Effects of Nd:YAG (1.06 µm) and CTH:YAG (2.1 µm) laser irradiations on dentin permeability in vitro

Summary

In the current study the characteristics of two experimental lasers (Nd:YAG: 1.06 µm and CTH:YAG: 2.1 µm) in modifying the permeability of dentin was investigated. Forty dentin disks were prepared from human molars and measured for permeability using a filtration technique. Half of the disks were acid-etched and the other half was covered by smear layer. Dentin permeability was measured after lasing and compared to baseline value. The temperature was monitored during irradiation and the morphological changes observed on the lased dentin were evaluated under the S.E.M. For both smear covered and acid etched dentin disks, Nd:YAG laser irradiation significantly increased dentin permeability whereas CTH:YAG laser energy reduced dentin permeability. The highest increase in temperature during lasing was produced by the Nd:YAG laser. S.E.M. observations of the Nd:YAG lased surfaces showed a characteristic appearance of melted dentin with the presence of large resolidified bubbles of dentin magma. Structural changes produced by CTH:YAG were mostly characterized by the presence of multiple layers of dentin material covering the underlying unaffected dentin, in absence of a melting process. The results showed that scanning electron observations of the dentin cannot accurately predict the functional changes produced by the laser beams. Since the efficiency of the laser beams seems mostly related to their surface absorption, research should continue with wavelengths more adapted to the dentin structure.

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Introduction

A number of clinical and histological studies have acknowledged the importance of the tubular structure of the dentin in pulpo-dentinal diseases. Morphologically, dentinal tubules run across the dentin mass, and both their diameter and density increase from the dentin-enamel junction to the pulp chamber. Therefore, dentin is a permeable tissue that can transport substances by diffusion and also permits bulk fluid movements (Pashley 1996). Both mechanisms can occur once the enamel or the cementum are lost by erosion, abrasion, attrition or dental caries. As a consequence of the exposed dentin to the oral environment, the tooth responds to chemical or physical stimuli with morphological changes. Such stimuli usually are also accompanied by pain.

Bergenholtz & Lindhe (1975) reported that pulpal inflammation can be elicited by placing bacterial components on freshly cut dentin because bacterial substances like lipopolysaccharide (LPS) can diffuse across dentin down to the pulp. Other studies have shown that some components of restorative materials can diffuse through dentin and subsequently pose a risk for pulpal irritation because of their toxicity (Hanks et al. 1988, Bouillaguet et al. 1996). Fluid movements down a hydrostatic or osmotic pressure gradient can elicit dental sensitivity. Brännström & Aström (1964) proposed a hydrodynamic mechanism for the transmission of painful stimuli through dentin. Recently Matthews & Vongsavan (1994) validated this hypothesis and demonstrated that intradental nerve endings can be excited by means of either outward or inward fluid movements within the dentinal tubules. It was also reported that air-blowing the surface of an exposed dentin area can increase dentinal fluid filtration from the pulp toward the periphery (Matthews et al. 1993). Therefore, it is currently accepted that closing tubules at the dentin surface has a protective role for the underlying living tissues.

During the last decade, laser technology has been progressively introduced into the field of dentistry for a wide variety of clinical applications. Despite well documented uses of lasers in oral surgery, much research concerning dental hard tissue treatment has focussed on the morphological changes produced after laser irradiation. Previous studies have demonstrated that laser beams interact with dental hard tissue by thermally producing a surface microfusion of the hydroxyapatite crystals (Kantola 1972). Depending on the energy absorbed, vaporization of the smear layer, melting and glazing of the dentinal surface or crater-like formations were reported. However, the temperature rises recorded during laser irradiation generally exceeded the 5.5 °C biological tolerance of the dental pulp (Zach & Cohen 1965). Other evidence showed that both optical properties and chemical content of the target tissue can influence the absorption of the laser energy and therefore the effects produced. For these reasons, specific laser beams have been developed for the different clinical applications and irradiation parameters have been more precisely defined to avoid thermal damage to the dental pulp.

Among the most promising lasers for dentin treatment are the systems emitting in the mid infrared region. Many reports have confirmed that these wavelengths can produce tissue ablation since they are well absorbed by the dental hard tissues (Koort & Frenzen 1992). Further, the addition of an air-water cooling system to the laser beam has been shown to substantially reduce increases in temperature observed during laser irradiation and the risk for thermal injury (Burkes et al. 1992). When applying a pulse mode during lasing, less heat is produced compared to the continuous mode. Finally, the depth of penetration into the substrate can be controlled by selecting the energy density delivered, the pulse duration and the repetition rate. As reported in previous studies, laser settings must be carefully controlled especially when using the non selective and very penetrating wavelength of Nd:YAG laser (Dederich et al. 1984, White et al. 1992)

Therefore, laser settings used in this study were selected from pilot studies which determined the best ratio between efficiency and side effects. The hypothesis tested in the current study was that lasing of the dentin could reduce its permeability by structurally altering the dentinal surface and tubules. To support this hypothesis, the flow rates of water through dentin before and after laser irradiation were measured in vitro under carefully controlled temperature conditions.

Materials and methods

Forty dentin disks were prepared from human third molars without carious lesions. They were cut in cross sections at the mid level of the crown using a thin sectioning machine under constant water coolant (Chillings-Hamco Scotland). The thickness of each disk was 1 mm and verified with a digital caliper (+/– 0.01 mm). The disks were treated with 37% orthophosphoric acid gel for 10 seconds to remove the smear layer resulting from sectioning and stored at 4 °C in a 0.1% sodium azide solution to prevent bacterial growth. Then, a standard smear layer was created on the occlusal surface of 20 dentin disks by rubbing the disks on a wet 600 grit SiC abrasive paper. For the experimental procedures, half of the disks were smeared and the other half acid-etched.

For the permeability measurements, the disks were placed into a modified split chamber previously described by Pashley et al. (1983). Briefly, the dentin disk was held between two squares of dental rubber dam defining a surface area of 2×2 mm^2. A temperature probe was placed in the lower part of the chamber, 1 mm below the dentin disk to monitor the temperature changes during and after lasing. The chamber was then connected through polyethylene tubing to an automated flow recording device (Flodec, De Marco Engineering, Geneva, Switzerland) and the hydraulic circuit filled with double-distilled water. The device automatically converted the linear displacement of an air-liquid meniscus located inside a precision glass capillary tube into flow data at a rate of one measurement per second (Ciucchi et al. 1995). Simultaneously, flow data and temperature changes were recorded by the computer (Fig. 1). The flow rates of water were measured in filtration conditions for 10 minutes, in presence of an internal pressure of 15 cm of water inside the lower chamber and the upper side of the disk covered with water. Fluid flows through dentin were recorded before and after lasing and expressed in microliters per minute per square centimeter. Then, the hydraulic conductance of each specimen was calculated by dividing fluid flow by water pressure. Care was taken to carefully control the ambient humidity and temperature since both of these variables influence permeability measurements (Pashley et al. 1983).

Both types of lasers used for this in vitro study were prototypes developed by the Applied Optics Laboratory at the Swiss Federal Institute of Technology in Lausanne. Ten etched and ten smear-covered dentin disks were treated using a Nd:YAG laser using a 1.06 µm wavelength with an energy of 300 mJ/pulse at a repetition rate of 20 Hertz. The laser beam was delivered to the dentin surface through a special optical handpiece incorporating a water cooling system. It produced a spot size of 0.12 mm at focal length. Under these conditions, the fluence of the laser beam i.e. the energy density by surface area was 2700J/cm^2. Multiple laser exposures were made perpendicularly to the dentinal surface to provide the maximum energy absorption over the entire surface to be treated. The other 20 dentin disks were treated with a CTH (Chromium Tolmium Holmium) :YAG laser working at 2.1 µm wavelength with an energy of 300 mJ/pulse at a repetition rate of 1 Hertz. The beam was delivered through an optical fiber with a spot size of 0.45 mm at focal length incorporating a water cooling system. The fluence of the laser beam was 190 J/cm^2.

At the end of the laser treatment, each specimen was fractured longitudinally and prepared for SEM observation. Briefly, each

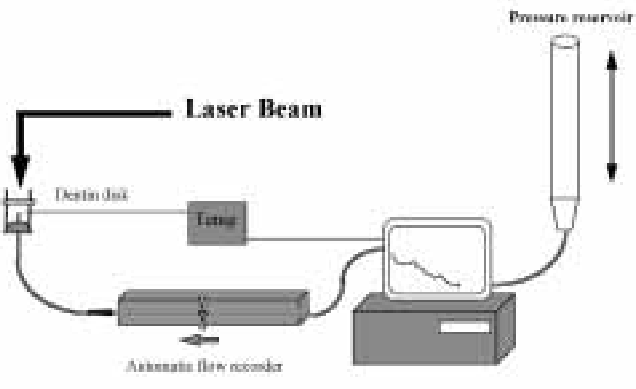

Fig. 1 Schematic representation of the experimental setup used in this study. The dentin disk is positioned into the split chamber for permeability measurements. The temperature probe is located 1mm below the dentin disk and the hydraulic circuit filled with double-distilled water under pressure. Fluid flow and temperature changes are simultaneously recorded by the computer before and after lasing.

dentin disk was dehydrated by immersion into ascending ethanol concentrations up to a 100% solution and critical point-dried. The dried samples were then mounted onto aluminum stubs and ion sputtered with gold. Scanning electron microscopy was carried out using a Phillips XL 20 scanning electron microscope. Observations at low magnification were performed to evaluate the percentage of treated areas on each specimen as well as the morphological changes observed on the dentinal surfaces. Finally, the reduction in dentin thickness resulting from the ablation of the dentin after laser treatment and the appearance of the dentinal tubules were controlled at higher magnification. Statistical differences in dentin permeability measurements before and after laser treatment were assessed using a paired t-test ($p<0.05$).

Results

Table I shows the mean hydraulic conductances for both smear-covered and acid-etched dentin discs measured before and after Nd:YAG laser irradiation. For the non-etched dentin disks, the mean pretreatment permeability value was 5.6×10^{-3} µL cm^{-2} min^{-1} cm^{-1} H_2O and increased to 17×10^{-3} µL cm^{-2} min^{-1} cm^{-1} H_2O after laser treatment. For the acid etched dentin discs, the post-treatment permeability value increased to 10.4×10^{-3} µL cm^{-2} min^{-1} cm^{-1} H_2O whereas the mean pretreatment permeability value was 6.1×10^{-3} µL cm^{-2} min^{-1} cm^{-1} H_2O. The increase in dentin permeability was statistically significant for both smear-covered ($p = 0.012$) and acid-etched dentin disks ($p = 0.02$). When expressed as a percentage of pre-treatment value, an increase of 300% and 170% in hydraulic conductance was observed for both groups, respectively.

When the CTH:YAG laser energy was applied to the dentin, the changes in dentin permeability were markedly different from those observed after Nd:YAG laser irradiation (Table II). For the non-etched dentin disks, dentin permeability was significantly reduced from 9.3×10^{-3} µL cm^{-2} min^{-1} cm^{-1} H_2O before lasing to 6.3×10^{-3} µL cm^{-2} min^{-1} cm^{-1} H_2O after treatment ($p = 0.02$). For the acid-etched dentin disks, the post-treatment permeability was 7.1×10^{-3} µL cm^{-2} min^{-1} cm^{-1} H_2O whereas the pretreatment permeability value was 8.4×10^{-3} µL cm^{-2} min^{-1} cm^{-1} H_2O. Respectively, a 32% and a 15% reduction in dentin permeability were reported for the smear-covered and the acid-etched dentin disks after CTH:YAG laser treatment.

Table I   Effect of Nd:YAG laser irradiation on the permeability of human dentin

Dentin conditions	**Dentin permeability***		**P value**
	Before treatment	After treatment	
Smear-covered	5.6 (3.1)	17 (1.2)	p = 0.01
Etched	6.1 (2.2)	10.4 (5.9)	p = 0.02

* reported as mean (stdev) Units: $\times10^{-3}$ µL cm^{-2} min^{-1} cm^{-1} H_2O (n=10)

Table II   Effect of CTH:YAG laser irradiation on the permeability of human dentin

Dentin conditions	**Dentin permeability***		**P value**
	Before treatment	After treatment	
Smeared dentin	9.3 (5.8)	6.3 (3.6)	p = 0.016
Etched dentin	8.4 (3.9)	7.1 (3.2)	p = 0.076

* reported as mean (stdev) Units: $\times10^{-3}$ µL cm^{-2} min^{-1} cm^{-1} H_2O (n=10)

Depending on the laser used, the temperature changes produced after lasing were variable. The mean increase in temperature resulting from the application of Nd:YAG laser irradiation was 9 °C. Changes in temperature did not exceed 4 °C after CTH:YAG laser application.

Scanning electron examination of the lased specimen revealed that only 40% of the total area exposed to the Nd:YAG laser radiation showed morphological changes. Multiple impacts created by the laser beam were observed on the dentinal surface adjacent to non-lased areas. The diameters of the craters ranged from approximately 100 to 300 µm and some of them were surrounded by a charring zone. The observation of the fractured specimen revealed that Nd:YAG laser application caused a significant removal of dentin substrate with an average ablation depth of 250 µm (Fig. 2). For some specimens, however, lased holes of 600 µm deep were measured. At higher magnification (Fig. 3), the lased dentinal surfaces showed a characteristic appearance of melted dentin with craters covered by large resolidified bubbles generated during the melting and recrystallization

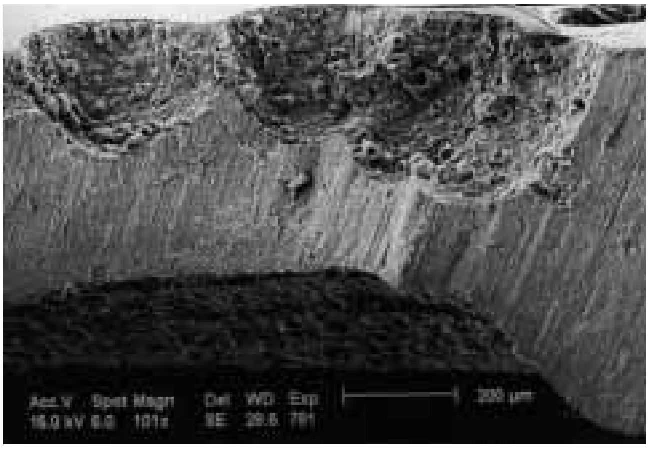

Fig. 2   Scanning electron micrograph (Original magnification × 100) of a smear-covered dentin surface after Nd:YAG laser irradiation showing area of undisturbed smear layer adjacent to lased surfaces. A mean ablation depth of 250 µm was reported.

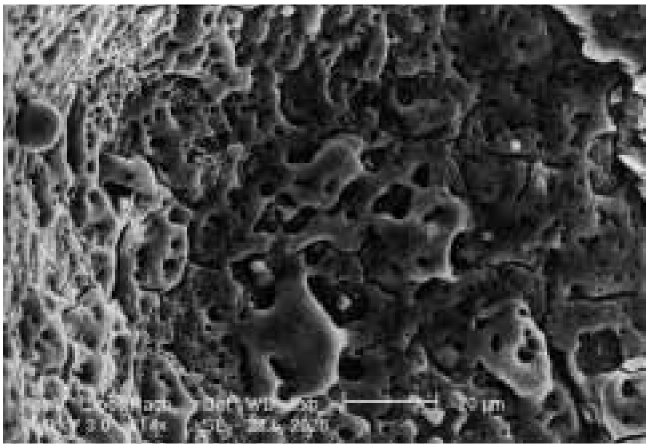

Fig. 3   SEM appearance of the lased dentin surface showing the presence of recrystallised hydroxyapatite in the center of the lased area (Original magnification × 800). Despite the presence of dentin magma covering the surface, the permeability of the dentin significantly increased after lasing.

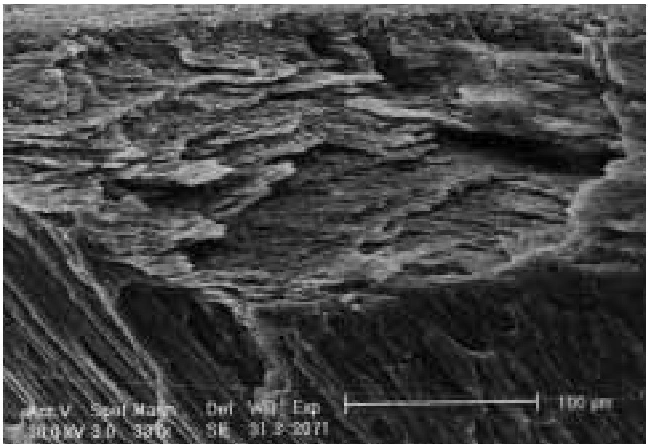

Fig. 4 SEM appearance of an acid etched dentinal surface after CTH:YAG laser irradiation (Original magnification × 100). A mean ablation depth of 200 µm was reported from the SEM observation of the fractured specimen. The ablated lesion is uniform and covered by multiple layers of dentin material.

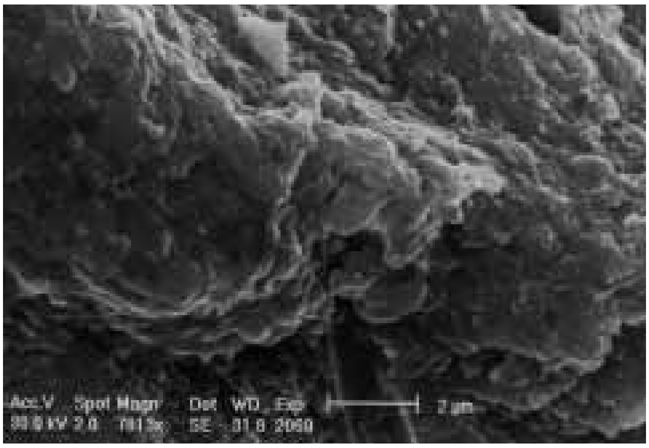

Fig. 5 Higher magnification (× 7800) reveals the presence of amorphous material occluding the dentinal tubules. The dissolution of the basic components of the dentin during lasing might have promoted the formation of mineral deposit responsible for the reduction in dentin permeability.

process (approximately 20 µm in diameter). The smear layer appeared to be removed and resolidified melted dentin occluding tubule orifices was frequently observed. Morphological changes and ablation pattern observed on smear-covered dentin were similar to those observed on acid-etched dentin.

In contrast, structural changes produced by CTH:YAG laser irradiation on dentinal surfaces were different from those observed after Nd:YAG treatment. Laser-treated area represented approximately 70% of the total surface area exposed to the laser beam with the persistence of unaltered zones. The irradiated surface showed morphological alterations revealed by the presence of ablation craters at the impact zone. The diameters of the craters ranged from approximately 400 to 600 µm and had roughened surfaces. The observation of fractured specimen (Fig. 4) showed a mean depth of ablation of approximately 200 µm but the ablation pattern was more uniform compared to the Nd:YAG-treated surfaces. At higher magnification, the irradiated surface presented an irregular appearance with the presence of craze lines around the ablation crater without melted dentin. These area were mostly characterized by the presence of multiple layers of dentin material covering the underlying unaffected dentin (Fig. 5). In the periphery of the interaction zone, the presence of compacted debris partially occluding dentinal tubules was evidenced by the SEM. Morphological changes and ablation patterns observed on smear-covered dentin were similar to those observed on acid-etched dentin.

Discussion

There is a growing interest in laser technology for dental applications such as preventive and restorative dentistry, endodontic procedures, and treatments of dentinal hypersensitivity. Most of these in vitro studies have attempted to demonstrate that the ultrastructural changes produced on dental hard tissues observed under the SEM were responsible for a sealing of the dentin (Stabholz et al. 1993). However, there is more evidence today that direct permeability measurements on treated samples are more valuable than SEM observations when considering changes in dentin permeability (Pashley et al. 1992). Therefore, this in vitro study focussed on the functional changes produced by laser energies on human dentin as a result of modification in dentin permeability measured by filtration technique. The dentinal tubules were filled with double-distilled water and there was no attempt to simulate in vivo conditions by using protein-containing fluids such as horse serum or cell-culture medium. Goodis et al. (1994) reported that the elevation in temperature occurring during lasing can be responsible for the coagulation of proteins inside dentinal tubules. Consequently, the dentin would appear sealed by precipitates in dentinal tubules. However, such a sealing may be less effective and permanent than the sealing resulting from the reorganization of the dentin hydroxyapatite after its microfusion. Therefore, changes in dentin permeability measured in the current study were attributed to structural modifications of the dentin and not to the coagulation of the proteins by heat. The use of perfused samples also permitted the reproduction of the natural water content of the dentin which plays a major role in the absorption of laser energy (Koort & Frenzen 1992). Before lasing, the dentin was either smeared or acid-etched to compare the absorption of the laser energy under two clinical conditions of treatment. Our data suggest that smear-covered dentin was more effective in absorbing laser energy compared to acid-etched dentin. This is in agreement with other reports that demonstrated the need for coating the dentin surface prior to lasing to reduce the loss of energy caused by reflection (Hess 1990).

The minimal temperature changes observed after laser irradiation supports the concept that laser energy can be used for dental treatments on vital teeth in presence of appropriate cooling mechanisms. This is in agreement with previous studies (Hoke et al. 1995, Anic et al. 1996). However, temperature changes reported in this study do not completely reflect the risk for thermal injury to the dental pulp since the heat generated during lasing was dissipated into the 1 mL content of the lower chamber.

It was clear from the present study that Nd:YAG and CTH:YAG laser treatment both affected the functional and morphological characteristics of the dentin. The Nd:YAG treatment significantly increased dentin permeability on both acid-etched and smear-covered dentin. For the smear-covered samples, the disruption of the smear layer was clearly a factor since the presence of smear layer is very effective at reducing dentin permeability

(Pashley 1996). The reduction in dentin thickness evidenced by the SEM pictures (Fig. 2) could also explain the increase in dentin permeability observed for all specimen. Since the filtration rate is inversely proportional to the length and the radius (raised to the fourth power) of the tubules, any procedure that decreases dentin thickness such as the ablation of the dentin by laser energy will in turn increase filtration rate. In addition, it can be assumed that the multiple spot overlapping observed on the lased surfaces has resulted in the formation of deep craters located over higher permeability areas. This is somewhat confirmed by the SEM pictures showing unaffected areas alongside deep ablation craters (Fig. 2). This non-uniform distribution of the laser energy could have resulted from a selective absorption of the laser beam by the dentin already irradiated. Local carbonization of irradiated zone might have increased the coefficient of absorption into dentin and favored additive ablation effects. At higher magnification (Fig. 3), SEM pictures of the Nd:YAG treated surfaces clearly showed the presence of dentin plasma created during the thermal interaction between the dentin structure and the laser beam (Frenzen & Koort 1990). However, these magma do not totally cover the craters and many orifices in continuity with the underlying dentinal tubules were observed on the fractured specimen. Filtration measurements confirmed that the presence of this melted dentinal structure covering the lased surfaces was not effective in reducing the permeability of the dentin.

The CTH:YAG laser treatment reduced the permeability of the dentin by 30% without excessive temperature changes. This partial sealing of the dentinal surface could be attributed to the formation of a superficial layer less permeable to water and confirms the good absorption of this wavelength into the dentin substrate in presence of water (Anic et al. 1996). Surprisingly, no sign of a melting process was detected on dentinal surfaces exhibiting reduced filtration rates after lasing (Fig. 4). For this reason, artifacts resulting from the preparation of the samples for SEM observations cannot be ruled out. However, the low raise in temperature recorded after lasing and the absence of melted material suggest a more complex interaction than a simple thermal effect. An ablation process occurs when the thermal energy generated by the laser beam can convert water into gas that increases the pressure inside the dentin and produces microexplosion (Keller & Hibst 1989). Such phenomenon is responsible for the ablative effect produced on the dentinal surfaces in absence of a melting process. A similar pattern has been previously described by LI et al. (1992) when using a Erbium:YAG laser on human dentin. As a result of the interaction between this wavelength and the dentin structure, changes in chemical composition of the irradiated surfaces have been also reported (Cechini et al. 1997). Therefore, it can be speculated that the dissolution of basic components of the dentin during lasing might have promoted the formation of mineral deposit inside in dentinal tubules. As reported by Pashley (1996), the presence of debris inside the tubules or covering the dentin surface significantly reduces the permeability of the dentin. This hypothesis is somewhat confirmed by SEM pictures showing the presence of amorphous material partially occluding the dentinal tubules (Fig.5). Although none of the specimen exhibited a complete sealing after lasing, this study suggests that the well absorbed wavelengths such as the CTH:YAG laser energy present interesting properties for hard tissue applications in dentistry. However, modifications in the delivery system through optical fibers are required to improve the efficiency of the laser beams and their clinical application.

Conclusion

In the course of this experiment, both laser treatments provided changes in dentin permeability. Nd:YAG laser treatment significantly increased dentin permeability whereas CTH:YAG laser energy generally reduced the permeability of the dentin. Different structural changes were observed under the SEM, but none of these observations can accurately predict the permeability characteristics of the irradiated area. Since the effects of the laser beams on the dentin seem mostly related to the surface absorption, more research must be done with wavelengths more adapted to the dentin structure.

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Résumé

L'étude in vitro visait à étudier les modifications structurelles et de perméabilité produites sur la dentine humaine par deux sources de rayonnement laser (Nd:YAG: 1.06 µm et CTH:YAG: 2.1 µm). Quarante tranches de dentine ont été préparées à partir de molaires extraites, la moitié a été mordancée et l'autre moitié a été recouverte de smear layer. La perméabilité de chaque échantillon a été mesurée avant et après irradiation et l'élévation de température induite par le rayonnement laser également enregistrée. L'irradiation à l'aide du laser Nd:YAG a entraîné une augmentation significative de la perméabilité alors que le laser CTH:YAG a diminué la perméabilité dentinaire. L'élévation de température a été plus importante avec le laser Nd:YAG. L'observation au MEB a mis en évidence des modifications morphologiques différentes pour chaque type de laser. Les résultats démontrent que la seule observation au MEB ne permet pas de prédire les modifications de perméabilité dentinaire observées après irradiation au laser. Si les lasers testés dans cette étude ont démontré d'intéressantes propriétés pour l'ablation et le scellement de la dentine, d'autres travaux utilisant des longueur d'onde mieux absorbées sont nécessaires pour confirmer leur intérêt en médecine dentaire.

Zusammenfassung

In der vorliegenden Studie wurden die Auswirkungen zweier experimenteller Laser (Nd:YAG: 1.6 µm und CTH:YAG: 2.1 µm) auf die Permeabilität von Dentin untersucht. Vierzig Dentinscheiben wurden aus menschlichen Molaren präpariert und die eine Hälfte geätzt, die andere mit smear layer bedeckt. Mittels einer Filtertechnik wurde die Permeabilität der Präparate vor und nach der Laserbestrahlung gemessen. Ausserdem wurden die Temperaturveränderungen während der Bestrahlung aufgezeichnet, und danach die morphologischen Veränderungen des bestrahlten Dentins im REM überprüft. Die Bestrahlung mit dem Nd:YAG-Laser erhöhte die Permeabilität aller Dentinproben signifikant, während die CTH:YAG-Laserenergie sie verringerte. Der höchste Temperaturanstieg während der Bestrahlung wurde vom Nd:YAG-Laser erzeugt. Im REM zeigten die mit Nd:YAG bestrahlten Oberflächen charakteristische Spuren geschmolzenen Dentins mit grossen, wiedererstarrten Blasen aus Dentinmagma. Die Strukturveränderungen durch den CTH-YAG-Laser bestanden vorwiegend in mehreren Schichten Dentinmaterial, das das unveränderte Dentin ohne Schmelzspuren

überdeckte. Die Ergebnisse zeigten, dass die durch Laserbestrahlung hervorgerufenen funktionalen Veränderungen des Dentins allein auf Grund der Beobachtung im REM nicht korrekt vorhergesagt werden können.

Da die Wirksamkeit der Laserstrahlen vor allem mit ihrer Absorption durch die Oberfläche zusammenzuhängen scheint, sollten zur weiteren Erforschung Wellenlängen verwendet werden, die der Oberflächenstruktur des Dentins besser entsprechen.

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