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Effect of Nd: YAG Laser on Human Dentin Fluid Flow

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ABSTRACT

Objectives: The aim of the current investigation was to assess the rate and magnitude of dentin fluid flow of dentinal surfaces irradiated with neodymium-doped yttrium aluminum garnet (Nd:YAG) laser.

Materials and Methods: Thirty extracted intact molars were sectioned, mounted and irradiated with Nd:YAG laser at 2-W power setting. The samples were automatically irradiated at sequential runs until dentin surfaces were completely irradiated. Samples were divided into 3 groups; group 1: the dentinal-fluid flow was measured over 5 minutes after irradiation, group 2: dentinal-fluid flow was measured over 10 minutes after irradiation and group 3: teeth were immersed in 1% sodium hypochlorite, then irradiated and dentinal-fluid flow was measured over 10 minutes after the end of irradiation. Dentinal-fluid flow was measured using fluid-flow apparatus. The rate, magnitude and direction of dentin-fluid flow were recorded at baseline and after irradiation.

Results: Non-parametric Wilcoxon Signed Ranks repeated-measure t-test and Kruskal-Wallis ANOVA test revealed a statistically significant increase in fluid flow after laser treatment. However, no significant differences were found between the groups. The samples demonstrated a baseline outward flow followed by inward flow due to irradiation followed by increased outward flow.

Conclusions: Nd:YAG laser at 2-W power setting has significantly increased dentinal-fluid flow rate. The removal of smear layer with sodium hypochlorite prior to irradiation did not have any effect on the fluid flow rate.

Keywords: Irradiation, Nd:YAG laser, Dentin, Fluid flow, Flodec.

1. Introduction

Dentin hyper-sensitivity is a common clinical condition (1). The most widely accepted theory on the transduction mechanism by which potentially painful stimuli are causing sensitivity is the hydrodynamic mechanism (2). The theory attributes dentin sensitivity to stimulation of mechanoreceptors located in dentinal tubule or at the pulp dentin junction, provoked by movement of tubule-fluid contents in either direction. The degree of sensitivity is proportional to dentin permeability and dentinal tubules' number. The number of tubules in sensitive teeth is remarkably greater than in non-sensitive teeth and the diameter of tubules is also considerably larger (3). Based on the former assumptions, agents or techniques capable of decreasing dentin permeability and reducing the rate of dentinal-fluid flow are hypothetically effective in lessening dentin hyper-sensitivity (4,5). Therefore, treatment strategies that adopt the concept of tubule occlusion are supportive of the hydrodynamic theory.

The methods available for treatment of dentin hyper-sensitivity include the professional application of adhesive resins, varnishes and dentin bonding agents or restorative materials able to provide tubule obturation

(5-7), in addition to the use of desensitizing tooth pastes that contain potassium, fluoride or strontium salts, bioactive desensitizers and the application of topical fluoride (6,8,9). Unfortunately, no single treatment strategy for this problem is consistently successful or has a long-term effect. Therefore, lasers were considered as a treatment option. For this particular purpose, lasers, such as neodymium-doped yttrium aluminum garnet (Nd:YAG) and erbium-doped yttrium aluminum garnet (Er:YAG), were proposed (10).

It has been found that low-level laser was effective in reducing dentinal hyper-sensitivity (11). However, various wavelengths of Nd:YAG laser produce varying amounts of fusion, glazing, melting, resolidification and partial closure of dentinal tubules. More surface topography, structure and heating effects appeared as Nd:YAG and Ho:Ytterium-Alumium Garnet lasers' power, frequency and laser-probe diameter increased, with an observed decrease in dentinal-fluid flow correlated with the degree of tubule closure (11). On the contrary, another study reported inconsistently partial closure of dentinal tubules and mostly accompanied with an increase in dentinal-fluid flow (12). The authors concluded that the use of Nd:YAG lasers system to seal exposed dentin does not seem to be advantageous. Furthermore, both Nd:YAG and Er:YAG lasers increased the permeability of radicular dentin when used with irrigating solutions (12). However, Nd:YAG laser reduced the permeability of root dentin and potentiated the effect of fluoride varnish (14-17). Additionally, Nd:YAG laser was reported to induce analgesic effect *via* blocking-nerve conduction (18). Clinically, the efficacy of Nd-YAG laser was proven in reducing dentinal hyper-sensitivity after 6 months of treatment and combining laser and adhesive desensitizer demonstrated immediate and long-lasting effects (19-20).

Furthermore, deproteinization of dentin with agents like sodium hypochlorite (NaOCl) to remove collagen matrix was suggested to improve infiltration of resin-based desensitizers and to consequently improve the sealing of dentinal tubules (21). The association of NaOCl-induced deproteinization with Nd:YAG laser produced the lowest dentinal permeability rate (22).

The aim of the current study was to explore the effect of Nd:YAG laser on coronal dentin-fluid flow with or without NaOCl-induced deproteinization.

2. Materials and Methods

2.1 Sample Preparation

The research protocol was reviewed and approved by the institutional review board. Thirty extracted intact third molars were selected for this study. The teeth were cleaned to remove soft tissues and stored in a 1% Chloramine T solution in distilled water at 4ºC until further processing.

The roots of the teeth were sectioned 2 mm apical to the cemento-enamel junction (CEJ) using a slow-speed diamond saw (Struers, Ballerup, Denmark), with continuous water coolant to minimize heat generation.

Dentin discs were then prepared by horizontally sectioning the occlusal surface by removing one-third of the crown using the slow-speed diamond saw and water coolant resulting in a crown segment containing a portion of the pulp chamber at the inferior portion and a flat dentin surface at the superior portion. The surface was carefully examined under X15 magnification using light microscopy to affirm the complete removal of enamel. Each dentin disc was then finished with 600-grit silicon carbide (SiC) paper under running water. The final thickness of each dentin disc ranged from 3 to 4mm.

A square plastic block (4 cm square, 5 mm thick) with a hole drilled at its center was used to mount each tooth. An 18-gauge needle was inserted into the center of the hole and fixed with cyanoacrylate cement (M-Bond 200, Micro-Measurement Group INC., Raleigh, NC, USA). The assembly was then covered with epoxy resin (Araldite, Vanito AG, Basel, Switzerland) to ensure airtight seal. Each tooth was then positioned on the plastic square, with the pulp chamber placed on the central hole and the needle and then the peripheries were sealed using epoxy resin.

2.2 Flow Measurement

Each tooth was connected to a precision glass capillary tube (30 cm long, internal diameter = 0.84 mm) by silicon tubing filled with phosphate-buffered saline. The precision capillary tube was positioned horizontally in an automated fluid-flow measurement apparatus (Flodec, De Marco Engineering, Geneva, Switzerland) (Figure 1). The linear displacement of an air bubble was measured by an infrared-emitting diode and sensing element located opposite to the diode attached to a step-motor that allows the diode to follow changes in the position of the air bubble.

Flodec device is designed to sense the linear displacement of the air bubble as small as 5 µm (2.8 nL). The device was adjusted to allow dentinal fluid-flow measurement over very short intervals with a sampling rate set at 20 points per second. Data was processed in a spreadsheet software (Microsoft, Excell 2003). Each mounted pulp chamber was filled with phosphate-buffered saline (PBS). A reservoir of PBS was employed to monitor intrapulpal pressure by varying the height. A hydrostatic pressure of 1.3 kPa was maintained throughout the experimental procedures to replicate the physiological pulp pressure (22). Fluid flow was recorded as negative (outward fluid flow) if the air bubble moved towards the tooth and positive (inward fluid flow) if the air bubble moved away from the tooth.

Fluid-flow volume (in nl) was calculated using the formula below:

Fluid-flow volume (nl) = $\pi r^2 \times D \times 10^{-3}$

where r = radius of the glass tubule (0.42 mm), and D = linear displacement (mm) of the bubble in the glass tube. Fluid-flow rate (in nl s^{-1}) was obtained from the volume (in nl) divided by measuring time (in seconds).

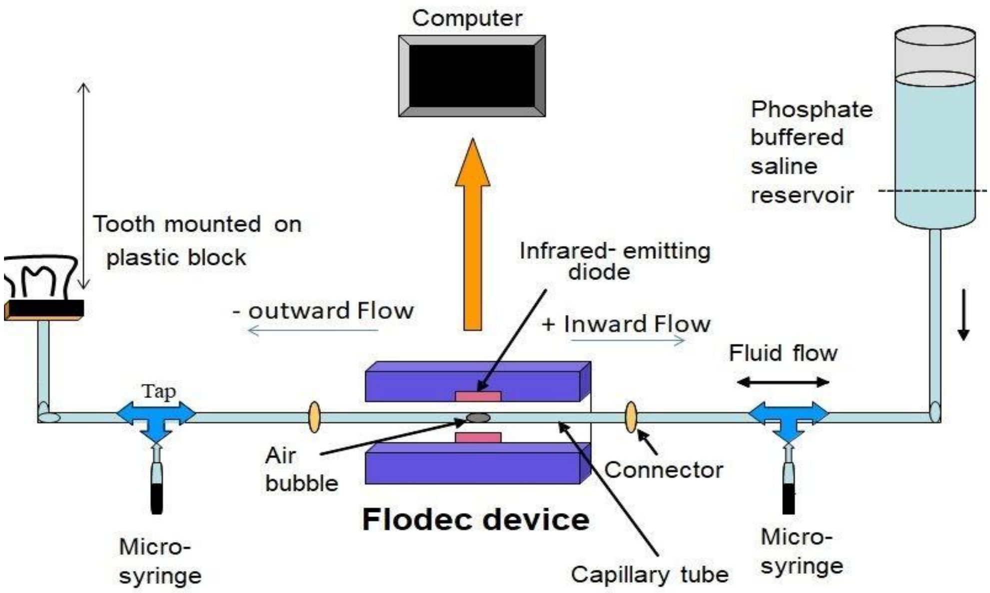

Figure 1: Diagrammatic representation of the test apparatus for measuring fluid flow.

2.3 Laser Irradiation

The laser used for this study was an Nd:YAG laser (American Dental Laser dLase 300, Sunrise Technologies Inc., Ca, USA) equipped with a 320-µm diameter optical fiber. The repetition rate was set to 20 Hz with a power output of 2W. To enhance energy absorption, black ink was applied to the tooth surface prior to each laser application. During irradiation, each tooth was clamped on a stationary stand, while the laser handpiece, held at a distance of 1 mm from dentin surface, was secured on a movable stand connected to a step-motor. Irradiation was automatically directed back and forth until the dentin-surface entire is fully exposed.

The laser handpiece was automatically moved along the horizontal mesio-distal plane of the tooth. At the end of each laseing line, the handpiece was vertically raised in a bucco-lingual plane at an interval of 300 µm prior to proceeding at the disto-mesial direction. Baseline flow was measured and recorded for 5 minutes. Prior to laser application, the optical tip was regularly inspected under light microscopy to inspect any existing debris. The tip was polished with 2000 and 4000 grit SiC paper under running water after each 5 laser passes. Dentinal-fluid flow was measured before and after each laser pass, and data was recorded on a separate Excel spread sheet.

The specimens were divided into 3 experimental groups (groups 1, 2 and 3). In group 1 (n=10), the pulp tissue was removed with tweezers, and the teeth were sonicated in distilled water for 5 minutes and dentinal-fluid flow was recorded for 5 minutes after laser application. In group 2 (n=10), the pulp tissue was removed, the teeth sonicated in distilled water for 5 minutes and dentinal-fluid flow was recorded for 10 minutes after laser treatment. In group 3 (n=10), the pulp was removed, then the teeth were immersed in 1% sodium hypochlorite solution (Milton's solution, Procter

& Gamble, Paramatta, NSW, Asutralia) and sonicated for 5 minutes for complete removal of the remaining pulp tissue and dentinal-fluid flow was measured for 10 minutes after each laser irradiation. Each tooth took approximately 3 hours to complete irradiation of the whole dentin surface and measuring the dentinal-fluid flow.

The flow was expressed as the percentage of the baseline flow. The amount of increase/decrease in dentin-fluid flow was calculated by dividing the flow after laser irradiation (L) by the baseline flow before irradiation (B). To minimize desiccation or over-drying, each tooth was surrounded with a moistened cotton wool throughout the measurement period.

2.4 Statistical Analysis

Non-parametric Wilcoxon Signed Ranks repeated-measure t-test was used to compare the effect of the laser irradiation on fluid flow, where values before and after laser application were compared for each experimental groups. Kruskal-Wallis ANOVA test was used to compare the dentin-fluid flow between the different groups for the baseline data and the data after laser application. All analyses were performed at the 0.05 level of significance.

3. Results

The means and standard deviations of dentinal-fluid flow volume values were calculated and the change in dentin-fluid flow after laser application was calculated by dividing the baseline dentin-fluid flow after laser application (L) by the dentin-fluid flow before laser application (B). Because each tooth has its individual standard, different teeth were comparable regardless of their anatomical differences. In this way, each smear layer that covered dentin surface represented its own control of that specimen.

The means and standard deviations for fluid-flow measurements with the statistical-analysis results are shown in Table 1. All smear layers that covered the samples showed outward flow. During laser irradiation, a sharp peak increase in outward flow was recorded. In the majority of the samples, this peak increase in outward flow was demonstrated in the first laser pass, or within the first 5 passes. However, the successive laser passes also showed an increase in outward flow, but not in similar pattern and magnitude. The peak was demonstrated in 70% of the samples in group 1 (7/10), 70 % of the samples in group 2 (7/10) and 100% of the samples in group 3 (10/10). Due to peak increase in the first laser pass, the data was not normally distributed; hence, non-parametric analysis was performed. The pattern of change in fluid flow was usually demonstrated as transient inward dentinal flow during the laser application which lasted from 5-10 seconds. Then, it was followed by a sharp increase in outward flow (Figure 2). The peak outward flow lasted from 2-7 minutes after each completed laser application, and then, the flow was leveled off. The baseline dentin-fluid flow rate showed non-significant differences between the experimental groups. Laser application has significantly increased the dentinal-fluid flow in all experimental groups (Table 1). However, there were no significant differences between the groups for either the rate of dentin-fluid flow measured at baseline and after laser application, as revealed by Kruskall-Wallis test ($P > 0.05$). Moreover, when the skewed values of the high peak dentin-fluid flow laser passes were excluded for each sample from statistical testing, the statistical differences between the groups and between the baseline and after laser application per each group remained unchanged. Additionally, once baseline flow rate (before laser application) was not considered and only the flow rate after the last laser application was only considered for each sample, the statistical differences remained also unchanged.

Table 1: Means (SD) of dentin flow (nl/s) after laser treatment compared to baseline

Group	Baseline (B)	Laser Treatment (L)	Wilcoxon Signed Rank Test (P-value)
Group1	0.23 (0.12)	0.45 (0.32)	0.013
Group 2	0.21 (0.15)	0.36 (0.17)	0.018
Group 3	0.22 (0.16)	0.39 (0.13)	0.008

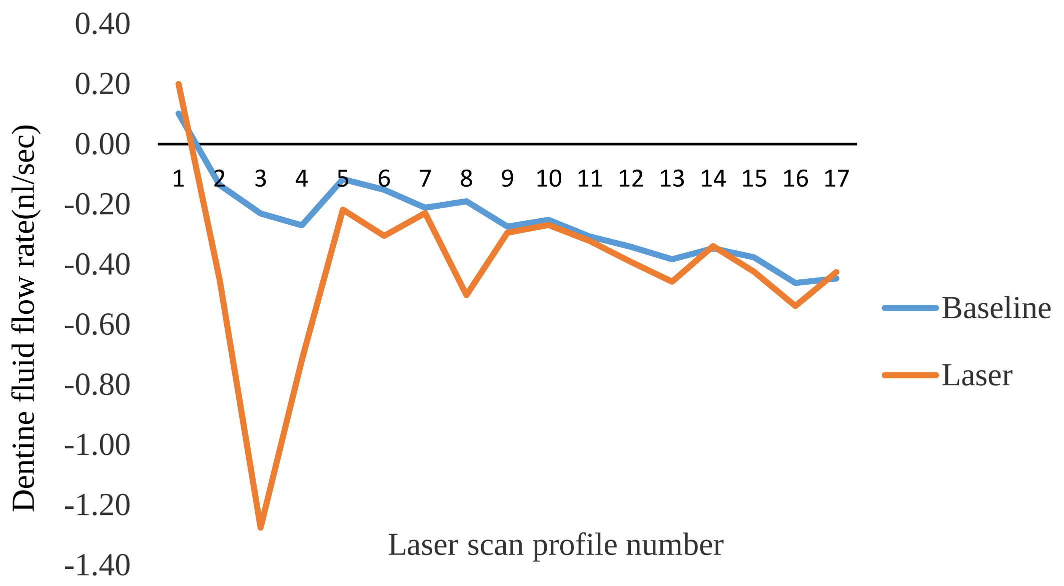

Figure 2: Dentin-fluid flow pattern before and after laser application. Positive readings indicate an inward flow, while negative readings indicate an outward flow

4. Discussion

The experimental design of the current investigation allowed the determination of the effect of Nd:YAG laser on dentin-fluid flow under a constant hydrostatic pressure of 1.3 kPa which causes continuous movement of dentinal fluids from the pulp to dentin, simulating the physiological pulp pressure (22). The effect of laser irradiation mainly depends on the distribution and amount of absorbed energy within the target tissues. However, regardless of dentin ability to disperse the irradiation energy of Nd:YAG laser, the extreme change in temperature at the irradiated spot can cause charring or melting. The automated laser irradiation was carried out in a standardized model which could be of minor clinical relevance, but was more controlled to render the samples and groups more comparable. That was also undertaken to avoid the variability produced by manual irradiation which usually results in inconsistent lased surfaces, because areas could be exposed more than once while others remain under-exposed (13-16). Furthermore, the laser optical tip was inspected and cleaned off any precipitating debris using 2000 and 4000 grit SiC paper after each 5 laser runs in order to reduce the variation between the various cutting tips, which may, if remaining unpolished, provoke new characteristics of the emitted laser beam.

To promote absorption and laser-tissue interaction, the dentinal surfaces were conditioned using black ink before each lasing (23). That also served to reduce the varying effect of laser irradiation caused by differences in adsorption resulting from natural color nuisance of the dentin samples. This measure was also undertaken to reduce variability between samples and reduce the influence of confounding factors.

Despite the assumption that postulates that deproteinization may contribute to partial elimination of collagen fibers and render affected dentin more porous, this statement was not confirmed by the results of the current study, because no significant differences in permeability as expressed in fluid-flow rate were detected compared to non-deprotenized specimens. This could not be compared with a previous study by Esteves et al. (2016), because in the latter investigation, deproteinization was assumed to have increased porosity, therefore enhancing deeper penetration of desensitizing adhesives. In the current study, low concentration of NaOCl was employed to remove the remnants of the pulp tissues only. This procedure proved to be effective without detrimental influence on dentin permeability (21).

Since fluid flow is directly proportional to the radius of tubules to the power of four, reducing the radius of the tubules by partial closure should reduce or eliminate the dentinal flow and subsequent dentinal hyper-sensitivity (25). It has been suggested that exposure to Nd:YAG laser commonly results in effects, such as melted dentin, surface crazing, slight debris formation and modifications of dentinal-tubule structure, such as partial decomposition of hydroxyapatite and carbonization of collagen in dentin (24). Melting and resolidification of dentin by Nd:YAG laser and the possible resultant permanent closure of dentinal tubules may hold the potential for permanent reduction of dentin sensitivity (12-15,26). A considerable decrease in dentinal permeability was reported as a result of Nd:YAG laser treatment (12-16). On the contrary, another report found that Nd:YAG laser has actually increased dentin permeability (13).

Whilst Goodis et al. (1997) reported that the documented decrease in fluid flow was correlated with blocking of the dentinal tubules as observed by SEM examination, complete closure did not occur, which explained the continued fluid flow at lower level (12). Schaller et al. stated that a nearly complete fusion of dentin resulting in a homogenous and plain dentin surface after irradiation with Nd:YAG laser could only be observed in one sample, and there was a trend towards partial closure of dentinal tubules at higher power settings (13).

The above quoted studies used manual laser irradiation, which could have resulted in inconsistent, incomplete and inhomogeneous irradiation of the surface, as many of the areas were unaffected and thus did not produce uniform modification of the irradiated surface. The current investigation used an automated protocol in order to produce uniform modification of dentin and examine the effect of resolidification and closure of dentinal tubules on the fluid flow. However, the result was unexpectedly an increase in fluid flow after laser irradiation. Even when only the baseline fluid flow was compared to the fluid flow measured after complete laser irradiation, still the net result was a noticeable increase in fluid-flow rate. Although complete elimination of dentin flow was not expected, a reduction in permeability could be predicted after dentin melting and resolidification. Because the cooling rate of melted hydroxyapatite after dentin irradiation could be very rapid, the dentin rearranged and recrystallized imperfectly and the laser irradiation could have vaporized the organic matrix and left voids or porous structure during the quenching phase, in addition to phase transformation (27-29).

Fluid movement within the tubules is primarily influenced by the direct effect of the stimuli. Application of air stream was found to incite outward fluid movement, attributed to possible evaporation of dentin fluid at the tubule orifice. However, thermal stimuli cause expansion (heat) or contraction (cold) of the fluid, which results in a corresponding inward or outward fluid movement (2). The laser irradiation may produce a profound heating effect (27). The pattern of change in flow demonstrated in our study confirmed the rapid cooling rate of thermally-affected dentin by laser irradiation. The flow was demonstrated as a transient inward dentinal flow during laser irradiation which lasted only from 5 to 10 seconds, followed by an immediate sharp increase in outward flow.

The partial closure of dentin tubules was previously reported to be accompanied by massive cratering on the dentin surface (13). Additionally, continued leakage and flow of fluids was attributed to the presence of minute cracks seen in the areas of resolidification (12). In a previous study (30), the permeability of dentin was reported to decrease immediately after laser irradiation, but it increased significantly after storing dentin samples for 72 hours and thus, it was suggested by the investigators that another mechanism rather than tubule occlusion could be responsible for initial decrease.

Based on the hydrodynamic theory, occlusion of dentinal tubule orifices should diminish dentinal-fluid flow. Seemingly, other mechanisms are involved in inducing dentin fluid flow otherwise the closure of dentinal tubules by resolidification of melted dentin should have solely at least reduced the dentinal flow in this study, provided that all exposed dentinal surfaces were irradiated uniformly and consistently. The initial dentin-fluid flow in intact teeth was previously observed before the temperature change reached the dentin, where the dentinal fluid is located, and was in the opposite direction to what would be expected with thermal expansion of fluid (31). Additionally, a thermally-induced structural deformation (expansion/contraction) was also reported to occur prior to any temperature changes at the dentin-enamel junction (DEJ). The mechanical stresses created by thermal deformation may have generated physical forces that stimulated a mechanically-induced dentinal-fluid flow. The study hypothesized that dentinal-fluid flow change is caused by the combined effect of thermal expansion/contraction of dentinal fluid and the mechanically-induced fluid flow triggered by tooth deformation (32). However, when dentinal tubules are exposed during cavity preparation, the net fluid shift is the result of complex interactions of several stimuli, and mechanical stress may be a minor component of fluid flow (33).

If melted and resolidified dentin deposit is assumed as the expected result of Nd:YAG laser irradiation and an assumption of consequent closure of dentinal tubules orifices is considered, then mechanisms other than those postulated by the hydrodynamic theory might be responsible for the increase in dentinal flow. Hence, further studies are warranted to correlate temperature change, tooth deformation and dentinal-fluid flow in laser-irradiated dentin slices.

5. Conclusions

Within the limitations of the current study, the Nd:YAG-laser irradiation increased the dentinal-fluid flow and thus, the use of this laser system to seal dentinal tubules and reduce dentinal-fluid flow could be challenged. Further studies are warranted to investigate the contribution of different potential mechanisms that may increase fluid flow within dentinal tubules.

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Conflict of Interests

The authors have no conflict of interests to declare.

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