Low-power pulsed Nd:YAG laser irradiation for pre-emptive anaesthesia: A morphological and histological study

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Background and aims: To determine if tooth structure or dental pulp of normal healthy human premolar teeth to be extracted for orthodontic reasons exhibit morphological or histological changes following dental anaesthesia by pulsed Nd:YAG laser and subsequent cavity preparation (CP).

Materials (Subjects) and Methods: 54 bilateral paired of human, healthy premolar teeth identified for inclusion in a trial of Nd:YAG-induced anaesthesia and subsequently extracted for orthodontic reasons, were randomly divided into 4 treatment groups: Group 1 - teeth (n=44) were irradiated with 150 µs pulsed Nd:YAG laser-1064 nm (American Dental Laser, dLase300, Sunrise Technologies Inc., Folsom, CA, USA; Average power: 1.1 ± 0.2 W, power density: 3.9+ 0.7 W/cm², area 0.28 cm², 15 Hz; energy density: 0.260± 0.047 J/cm²) +Sham EMLA (cream without active component) followed by cavity preparation (CP); Group 2 - Teeth (n=44) - were treated with EMLA + Sham Laser (1 mW 632.8-nm He:Ne laser aiming beam only) with CP; Group 3 Teeth (n=10) - were irradiated with pulsed Nd:YAG laser as above but minus CP; Group 4 (n=10) - was a Control group with teeth untreated (no Laser, EMLA or CP).

Clinical anaesthesia was assessed by electric pulp testing (EPT) and CP. Teeth in each of the 4 groups were processed for examination by i) scanning electron microscopy (SEM); ii) longitudinal undecalcified ground sectioning (LUGS); iii) light microscopy of pulpal tissues or iv) dye penetration.

Results: Both Laser and EMLA groups demonstrated no alteration to mineralized tooth structure and dentinal permeability. Mild superficial pulpal changes were found in both groups (3/18 teeth) and of no statistical difference (p>0.99, the McNemar test). Neither Laser nor the Control groups minus CP, showed pulpal changes.

Conclusions: Low-power pulsed Nd:YAG laser dose, as used in the clinical trial to induce anaesthesia, does not cause morphological damage to the mineralized tooth structure. Both Laser and EMLA groups showed minor superficial pulpal change following cavity preparation which was not statistically significant. Laser and Control groups minus preparation had no pulpal changes.

Key words: Pain • Laser • Anaesthesia • Dentistry
have an unpleasant taste and produce a numb sensation\(^5\). An alternative non-invasive, painless low power laser-induced anaesthetic with no side-effect, is now being used in increasing numbers of dental practices and has been shown to be effective in several clinical studies using low-power visible\(^6\) and infrared\(^7\) lasers to induce post-surgical and pre-operative dental anaesthesia\(^8\). Walsh\(^9\) showed Nd:YAG laser 50-100 mJ/pulse (defocused, without water spray), for 2 mins, can lead to analgesic effects (with a duration clinically of some 10-15 mins) which allowing minimal intervention treatment in several teeth, in one appointment, without the need for injected local anaesthesia. Whitters et al.\(^10\), in a crossover clinical study of pulsed Nd:YAG irradiation of maxillary teeth from 21 patients, reported a statistically significant increase in pain threshold to electrical stimulus assessed by EPT (Electric Pulp Test). In our randomized, double-blinded, comparative clinical trial\(^11\) of healthy human pre-molar teeth in the mandible or maxilla, prior to their extraction for orthodontic reasons, we reported that low power pulsed Nd:YAG laser irradiation, at average power: 1.1 ± 0.2 W, energy density: 0.260 ± 0.047 J/cm\(^2\) and duration 4 mins induced significant dental anaesthesia sufficient to permit cavity cutting.

This is consistent with conduction block of the intradental nerve as tested by EPT with a statistically significant decreased response to mechanical stimulation. The depth of laser-induced anaesthesia was not statistically different from anaesthesia induced by EMLA.

The anaesthesia induced by low-power infrared Nd:YAG laser generates high peak power pulses with deep tissue penetration and minimal heat generation\(^12\) and the consistency of such laser-induced anaesthesia reported by our clinical trial and the studies\(^7\) above has led to considerable interest in the use of such laser pulpal anaesthesia during restorative dental procedures\(^13\) – a procedure which does not induce injection-related anxiety, numbness or unpleasant tastes. The strong evidence of its effectiveness shown in our clinical trial and from animal studies is consistent with a neural basis resulting in conduction block, the mechanism by which laser-induced photo-bio-inhibition achieves dental anaesthesia.

However as photons of pulsed infrared Nd:YAG laser are negligibly absorbed by enamel and water\(^15\) they pass through the enamel to the dentine and pulp\(^16\). It is important and particularly relevant to dental procedures to ensure that this does not result in any adverse change to the tooth structure. The aims of this study were to investigate any morphological or histological changes following dental anaesthesia by low power pulsed Nd:YAG laser, at specific parameters as used in the clinical trial, and subsequent cavity preparation (CP).

### Materials and Methods

#### Ethics approval

The clinical trial\(^11\) and subsequent histological/morphological studies were carried out with Human Ethics approval # HREC/93/8/4.2 (Westmead Hospital, NSW, Australia) and informed patient consent.

#### Information on the Clinical trial

In the clinical trial\(^11\), 54 bilateral pairs of healthy (caries-free) premolar teeth, from 50 patients, were randomly assigned to one of 4 groups. Teeth in Group 1 (n=44) teeth were irradiated with 150 µs pulsed Nd:YAG laser-1064 nm.(American Dental Laser, dLase300, Sunrise Technologies Inc., Folsom, CA, USA; Average power: 1.1 ± 0.2 W, power density: 3.9± 0.7 W/cm\(^2\), area 0.28 cm\(^2\), 15 Hz; energy density: 0.260 ± 0.047 J/cm\(^2\)) +Sham EMLA (cream without active component) followed by cavity preparation (CP); Teeth in Group 2 (n=44) - were treated with EMLA + Sham Laser (1 mW 632.8-nm He:Ne laser aiming beam only) with CP; Teeth in Group 3 (n=10)- were irradiated with pulsed Nd:YAG laser as above but minus CP; Group 4 (n=10)- was a Control group with teeth treated without Laser, EMLA treatment or CP.

EMLA or Sham EMLA cream was applied onto the buccal sulcus of the test tooth via a syringe and the area isolated with an Orahesive bandage for 20 mins and Sham Laser or Laser, was applied at a scanning speed of 3mm/sec mm (spot diameter approximately= 6 mm) over the cervical half of the buccal and lingual/palatal aspects for 2 mins/surface. Anaesthetic effects were assessed by i) the changes in EPT readings from the baseline: an 15-300 volts electrical stimulus was applied via an electrode placed against the centre of the buccal tooth surface (after each tooth was dried and coated with a conducting gel -Colgate 1.23 % APF) and ii) the number of cavity completions: This involved cutting a cavity, on the cervical half of the buccal surface, which was standardized by using a 1mm x 2mm diamond flat-fissure bur and a high-speed turbine (> 200,000 rpm), under perfused water/air cooling. Cavity preparation was terminated if subjects indicated sensitivity (Fig 1a).
Sampling

Following the trial, each tooth was immediately extracted by forceps following 2% xylocaine local anaesthetic, stored in Karnovsky's fixative solution (2% formaldehyde and 2.5% glutaraldehyde in 0.1M sodium cacodylate at pH of 7.4) for two days. Each tooth was coded and randomly assigned for scanning electron microscopy (SEM), longitudinal undecalcified ground sectioning (LUGS), dye penetration or pulp histology studies. The central cervical areas of all teeth in the study, whether Laser irradiated (LI) or Sham-laser irradiated (SL), were rated by 2 independent examiners following the criteria set out below (Fig 1b).

A) Scanning Electron Microscopy (SEM)

The six bilateral paired of premolar (Laser, n=6; EMLA, n=6) selected for SEM were critically point dried, coated with gold and mounted on stubs with the long axis orientated vertically were imaged by SEM (JSM-840A-SEM-15kV) and rated for:
1. Absence of surface cratering and melting.
2. Presence of impact craters, cracks and melting surfaces.

B) Longitudinal Undecalcified Ground-Sectioning (LUGS)

The ten bilateral pairs of premolar teeth (Laser, n=10; EMLA, n=10) had their root apices removed to permit entry of fixative to the pulp and to minimize shrinkage artefact, dehydrated in graded alcohols, infiltrated with London White resin and polymerized at 60°C for 48hrs. 100-200 µm longitudinal mineral sections were prepared, polished and stained with 2% Toluidine-blue. Sections were rated by light microscopy (Wild Leitz) for:
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0- Disruption of the enamel and dentine layers.
1- Disruption of the enamel surface, including areas of carbonization and necrosis.
2- Disruption of the dentine surface, including areas of carbonization and necrosis.

C) Dye penetration-dentinal permeability
A further ten bilateral pairs of premolar teeth (Laser, n=10; EMLA, n=10) were fixed and sectioned longitudinally, the pulp chamber thoroughly cleaned by soaking in 17% EDTA followed by immersion in 5.25% sodium hypochlorite for 5mins and each tooth filled with 5% methylene blue, avoiding the tooth surface and stored for 24hr in an antiseptic container. The degree of dye penetration from the pulp chamber was the imaged by light microscopy and rated as follows:
0 - No dye penetration or penetration greater than half the depth of the dentine.
1- Dye penetration beyond half the depth of the dentine.
2- Dye penetration the full depth of the dentine.

D) Pulpal histology study–This study was further divided into two sections
a. teeth subjected to Laser or EMLA with CP (Laser, n=18; EMLA, n=18);
b. teeth subjected to Laser or Control without CP (Laser, n=10; control, n=10).
All teeth in the pulpal histology study were radiographed before and following decalcification (10% EDTA) to measure the dentine thickness (DT) at the central cervical area, and to confirm that decalcification was complete. Specimens were then sectioned at 5um and stained with H&E and at least 3 sections from each specimen examined by light microscopy and rated using the criteria modified from other pulpal studies.
0- Normal pulpal elements and cell layers underlying the zone of laser irradiation.
1- Mild superficial pulpal changes underlying the zone of laser irradiation, including: mild odontoblastic elongation and vascularization.
2- Severe superficial pulpal changes underlying the zone of laser irradiation, including loss of stroma and viable epithelial root sheath of the odontoblastic layer, severe cytoplasmic changes, and aspirated odontoblasts.

Results
Laser irradiated and EMLA-treated teeth examined by SEM, LUGS showed no alteration (Fig. 2 a & b; Fig. 3 a & b) to the mineralized tooth structure. Dye penetration studies showed no changes in dentinal permeability following treatment with LI or EMLA. A paired t-test

Fig. 2a: SEM (35x) Laser irradiated palatal enamel surface (cervical half) showed no melting or cratering. Surface roughness (SR). Bar = 1mm.
Fig. 2b: SEM (35x) EMLA-treated palatal enamel surface (cervical half) showed no surface melting, crater formation and surface roughness (SR) was possibly due to forceps extraction. Bar=1mm.

Fig. 3a: LM (10x), ULGS, Laser irradiated palatal tooth surface (cervical half) demonstrated normal enamel (E) and dentine (D) layer and enamel lamella (EL). Bar=500 μ.
Fig. 3b: LM (10x), ULGS, EMLA treated palatal tooth surface (cervical half) demonstrated normal enamel (E) and dentine (D) layer. Bar=500μ.
(p<0.001) showed no significant difference between two treatment groups.

Minimal superficial pulpal change such as mild odontoblastic elongation was found in 3/18 teeth of both groups (Fig. 4a & b) but this was not statistically significantly different between the two groups (CI: -2%-36%; P>0.99, McNemar test). Teeth from both groups but minus CP, showed no pulpal changes (Fig. 5a and b).

**Discussion**

This morphological and histological study demonstrated that pulsed Nd:YAG laser irradiation at a low-intensity, sub-ablative dose, used in the clinical trial to achieve effective dental anaesthesia, caused no alteration to mineralized tooth structure and are consistent with the findings of White et al.21) (where the irradiated dose was below the morphological threshold: 207 J/cm²) caused no detectable morphological changes in enamel and dentine.

The hypothesis that morphological blocking of the dentine tubules underlying the laser irradiated area, causes conduction block of intradental nerve resulting in dental anaesthesia, was first postulated by Nagasawa 13, 22). However, we have reported that laser-induced anaesthesia, using low-intensity laser irradiation (defined as using low laser power over a large focal area) neither damages the enamel nor causes dentine changes.

The non-significant mild superficial pulpal changes on some teeth from both Laser and EMLA groups where there was cavity preparation is consistent with studies showing that conventional high speed rotary CP can induce mild odontoblastic changes to severe superficial pulpal changes, such as aspirated odontoblasts 23). Also, consistent with this is that the mild pulpal changes of the Laser group and EMLA group teeth subjected to CP were indistinguishable.

It is interesting that White et al., 24) in an in vivo study, defined the pulpal safety dose for pulsed Nd:YAG laser irradiation of teeth (energy density<165 J/cm², dentine thickness (DT) >1mm, average power <2W, for 2 mins, total energy of 240J). This is consistent with the investigation by Parkins and Miller 8) of pulpal histology of human premolar teeth at 2days, 5days, and one week following pulsed Nd:YAG laser-induced pulpal anaesthesia. They found that a sub-ablative dose of pulsed Nd:YAG laser energy (average power 1.5W, at 15Hz, for 240 s, total energy of 360J) caused no adverse changes. In a 3 years follow-up clinical study, White et al., 25) confirmed that all Nd:YAG laser-irradiated teeth remained vital and healthy. In our study, the irradiated laser dose (low-intensity) and the recorded dentine thickness (DT...
There are significant differences in size, shape and the DT. For example, Tate et al. demonstrated low-intensity laser irradiation to induce reversible conduction block with no tooth damage and pain. In our study, we used a low-intensity irradiation of 90 times less than that used in the above studies. However, studies by Sunakawa et al. and Tokita et al. investigated the effects of high intensity pulsed-Nd:YAG laser used in the treatment of “hyper-sensitive dentine”, and compared “spot irradiation - fixed at one spot (irradiated area of 8x10^{-4} \text{cm}^2)” with “scan irradiation (moved to and fro)”. Unfortunately, the irradiated area and DT were not reported. They also examined intrapulpal nerve activity, pulpal blood flow and integrity on anaesthetized cat’s tooth crown and reported that Nd:YAG irradiation suppressed intrapulpal nerve activities, blocking nerve conduction, potentially causing pulpal ischemia and severe pulpal damage sequelae with such high laser intensity (power density ranged: 744-3730W/cm²). These studies examined feline teeth so that it is relevant to note that there are significant differences in size, shape and the DT between human and cat’s teeth. Nevertheless, they advocated that the use of high intensity to induce morphological blocking of the dentine tubule, should however be carefully considered, for the treatment of hyper sensitive dentine.

In our study, we used a low-intensity irradiation of 90 times less than that used in the above studies without adverse effect to the pulp, dentine and enamel. In addition, in the clinical trial, no patients reported untoward discomfort during laser irradiation. The lack of adverse effects, no reporting of numbness or pain and the lack of damage to the irradiated teeth are consistent with the Arndt-Schulz’s principle which states that for a given intensity below or above the therapeutic thresholds, there would be insignificant biochemical or cellular responses. Our findings are also consistent with our hypothesis that the mechanism of laser induction of dental anaesthesia results from reversible neural inhibition of the neuro-odontoblastic complex, at a sub-ablative dose which is a recognized low-intensity laser phenomenon with laser dose < 8mJ/cm².

For example Tate et al. demonstrated low-intensity laser irradiation induced blocking of the dentinal tubules due to the production of tertiary dentin by this photo-bio-stimulation of the odontoblastic cells, while Tengrungsun and Sangkla showed a direct anesthetic effect of the laser treatment of sensitive dentine which operates by reversibly blocking nerve conduction without the risk associated with intense laser irradiation. Such observations support the hypothesis that photo-bio-stimulation by low-intensity pulsed Nd:YAG laser irradiation effectively induces clinical pulpal anaesthesia with no structural changes.

While studies to investigate the effects of pulsed Nd:YAG laser on dental nerve function employing anti-P9.5 antibody - a marker for human neuron-specific protein gene product 9.5 which immune-stains sensory nerve has shown no change in P9.5 expression in sensory neurons. The current study clearly demonstrates the effectiveness of sub-ablative, low-intensity laser bio-modulation at the specific laser parameters used in the clinical trial for clinical induction of dental anaesthesia neither damaged nor changed the tooth structure or the dental pulp.

Consistent with this is the EPT evidence from our clinical trial that such pulsed laser irradiation provides anaesthesia consistent with transduction and conduction block of the dental primary afferent neurons of the neuro-odontoblastic complex at the dentino-pulpal interface. A similar scenario of conduction block has been corroborated in animal studies related to laser and pain. Our group have further shown that low intensity laser irradiation of neurons in cell culture and calibrated to deliver a comparable dose to that used in the clinical trial showed axonal varicosity formation and disruption of microtubule polymerization evidence of a direct effect on nerve conduction. In further studies we have reported a decrease in mitochondrial membrane potential and block of fast axonal flow – all significant events related to low-intensity laser induced reversible conduction block with no tooth damage.

Conclusions

Low-power pulsed Nd:YAG laser dose, as used in the clinical trial to induce anaesthesia, does not cause morphological damage to the mineralized tooth structure. Both Laser and EMLA groups observed mild superficial pulpal change in the presence of cavity preparation, but was not statistically significant. The Laser and Control groups without cavity preparation showed no evidence of pulpal changes. Thus photo-bio-modulation induced dental anaesthesia is a safe alternative to more conventional dental procedures and their associated side effects.
References


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