralization · Scanning electron microscopy · Intrapulpal temperature

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Introduction

Dentin hypersensitivity (DH) can be defined as a short, sharp pain resulting from dentin exposure in response to thermal, tactile, osmotic, or chemical stimuli, which is not associated with any other dental pathology [1–3]. It is one of the most common causes of dental pain encountered in clinical practice, and although its pathophysiology is not yet fully understood, the hydrodynamic theory [4] is the most widely accepted. This theory suggests that external stimuli causes fluid movement within the dentinal tubules, activating nerve endings at the dentin–pulp interface, which can result in pain and discomfort [4]. Despite its high prevalence, effective and lasting management of DH remains a clinical challenge, especially in cases involving structurally compromised teeth [5].

DH is frequently observed in hypomineralized teeth, a condition characterized by qualitative enamel defects, including reduced mineral content and quality, increased porosity, and decreased mechanical resistance [6]. In addition to the dentinal tubules being more susceptible to external stimuli, microorganisms may infiltrate the porous enamel and reach dentinal tubules, leading to subclinical inflammatory responses in pulpal cells, thereby contributing to hypersensitivity in hypomineralized teeth [7]. In children, the pain caused by dentin hypersensitivity can compromise essential functions such as eating and oral hygiene, negatively affecting quality of life [8].

Various therapeutic approaches have been proposed for the management of DH, aiming to block or narrow the dentinal tubules, thereby reducing their permeability and/or modulating pulpal inflammation. These approaches include desensitizing agents, fluorides, adhesives, sealants, and laser therapy [9–11]. High-power lasers are indicated for the treatment of dentin hypersensitivity due to its ability to promote superficial structural modifications on the exposed dentin surface, including partial melting and recrystallization of the mineral matrix - a phenomenon known as “melting” - which leads to the obliteration of dentinal tubules [11–13]. Currently, lasers used for this purpose include diode lasers (semiconductors), CO₂, Nd: YAG, Er: YAG, and Er, Cr: YSGG lasers [11–14].

The Er: YAG laser, in particular, has gained prominence in the dental market due to its wide range of clinical indications [15] and its ability to reduce pain in a minimally invasive manner [16]. In addition to its use in the management of DH, the Er: YAG laser has also been studied for the prevention of dental caries, based on its ability to alter the surface morphology of enamel and dentin, thereby reducing acid solubility and increasing resistance to demineralization.

Preventive protocols based on its application have shown promising results, especially on surfaces with poor-quality enamel, as is often the case in hypomineralized teeth [17, 18]. These properties make the Er: YAG laser a potentially effective tool both for managing hypersensitivity and for preventing secondary complications in teeth affected by Molar-Incisor Hypomineralization (MIH).

Despite the promising features of the Er: YAG laser, there remains a significant gap in the literature regarding standardized and safe protocols, specifically targeting dentin hypersensitivity (DH) in hypomineralized teeth. Although the erbium laser is widely marketed and available for clinical use, there is a lack of robust scientific evidence supporting its safe and effective application for this indication. To date, only one randomized clinical trial has addressed its use in children with MIH, without establishing clear and standardized clinical guidelines [19]. The absence of consensus and limited available evidence pose a considerable challenge for clinicians seeking reliable, effective, and long-lasting treatments for hypersensitivity in structurally compromised hypomineralized teeth.

The present *in vitro* study aims to address this clinical gap by evaluating the effects of various Er: YAG laser irradiation parameters on hypomineralized permanent molars. The proposed protocol is distinguished by its conservative approach, specifically designed to preserve the structurally compromised dental tissues commonly observed in pediatric patients with enamel hypomineralization. Through a systematic assessment of morphological alterations and safety indicators, this research aims to establish a robust scientific basis for the development of effective, safe, and evidence-based clinical protocols, thereby contributing to improved management of dentin hypersensitivity in children with MIH.

Objective

The objective of this *in vitro* study was to test different high-power Er: YAG laser protocols for the management of dentin hypersensitivity in hypomineralized teeth.

Materials and methods

This study was approved by the Research Ethics Committee of the University of São Paulo School of Dentistry (approval number 5.994.828), and the teeth used were donated by the Biobank of the School of Dentistry at USP. The study was conducted and reported in accordance with the guidelines of the Checklist for Reporting *In Vitro* Studies (CRIS).

Experimental design

Twelve extracted human molars with hypomineralization were selected. The inclusion criteria were permanent human molars exhibiting enamel hypomineralization, characterized by white-cream or yellow-brown opacities, and with no signs of caries or prior restorative treatments. All teeth were thoroughly examined using a stereoscopic magnifying glass to identify and exclude any specimens presenting enamel fractures or cracks, restorations, or carious lesions. The study was carried out in two steps: (I) Testing of six different irradiation parameter protocols (with the variables: energy, power, repetition rate, and cooling) on extracted hypomineralized human teeth, and (II) Temperature variation testing within the pulp, as illustrated in the Flowchart (Fig. 1).

The qualitative response variable was the ability to cause surface melting of the irradiated dental surface, analyzed through micrographs obtained by scanning electron microscopy (SEM) in step I. The quantitative response variable was the intrapulpal temperature variation during irradiation, analyzed during the test in step II.

It is important to highlight that the teeth used in this study were obtained from a certified institutional Biobank, where samples are donated and stored anonymously for research purposes, making it impossible to identify the donors' age or confirm their pediatric origin. Molar incisor hypomineralization (MIH) is currently diagnosed predominantly in the

pediatric population, and this study was designed considering the clinical relevance of this condition in pediatric dentistry. Conservative Er: YAG laser parameters were chosen to simulate safe conditions for young permanent teeth, characterized by large pulp chambers and possible root immaturity. Nevertheless, it should be emphasized that the findings of this study may be extrapolated to hypomineralized permanent teeth in adult populations.

Step I - Irradiation parameter testing on hypomineralized human teeth

Tooth selection and specimen preparation In Stage I, six extracted human molars with white-cream or yellow-brown opacities on the buccal and palatal/lingual surfaces, suggestive of hypomineralization, without fractures, cracks, restorations, or associated caries lesions, were selected and cleaned with periodontal cures, polished with Robinson brushes mounted on low-speed handpieces, and soaked in pumice stone and water. The teeth were obtained from an institutional biobank, where they are anonymized and stored in deionized water under controlled conditions and refrigerated at 4 °C until use. The crowns were separated from the roots. The crowns were sectioned in half through the mesio-distal sulcus using a carborundum disc (22 × 0.12 mm – American Burrs), resulting in twelve samples.

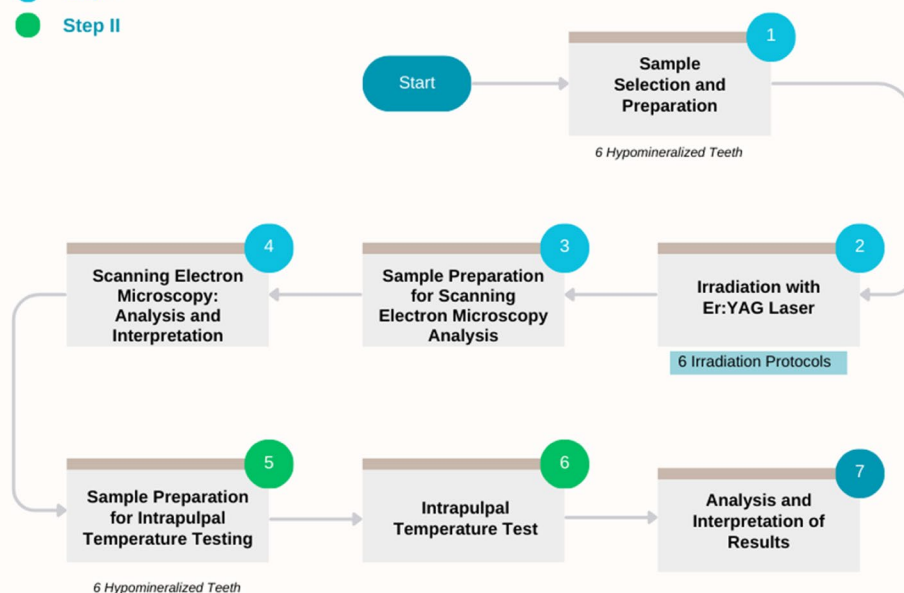
The samples were irradiated with the high-power Er: YAG laser (LiteTouch, Light Instruments, Israel – FAPESP

Fig. 1 Flowchart of the experimental steps

Flowchart of Experimental Steps

● Step I

● Step II



Process 2018/21696-7), with a wavelength of 2940 nm, using an AS7066X sapphire tip (1.3×14 mm), in pulsed mode with a pulse duration of approximately 100 to 400 microseconds, for 20 s, with linear movements in both vertical and horizontal directions at a speed of 2 to 4 mm/s. The irradiated area corresponded to the demarcated opacity on each sample.

Six irradiation protocols were tested, with two samples in each group ($n=2$). In Groups 1 and 2, the protocols suggested by the manufacturer (LiteTouch – Er: YAG), specifically for the treatment of dentin hypersensitivity, were applied. These parameters are pre-programmed in the device's software as clinical recommendations. The settings included an energy of 20 mJ and a repetition rate of 20 Hz, resulting in a power output of 0.40 watts (W). The distinction between the two groups lies in the application mode: Group 1 employed the focused mode, with the sapphire tip positioned approximately 0.8 to 1.0 mm from the surface, whereas Group 2 used the defocused mode, with the tip positioned at 5.0 mm.

In Groups 3, 4, 5, and 6, experimental variations were introduced to assess the influence of power output, repetition rate, application mode, and the presence or absence of water cooling on surface morphology and intrapulpal temperature variation. These protocols were designed to simulate a variety of potential clinical scenarios and to allow for isolated analysis of each variable.

Group 3 employed a power setting of 0.20 W, with an energy of 20 mJ and a repetition rate of 10 Hz. Irradiation was performed in focused mode, with the tip positioned approximately 0.8 to 1.0 mm from the surface, and with active water cooling at a flow rate of 60 mL/min. Group 4 used the same laser parameters—0.20 W, 20 mJ, 10 Hz—but in defocused mode, with the tip positioned 5.0 mm from the surface, also with active cooling. Group 5 utilized a power output of 0.20 W (20 mJ, 10 Hz) in focused mode (0.8–1.0 mm), but without water cooling. Group 6 followed the same energy and frequency settings as Group 5 (0.20 W, 20 mJ, 10 Hz) but with defocused application (5.0 mm) and no cooling. The complete set of parameters used in each

group is summarized in Table 1 and was carefully selected to ensure precise control over the experimental variables.

After irradiation, the samples were separately stored in a sealed container with distilled water at room temperature (20 °C) for 24 h.

Specimen processing for SEM The specimens were dehydrated through a graded ethanol series with immersion times of 5 min each of 30%, 50%, 70%, 80%, 90%, and 95% ethanol, followed by two immersions in absolute ethanol for 10 min each. Subsequently, specimens were air-dried, mounted on aluminum stubs using a colloidal silver adhesive, and sputter-coated with gold using a Balzers SDC-050 apparatus (Bal-Tec AG, Liechtenstein). The samples were then examined under a Leo 430i scanning electron microscope.

SEM analysis - image acquisition The samples were examined in the LEO 430i scanning electron microscope (operating to 10–15 kV) and then representative images were acquired at various magnifications (50x, 100x, 350x, 1000x, 1500x, 2500x). The morphological changes induced by the treatment on the enamel surfaces were ultrastructurally analyzed.

Upon analyzing the micrographs, the 20 mJ, 10 Hz, 0.20 W protocol was the most effective in modifying the exposed surface structure, including partial fusion and recrystallization of the mineral matrix. The absence of water irrigation resulted in a greater manifestation of this effect. However, the use of cooling during irradiation mitigates the temperature rise in the tissues, minimizing the risk of thermally induced pulp injury. Therefore, a test was necessary to assess whether treatment without cooling could cause thermal damage to the tissues.

Step II - Temperature test

Selection of teeth and specimen preparation Six human extracted molars with white-cream or yellow-brown opacities, suggestive of hypomineralization, without fractures,

Table 1 Irradiation parameter protocols

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5	GROUP 6
Power	0.40 W		0.20 W			
Energy	20 mJ					
Repetition Rate	20 Hz		10 Hz			
Beam Area	0.01326 cm ²	0.0415 cm ²	0.01326 cm ²	0.0415 cm ²	0.01326 cm ²	0.0415 cm ²
Energy Density	1.508 J/cm ²	0.482 J/cm ²	1.508 J/cm ²	0.482 J/cm ²	1.508 J/cm ²	0.482 J/cm ²
Power Density	30.15 W/cm ²	9.64 W/cm ²	15.08 W/cm ²	4.81 W/cm ²	15.08 W/cm ²	4.81 W/cm ²
Cooling	With cooling (60 ml/min)			Without cooling		
Application Mode	Focused (0.8–1.0 mm)	Defocused (5.0 mm)	Focused (0.8–1.0 mm)	Defocused (5.0 mm)	Focused (0.8–1.0 mm)	Defocused (5.0 mm)

*Only one repetition per condition was performed ($n=2$ per group)

Fig. 2 Thermocouples positioned in the pulp chamber of the dental elements

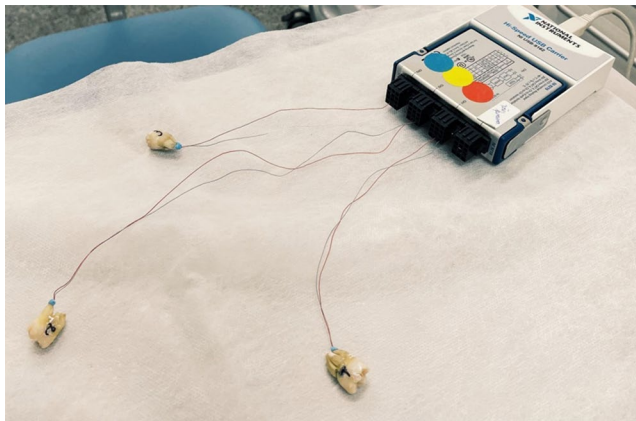
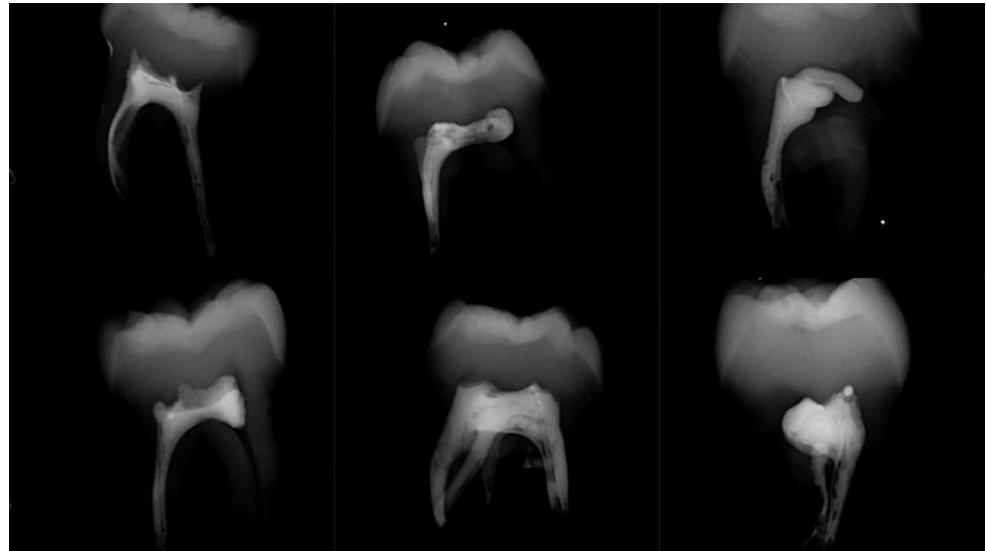


Fig. 3 Thermocouples connected to the temperature monitoring system

cracks, restorations, or associated caries lesions, were selected and then cleaned with periodontal curettes, polished with Robinson brushes mounted on low-speed handpieces, and immersed in pumice and water. They were then washed and kept in distilled water at room temperature.

The pulp chambers of the teeth were accessed through retro-instrumentation via the apical foramina. These were opened with a Gates-Glidden rotary instrument, and the removal of pulp remnants and cleaning of the root canals and pulp chambers was performed using K-type endodontic files #40 with the aid of distilled water.

Through the apical opening, a thermally conductive paste based on water (Implastec, Votorantim, Brazil), with a thermal conductivity equivalent to $0.4 \text{ cal s}^{-1} \text{ m}^{-1} \text{ K}^{-1}$, was introduced using disposable syringes, which allowed filling of the pulp chamber and root canals of the teeth. Then, K-type thermocouples (chromel-alumel – NiCr-NiAl,

Omega Eng. Inc., Stamford, USA), $127 \mu\text{m}$ in thickness with a resolution of $0.2 \text{ }^\circ\text{C}$, were placed so that their ends coincided with the ceiling of the pulp chamber, which was monitored radiographically (Fig. 2). The apical portion of the roots was sealed with a gingival barrier (Top Dam, FGM, SP, Brazil), which was photoactivated for 40 s (Opti-light Plus, Gnatus, SP, Brazil) to prevent the entry of water into the root canals.

The thermocouple wires were connected to a temperature monitoring system consisting of an amplifier, a signal converter, a recorder with a time resolution of 0.05 s (Lock-in Amplifier SR510, Stamford Research Systems, CA, USA), and a computer (Fig. 3).

Irradiation and data collection The irradiated area corresponded to the marked opacity of each hypomineralized tooth, covering the occlusal, buccal, and palatal/lingual surfaces, simulating a clinical procedure. Each tooth was irradiated using the high-power Er: YAG laser (LiteTouch, Light Instruments, Israel), with a sapphire tip AS7066X of $1.3 \times 14 \text{ mm}$, with the same parameters of energy, repetition rate, and power (20 mJ, 10 Hz, 0.20 W), for 20 s, without cooling. The experiments were conducted at room temperature ($20 \text{ }^\circ\text{C}$). The measured temperature was recorded before, during, and after the irradiation, but only the temperature variation during the irradiation time of each tooth was considered.

Statistical analysis For the quantitative analysis of temperature variation data, the software Origin 2022b (64-bit) SR1, version 9.9.5.171 (Academic) was used. The Shapiro-Wilk normality test showed that the data was non-parametric. Therefore, the Mann-Whitney test was applied to compare

the samples in pairs. All analyses were performed with a significance level of $\alpha=0.05$.

Results

Step I - SEM qualitative analysis of the tested irradiation parameters

The morphological analysis of the surfaces irradiated with the Er: YAG laser, using scanning electron microscopy (SEM), revealed significant variations between the groups, related to the modulation of parameters such as frequency, energy, application mode, and the presence or absence of cooling. Representative images at two magnification levels allowed the identification of distinct patterns of surface alteration, indicative of thermal, ablative, and mineral fusion effects, as described below and summarized in Table 2. Figure 4 depicts the surface of a hypomineralized enamel sample that was not subjected to Er: YAG laser irradiation or any other treatment, serving as a reference for comparison with the treated teeth.

Group 1–20 mJ, 20 Hz, 0.40 W, focused mode, with cooling

The surface showed localized signs of mineral matrix fusion, characterized by smooth and continuous areas, compatible with superficial melting. The application appeared irregular, suggesting instability in the manual movement or focusing, but without evidence of carbonization or thermal damage to the adjacent enamel, which remained morphologically intact.

Group 2–20 mJ, 20 Hz, 0.40 W, defocused, with cooling

A pronounced surface roughness was observed, with the presence of micro-explosions, micro-cracks, and areas of superficial fracture. The beam dispersion in defocused mode contributed to a more heterogeneous pattern, with irregular superficial loss and crater formation. At higher magnification, zones of melting alternate with disorganized regions, compatible with mild ablative effects, but with low morphological predictability.

Group 3–20 mJ, 10 Hz, 0.20 W, focused mode, with cooling

This group presented a highly heterogeneous topography, with alternating areas of melting and partial preservation of the tubular architecture. Dentinal tubules appeared

obliterated or transversely cut. The reduction in frequency and power promoted less aggressiveness, with selective thermal action and no evidence of carbonization. The pattern suggests the beginning of the occlusive process of the tubules, which is important in therapeutic approaches for dentin hypersensitivity.

Group 4–20 mJ, 10 Hz, 0.20 W, defocused, with cooling

The surface exhibited a more homogeneous morphology compared to the previous groups, with an intermediate texture between melting and ablation. Partial exposure of the dentinal tubules was observed, indicative of mild ablation of the superficial layer, without deep removal. The laser beam diffusion in defocused mode, combined with the lower frequency, favored a transitional pattern.

Group 5–20 mJ, 10 Hz, 0.20 W, focused mode, without cooling

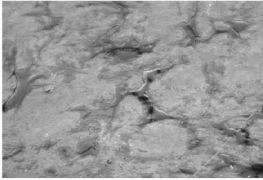
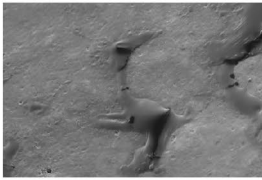
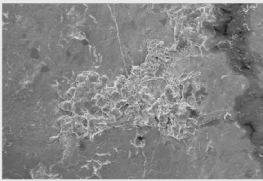
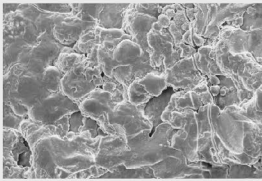
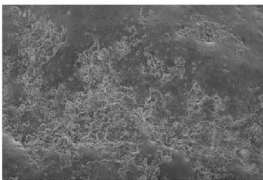
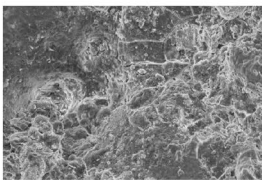
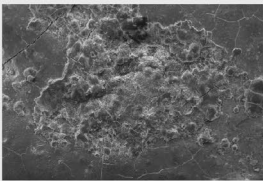
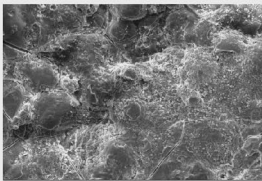
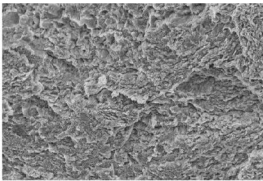
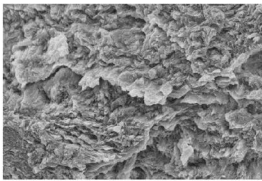
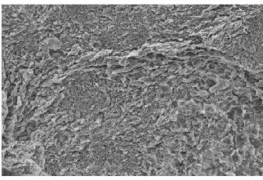
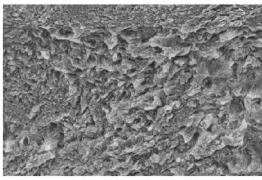
In this group, the absence of cooling promoted more pronounced and continuous melting of the surface. The visualization of enamel prisms was impaired, indicating fusion of the mineral matrix and absence of distinct prismatic structures. The intense thermal action created a vitreous and compacted surface, compatible with the occlusion of dentinal tubules and the formation of a protective layer, potentially useful in dentin desensitization protocols.

Group 6–20 mJ, 10 Hz, 0.20 W, defocused, without cooling

The surface revealed a homogeneous and well-distributed melting pattern, without zones of explosion or cracks. General morphology was similar to that of Group 5. The vitrified surface, without the visualization of prisms, suggests favorable action for sealing dentinal tubules with minimal risk of microfractures.

Furthermore, the micrographs revealed the structure of the hypomineralized enamel surface - characterized by disorganized enamel - areas with exposed and irregular dentinal tubules. In one hypomineralized area, bacterial accumulation was observed, suggesting that hypomineralized enamel is more porous and consequently more likely to retain microorganisms, which is an important factor for the risk of caries and contributes to the hypersensitivity of hypomineralized teeth (Fig. 5).

Table 2 Description of the micrographs regarding the enamel structure alterations after irradiation

Group	Protocol	Lower magnification micrograph (At 100x magnification)	Higher magnification micrograph (At 350x magnification)	Interpretation
1	20mJ, 20Hz, 0.40W, focused, with cooling			Clear evidence of superficial fusion of the mineral matrix at the application points (irregular application). No damage to the adjacent enamel surface (intact surface).
2	20mJ, 20Hz, 0.40W, defocused, with cooling			Presence of micro explosions and cracks, more evident at lower magnification. Surface roughness and micro irregularities, consistent with mild ablative action. At high magnification, signs of surface melting are observable.
3	20mJ, 10Hz, 0.20W, focused, with cooling			Heterogeneous surface alteration with areas of melting alternated with partially open or cut tubules.
4	20mJ, 10Hz, 0.20W, defocused, with cooling			The surface is more homogeneous, but still with areas of fusion and partial tubular exposure. The surface is in transition between melting and ablation.
5	20mJ, 10Hz, 0.20W, focused, without cooling			The enamel prisms are not easily observed; melting appearance.
6	20mJ, 10Hz, 0.20W, defocused, without cooling			The enamel prisms are not easily observed; the surface shows a regular and homogeneous melting appearance.

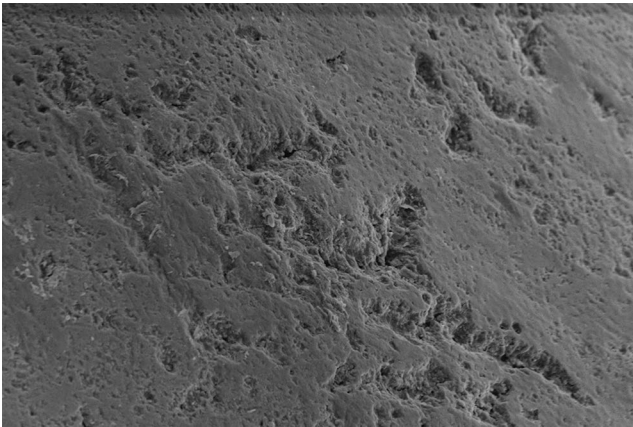


Fig. 4 At 350x magnification, a hypomineralized tooth not subjected to irradiation or any other treatment is observed

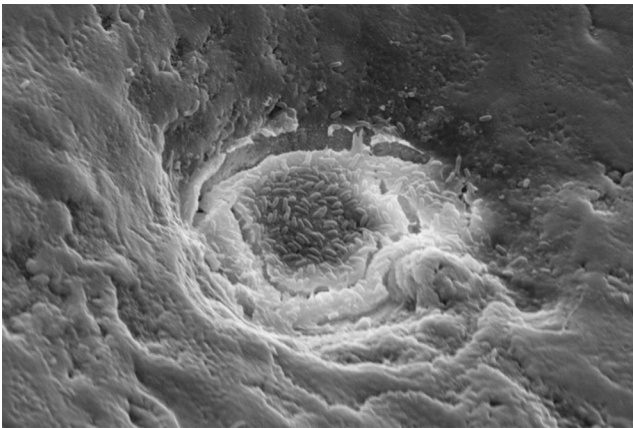


Fig. 5 At 2500x magnification, a hypomineralized enamel area where bacterial accumulation is present

Step II - Temperature test

Table 3 presents the median, maximum, and minimum temperature values, as well as the temperature range (difference between maximum and minimum values) recorded for each sample during irradiation. The minimum temperature values reflects the initial room temperature at the time of each measurement.

According to the Mann-Whitney test, there was a difference between all the samples, except for samples 2

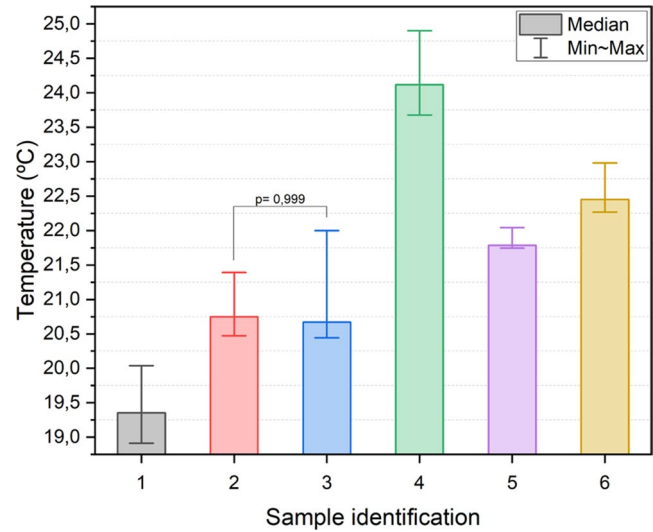


Fig. 6 Graph of the median, maximum, and minimum values for each sample

and 3, where the p-value was equal to 0.999, as shown in graph (Fig. 6).

None of the samples showed a temperature variation greater than 2 °C during the irradiation time.

Discussion

In this in vitro study, the Er: YAG laser with irradiation parameters of 20 mJ, 10 Hz, 0.20 W, applied in focused mode, without cooling, proved to be effective in sealing dentinal tubules in hypomineralized teeth, a phenomenon known as dentin melting, suggesting it is a promising treatment for dentin hypersensitivity.

These findings are consistent with previous in vitro studies [11, 22], although the parameters used in the present study differed from those employed earlier. Zhuang et al. (2021) [11] found success in obliterating dentinal tubules with the 50 mJ, 10 Hz parameter. Belal and Yassin (2014) [20] observed, in SEM analysis, areas of melting around exposed dentinal tubules and greater success in treatment with the Er: YAG laser at 40 mJ and 10 Hz, compared to other groups.

Table 3 Median, maximum, and minimum values, as well as the temperature range (maximum-minimum) for each sample

Sample	Minimum (°C)	Median	Maximum (°C)	Delta (°C)
1	18.913	19.355	20.038	1.125
2	20.471	20.750	21.391	0.920
3	20.442	20.670	22.000	1.558
4	23.678	24.117	24.903	1.225
5	21.744	21.784	22.043	0.299
6	22.266	22.452	22.980	0.714

The only randomized clinical trial that used the Er: YAG laser for the treatment of hypersensitivity in children with hypomineralization applied the 40 mJ, 10 Hz protocol and achieved success in its objectives, reducing dentin hypersensitivity and improving oral health-related quality of life [19].

The Er: YAG laser (2940 nm) is not the first-choice high-power laser for the treatment of dentin hypersensitivity, given high affinity for water and strong ablation capacity on hard tissues, which makes it more suitable for cavity preparation procedures [21]. Its mechanism of action involves the absorption of thermal energy by tissues at this wavelength, causing the phenomenon of surface ablation, which is the opposite of sealing dentinal tubules. However, in the present study, as well as in previous research [11, 19], the use of low irradiation parameters promoted surface melting instead.

In addition to the Er: YAG laser, other laser systems have been investigated for the treatment of dentin hypersensitivity (DH), each with distinct action mechanisms and clinical outcomes. Systematic reviews and meta-analyses have highlighted the efficacy of CO₂ and diode lasers with various wavelengths in reducing DH symptoms through different approaches, such as tubule occlusion, nerve desensitization, and biostimulation [22, 23]. CO₂ lasers, characterized by high absorption in water and hydroxyapatite, promote superficial melting and recrystallization similar to Er: YAG but with different penetration depths, providing effective tubule sealing. Diode lasers, commonly used at wavelengths between 660 and 980 nm, act mainly by modulating nerve responses and stimulating secondary dentin formation rather than inducing significant morphological changes on dentin surface. The choice of laser system should consider tissue interaction, patient comfort, and clinical practicality. While Er: YAG offers precise ablation and controlled morphological effects, CO₂ and diode lasers present alternative or complementary options, expanding the therapeutic arsenal for DH management [22, 23].

Although the clinical motivation of this study centers on managing dentin hypersensitivity in children with enamel hypomineralization, the teeth used were obtained from an institutional biobank and are anonymized, preventing identification of donor age or clinical diagnosis. Molar-Incisor Hypomineralization (MIH) is a developmental enamel defect affecting one to four permanent first molars, sometimes associated with affected permanent incisors [24]. This condition is prevalent in the pediatric population and carries significant clinical repercussions: children with MIH are up to three times more likely to seek dental care due to pain and six times more likely due to dentin hypersensitivity compared to unaffected children [25]. For this reason, despite the inability to confirm an MIH diagnosis in the specimens, the term “hypomineralized permanent molars” was used

since the teeth exhibited morphology compatible with this defect. This approach respects the methodological limitations of the study while allowing the findings to be interpreted within the pediatric clinical context that motivated the research, without excluding the possibility of extrapolating results to managing dentin hypersensitivity in adults with hypomineralized enamel.

The Er: YAG laser configurations used in dentin desensitization protocols are mostly based on applying low energies and frequencies, aiming to promote superficial morphological changes that reduce tubule permeability without compromising pulp vitality or causing discomfort to the patient [11]. In the context of this study, which focuses on children with enamel hypomineralization as the target population, it was crucial to consider both the structural fragility of the tissue and the susceptibility to pain and anxiety associated with dental treatment. For this reason, even more conservative parameters were chosen compared to those frequently reported in the literature for adults with dentin exposure [12, 14].

Additionally, there is growing evidence that low-energy Er: YAG laser protocols are effective in inducing physico-chemical changes in enamel, such as recrystallization and increased acid resistance, making them promising not only for the treatment of hypersensitivity but also as a preventive measure against dental caries [17, 26]. These effects result from the partial fusion of the mineral matrix and subsequent surface restructuring, leading to a barrier more resistant to the diffusion of ions and cariogenic agents [26].

Areas of enamel prism exposure and dentin tubule openings, characteristics indicative of ablation, were found more frequently in protocols that used higher power and higher repetition rates. This finding corroborates the literature and can be explained because higher powers generally cause faster evaporation of water, resulting in micro-explosions on the irradiated surface [11], and consequently, morphologically altering the surface, which appears bubble-like, with only a few dentinal tubules being blocked. Furthermore, when the power exceeds the ablation limit, it causes cutting of hard tissue. For this reason, some of the tested protocols exhibited surface heterogeneity: areas of melting while other areas presented exposure of enamel prisms or bubble-like features, illustrating the transition between the mechanisms of action of this laser at different powers.

The focal distance affects the power density delivered to the tissue. Since the power provided is preset at the beginning of the procedure, when increased, the power density area is reduced. The same process occurs when there is a change in the beam's angle of incidence, as ideally, it should be perpendicular to the application surface [27]. However, in the present study, no differences were found that could be attributed to the focal distance when comparing the same

protocols performed at different distances. As demonstrated by the scanning electron microscopy (SEM) images, both the focused and defocused application of the irradiation parameters (20 mJ, 10 Hz, 0.20 W, without cooling) produced satisfactory and noticeable alterations in the surface morphology of the hypomineralized teeth. Nonetheless, the focused mode offers greater precision and control during clinical application, which is critical for targeting specific areas while minimizing unintended effects on adjacent tissues. Consequently, the focused protocol (Group 5) was selected as the preferred approach due to its enhanced clinical applicability and reliability.

Recently, the Er: YAG laser has been investigated not only for its morphological and thermal effects on dentin structure modification but also for its bioactive properties, including significant antimicrobial potential. Studies indicate that Er: YAG irradiation reduces microbial viability, especially when combined with irrigants such as sodium hypochlorite, chlorhexidine, and hydrogen peroxide, which may enhance the decontamination of dental surfaces and improve clinical outcomes [28]. Moreover, Er: YAG exhibits effective antifungal activity against resistant microbial biofilms, suggesting additional benefits in the prevention and control of oral infections [29]. The ultrastructural examination of hypomineralized areas at high magnifications reveals porosities that facilitate bacterial accumulation, highlighting the importance of antimicrobial strategies. These characteristics expand the therapeutic potential of Er: YAG, making it a promising tool not only for the structural and reparative modification of dental tissues but also for reducing microbial load in vulnerable areas.

Regarding the use or absence of cooling, it is known that children with dentin hypersensitivity resulting from molar-incisor hypomineralization may show resistance to treatment due to anxiety and fear of painful stimuli [8]. Thus, water and air used during Er: YAG laser irradiation may complicate treatment, and therefore, a preference for clinical use of the laser without cooling may be expected to avoid causing painful stimuli to the patient.

When no irrigation with water was used, an increased effect of fusion and recrystallization of the dentinal tissue was observed, resulting in the obliteration of the dentinal tubules. However, the use of cooling during irradiation has the advantage of attenuating the rise in tissue temperature, minimizing the risk of thermally induced pulp injury. Matsumoto K [30] shows that a 5.5 °C increase in intrapulpal temperature can result in a percentage of pulp necrosis. However, in the present study, irradiation with the proposed parameters did not increase the intrapulpal temperature by more than 2 °C, making it a safe option. Furthermore, the variation observed in temperature rise among groups can

be explained by several experimental factors. The irradiated surfaces were selected according to the clinical location of the demarcated opacities on each tooth, resulting in differing distances between the irradiation site and the pulp chamber where the thermocouple was positioned, potentially influencing thermal measurements. Additionally, in the groups without water cooling (Groups 5 and 6), a higher temperature increase was expected; however, in Group 5, the focused mode likely provided a more controlled and concentrated energy delivery over a smaller area, reducing thermal diffusion and consequently the temperature variation. These experimental variations were intentional to reflect diverse clinical scenarios, allowing validation of the safe applicability of the proposed parameters on lesions with varying location and depth.

A limitation of this study was the small sample size, with only six teeth divided into twelve samples and two samples per group, resulting in only one repetition per condition. This limitation significantly restricts the statistical power of the study. The difficulty in obtaining teeth with enamel hypomineralization in suitable condition for research, without fractures, restorations, or caries, contributed to this sample size. Furthermore, it is important to highlight that the teeth used in this study were obtained from a certified institutional Biobank, where samples are donated and stored anonymously for research purposes, making it impossible to identify the donors' age or confirm their pediatric origin. In addition, it was not possible to verify a prior diagnosis of molar-incisor hypomineralization (MIH); however, all teeth were carefully examined to establish a clinical diagnosis of enamel hypomineralization. Nevertheless, as this was a pilot exploratory study, the findings provide relevant preliminary data on the morphological effects of Er: YAG laser irradiation, which may serve as a basis for future studies with larger sample sizes and clinical trials.

The clinical translation and scope of this study are important considerations. Specifically, the identification of safe and effective Er: YAG laser parameters for treating dentin hypersensitivity in hypomineralized molars provides a foundation for future clinical protocols. It is important to highlight the scarcity of studies addressing safe clinical parameters for Er: YAG laser use in this indication, which poses a challenge for clinicians using this technology. Since the laser is widely marketed and available for clinical use, the lack of well-established protocols may lead to risks associated with improper use. Therefore, this study bridges a gap between laboratory research and practical application, assisting clinicians seeking evidence-based approaches to manage dentin hypersensitivity with laser technology. Further *in vivo* studies and clinical trials are needed to validate and expand these results.

Conclusion

1. Among the tested protocols, high-power Er: YAG laser irradiation with parameters of 20 mJ, 10 Hz, and 0.20 W, applied in focused mode without cooling, yielded the best results, promoting — as demonstrated by scanning electron microscopy (SEM) — enamel melting and occlusion of exposed dentinal tubules, which are essential effects for managing dentin hypersensitivity in hypomineralized molars.
2. The use of the protocol without cooling, considered clinically relevant for patients sensitive to water and air stimuli, proved to be safe, as intrapulpal temperature variation tests indicated levels below the critical threshold for pulpal damage.

These findings are based on an *in vitro* experimental design with a small sample size, characteristic of a pilot study. Therefore, caution is advised when extrapolating the results to clinical scenarios, and further studies are necessary to confirm the outcomes under *in vivo* conditions.

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Author contributions All authors contributed to the conception and design of the study. Material preparation, data collection, and analysis were performed by Giovanna Bueno Marinho, Bruna Cordeiro Amarante, Patrícia Moreira de Freitas, and Marcelo Bönecker. Scanning electron microscopy analyses were conducted by Victor Elias Arana-Chavez. Thermal testing was performed by Daniela Fátima Teixeira Silva and Denise Maria Zzell. The first draft of the manuscript was written by Giovanna Bueno Marinho, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Clinical trial number Not applicable.

Competing interests The authors declare no competing interests.

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