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**Nd:YAP laser in the treatment of dentinal hypersensitivity: an *ex vivo* study.**

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## **Abstract**

**Purpose:** The aims of this *ex vivo* study were to evaluate the effectiveness of the Nd:YAP laser in the treatment of dentin hypersensitivity, to compare the temperature rise during laser irradiation at three different dentine thicknesses, and to analyse the composition of the dentine-lased surface.

**Methods:** A total of 33 teeth were used in this study. For scanning electron microscopy (SEM) observation, 24 teeth were transversely sectioned and divided into 4 groups: group A was irrigated with EDTA; group B was irradiated by Nd:YAP laser with 180 mJ energy/per pulse, 0.9 W average power, and 5 Hz frequency (power density [PD] = 229 W/cm<sup>2</sup>); group C was irradiated by Nd:YAP laser with 280 mJ energy/pulse, 1.4 W average power, and 5 Hz frequency (PD = 356 W/cm<sup>2</sup>); and group D was irradiated by Nd:YAP with 360 mJ energy/pulse, 1.8 W average power, and 5 Hz frequency (PD = 458W/cm<sup>2</sup>). Energy-dispersive spectroscopy (EDS) analysis was performed on the same teeth evaluated for SEM observations. For temperature increase evaluation performed with thermocouples, 9 teeth were transversely sectioned at 3 different thicknesses (3 for each group) of 1, 2, and 3 mm.

**Results:** Statistical analysis showed significant changes in the diameter of the dentinal tubule orifices among all groups; EDS did not show modification of the Ca/P ratio. Temperature increase under irradiation exceeded 5.5°C only in the group D samples.

**Conclusions:** This *ex vivo* study, based on temperature recording, SEM observation, and EDS analysis, demonstrated that Nd:YAP laser at a PD of 356 W/cm<sup>2</sup>, corresponding to an average power of 1.4 W, defines the best treatment for dentine hypersensitivity in terms of compromise between efficacy of the treatment and safety of the pulp.

**Keywords:** dentinal hypersensitivity; Nd:YAP laser; scanning electron microscopy; energy-dispersive spectroscopy; thermal evaluation.

## Introduction

Dentine hypersensitivity is one of the most frequent conditions causing sharp, short pain from exposed dentinal tubules following different thermal, evaporative, tactile, osmotic, or chemical stimuli that cannot be attributed to any other dental pathology [1-6]. The prevalence rate of dentine hypersensitivity ranges from 4% to 73% [2, 4, 7], and it can affect patients of different ages [2]. The prevalence and aetiology of dentine hypersensitivity can range from incorrect tooth brushing, gingival recession, acidogenic food (mainly fruits), and factors such as attrition, abrasion, and erosion, all of which are more commonly in association [1,3,4,8-9]. The mechanism of dentine hypersensitivity is related to different theories: the most accepted is the “hydrodynamic” theory, proposed by Brannstrom and Astrom in 1964, which defines it as “the pain caused by the movement of fluid in the dentinal tubules” [4,8-12]. Dentine hypersensitivity is more frequently observed in patients with periodontal procedures [2,7], and more than 90% of hypersensitive areas are situated at the cervical region on the buccal or labial surfaces of the teeth [3]. There are two ways to reduce dentine hypersensitivity: a passive mechanism, such as precipitation of salivary proteins and calcium phosphate inside dentinal tubules or accumulation of plasma proteins and saliva contents, and an active mechanism, such as accumulation of intratubular crystalline material and consequent creation of secondary, peritubular, and tertiary dentine [3]. According to microscopic and histologic studies, dentinal tubules in hypersensitive areas of dentine are greatly increased in number and are two times wider than in nonsensitive dentine [1,4,8,12]. In 1935, Grossman proposed some rules for the treatment of this disease that are still used today: the treatment must be faster acting, effective for long periods, easy to apply, not irritating to the pulp or causing pain, not causing pigmentation to the teeth, and constantly effective [1,2,4,13]. Subsequently, clinicians and researchers have discovered additional different treatment options, classified into two groups according to their mode of application: self-applied at home by the patient or applied at the dental clinic by a dental professional [2,4]. Desensitizing toothpastes and mouthwashes containing potassium salts and low-fluoride products are most commonly applied at home, whereas current in-office treatments by dental professionals include dentine adhesives, resin, varnishes, bonding agents, restorative materials, and laser irradiation, with the latter first applied for treating dentine hypersensitivity by Matsumoto et al [14].

Lasers may be used for treatment of dentine hypersensitivity in two ways: at low-output power (photobiomodulation) or at high-output power [1, 2,7,15].

The use of laser devices is described as very efficient for dentine hypersensitivity treatment, depending on the frequency, energy, and time of irradiation; it can effectively reduce dentinal hypersensitivity for at least 4 months [2,3,7,16]. Although several theories have been proposed for explaining the effect of laser irradiation on dentine, the most accepted theory indicates the sealing or occluding ability of dentinal tubules by melting and recrystallisation of dentine [3,7,17].

The aims of this *in vitro* study were to evaluate the effectiveness of the Nd:YAP laser as a dentine desensitiser, to compare the temperature rise during laser irradiation at three different dentine depths, and to analyse the composition of the dentine-lased surface.

## **MATERIALS AND METHODS**

### **Laser device**

The laser device was LOKKI Dt (LOKKI, LOBEL Medical, France), for which the active medium is represented by a crystal of yttrium-aluminium-perovskite with trivalent ions of neodymium as a doping material. The laser wavelength was 1340 nm, in the middle infrared range, and its emission mode was pulsed at a frequency of 5, 10, and 30 Hz, with an energy per pulse between 180 mJ and 400 mJ. The pulse duration was 150  $\mu$ s, and the peak power was 2.6 kW. The laser beam was transmitted by optical fibres of 200- $\mu$ m and 320- $\mu$ m diameter, which could be switched and wrapped easily from a handpiece. The device has several operating modes available with different pulse energies and power density, but the lasing characteristics for dentinal hypersensitivity in this *ex vivo* study were 0.9 W, 1.4 W, and 1.8 W output power.

### **Sample preparation**

A total of 33 extracted caries- and restoration-free human molars were selected and collected in accordance with a protocol that satisfied the ethical standards of the Centre Hospitalier Universitaire de Nice. Each tooth was prepared in the form of dentine disc by means of an immersion in a mixture of Epoxicure Resin and Epoxicure Hardener into a specific block for 24 hours. Subsequently, the crown of each tooth was removed by transverse sectioning using a high-speed disk (BUEHLER-ISOMET 2000) and coated with water at the level of the cusps to expose the dentin surface beneath the enamel surface. Then, all dentine specimens were horizontally abraded using a carborundum disk and water (DAP-U system) to obtain a polished surface. Finally, dentine specimens were immersed in EDTA 17% solution for 5 minutes to remove the smear layer and open the dentinal tubules, they were then rinsed with water.

### **Microscope observation**

Twenty-four teeth were used for scanning electron microscopy (SEM) observation under ambient conditions.

At first, all dentine specimens were graphited by a pencil to enhance the absorption of the laser beam and to reproduce the same treatment normally performed in *in vivo* conditions.

To observe the dentine surface by SEM, and particularly the possible changes after laser irradiation, the specimens were randomly divided into 4 groups:

- **Group A** (n = 6) control group (EDTA 17% irrigation)
- **Group B** (n = 6) irradiated by Nd:YAP laser by the following parameters: output average power = 0.9 W, energy/pulse = 180 mJ, frequency = 5 Hz (power density = 229 W/cm<sup>2</sup>), with 30 passages of 2 seconds each delivered in two different directions (vertically and horizontally) at a 30° angle in noncontact mode at a distance of 1 mm to the irradiated surface (Figure 1)

- **Group C** (n = 6) output average power = 1.4 W, energy/pulse = 280 mJ, frequency = 5 Hz, (power density = 356 W/cm<sup>2</sup>), with 30 passages delivered in the same way as for group B
- **Group D** (n = 6) output average power = 1.8 W, energy/pulse = 360 mJ, frequency = 5 Hz (power density = 458 W/cm<sup>2</sup>) with 30 passages delivered in the same way as for groups B and C

All specimens were observed with a JEOL scanning microscope (JSM-5310LV35, Jeol Ltd, Tokyo, Japan) before and after the laser irradiation to analyse the changes to the dentinal tubules.

### **Thermal Evaluation Tests**

For temperature increase evaluation performed with thermocouples, 9 teeth were transversely sectioned at 3 different thicknesses (3 for each group) of 1, 2, and 3 mm and then randomly distributed into three groups, on the basis of the power used, for measuring thermal evaluations: group A, 0.9 W; group B, 1.4 W; and group C, 1.8 W. Dentine ablation was performed transversely with a high-speed disk (BUEHLER-ISOMET 2000) to obtain three different dentine disk thicknesses of 1 mm, 2 mm, and 3 mm for each group; thermal evaluation tests were performed by thermocouples (Pico Technology, TC-08 USB, United Kingdom). Each thermocouple was put under the dentine disks and connected to a computer (Picolog Recorder Software). Finally, data were collected by applying the laser handpiece 30 times at a 30° angle in noncontact mode to the external surface of the dentine.

### **SEM Evaluation**

The SEM images (original magnification 500×, bar = 50 μm) were registered under ambient conditions, for each sample, without coating, and randomly selected: each was divided into 10 quadrants. Each SEM image was then measured by a ruler (50 μm = 3.9 cm) on the computer's screen (12.1 WXGA Acer CrystalBrite LCD). On the screen, a 50-μm bar corresponded to the size of 3.9 cm, so if the size of the tubule diameter on the screen was 0.3 cm, its real size was calculated as 3.84 μm, based on the following equation:

$$X \mu\text{m} : 0.3 \text{ cm} = 50 \mu\text{m} : 3.9 \text{ cm}$$

where X is the real diameter of the nontreated tubule, 0.3 cm is its size on the screen, 50 μm is the bar size, and 3.9 cm is the size of the bar on the screen.

The same equation was used to determine the real diameter of the treated tubule; for example, where its size was 0.1 cm, its real size was calculated as 1.28 μm on the basis of the same equation:

$$X \mu\text{m} : 0.1 \text{ cm} = 50 \mu\text{m} : 3.9 \text{ cm}$$

where X is the real diameter of the nontreated tubule, 0.1 cm is its size on the screen, 50 μm is the bar size, and 3.9 cm is the size of the bar on the screen.

### **Energy-Dispersive Spectroscopy (EDS) analysis**

EDS analysis was realised on the same teeth evaluated for SEM observations.

Three specimens were prepared as control group: the first specimen was only fresh dentine, the second was irrigated with EDTA 17% solution, and the third was graphited by a pencil to perform the microanalysis test of the dentine surface before and after laser irradiation.

By randomisation, three lased specimens of each group were selected to evaluate the micro-analysis test of the dentine-lased surface and also for to observe the chemical composition of dentine after laser irradiation. For performing the microanalysis, we used EDS associated with an SEM (PHILIPS XL30 ESEM) connected through INCA software.

In this test, the electrons inside the device collided with the electrons within the sample and caused some to be knocked out of their orbits. The vacated positions were filled by higher-energy electrons, which emit x-rays in the process, and by analysing the emitted x-rays, the elemental composition of the sample can be determined.

This test was conducted to compare the interstitial composition of the intertubular and peritubular dentine and the contents of the area of the melted bubbles before and after laser treatment. Quantitative element analysis of Ca, P, O, and C was carried out by EDS. On each specimen, one spot measurement was made in the peritubular dentine, intertubular dentine, and bubble area in two different ways: (1) qualitative analysis to determine all of the elements present on the surface of the dentine by obtaining a spectrum and (2) quantitative analysis to locally determine the composition of the target tissue (in weight % or atomic %) by obtaining a table of value.

### **Statistical analysis**

The average diameter of the tubules before and after laser irradiation was measured, and each specimen's photo was divided into 10 rectangles. In each rectangle, the average of the diameters of each tubule was measured in micrometres depending on the magnification of the SEM image (500×).

Statistical analysis was performed using GraphPad Prism Statistic Software using an analysis of variance (ANOVA) test for multiple comparisons.

## **RESULTS**

### **SEM observation**

Group A showed the opening of the dentinal tubules with an absence of smear layer due to the etching effect of the 17% EDTA solution (Figure 2a). Specimens of group B showed occlusion of a few dentinal tubules with narrowing of their diameter and cracking in the dentine (Figure 2b); moreover, most of the dentinal tubules showed micro-morphological features similar to those shown in the first group.

Group C specimens (Figure 2c) showed the obliteration or occlusion of more dentinal tubule orifices and showed melted bubbles on the dentine surface view, with more and wider cracks or fissures in comparison with group B specimens.

The specimens treated with the laser at 1.8 W power (group D; Figure 2d) showed the formation of more and wider cracks or fissures and a melted-bubbles areas with occluding of more dentinal tubule

orifices. However, most of the dentinal surfaces of group D showed micro-morphological features similar to those of the group C.

The mean diameters and standard deviations of the dentinal tubules of every group before (control) and after (laser) treatment are reported in Table 1.

| <b>Dentinal tubule diameter (<math>\mu\text{m}</math>)</b> |   |                                       |
|--|---|---------------------------------------|
|  | <b>Control (Mean<math>\pm</math>SD)</b> | <b>Laser (Mean<math>\pm</math>SD)</b> |
| <b>Group B (0.8 W)</b>                                     | 2.267 $\pm$ 0.175                       | 1.41 $\pm$ 0.169                      |
| <b>Group C (1.4 W)</b>                                     | 2.277 $\pm$ 0.241                       | 1.133 $\pm$ 0.083                     |
| <b>Group D (1.8 W)</b>                                     | 2.317 $\pm$ 0.155                       | 1.045 $\pm$ 0.111                     |

**Table 1:** Average diameter of different laser-irradiated groups compared with control groups

No difference was found among the different control samples ( $p = 0.8950$ ). ANOVA statistical test showed a very significant statistical difference between the laser and control groups ( $p < 0.0001$ ) and between the 0.9 W and 1.8 W laser-treated samples ( $p = 0.0004$ ), a significant difference between the 0.9 W and 1.4 W laser-treated samples ( $p = 0.0046$ ), and no statistical difference between the 1.4 W and 1.8 W laser-treated samples ( $p = 0.4668$ ; Figure 3).

### **EDS analysis**

EDS element analysis showed different Ca, P, O, and C contents in the peritubular and intertubular dentine surfaces. The relative Ca and P content increased from peritubular dentine toward intertubular dentine, whereas the relative C content increased accordingly with the graphited dentine surface. The Ca/P ratio in peritubular dentine was significantly higher than in intertubular dentine. The O/C ratio in the intertubular dentine was much higher than in peritubular dentine and the melted-bubble area, whereas the Ca content in the melted-bubble area was higher than in the peritubular and intertubular dentine (Table 2).

The different power densities did not affect the mass composition or the atomic components, with the exception of the small areas, where the melted dentine was observed.

Figure 4 shows the results of the EDS microanalysis test for detecting the composition of peritubular dentine (Figure 4 a-b), melted-bubble area (Figure 4 c-d), and intertubular dentine of freshly nontreated dentine (Figure 4 e-f).



|                       | Oxygen | Calcium | Carbon | Phosphorus |
|-----------------------|--------|---------|--------|------------|
| <b>Untreated PTD</b>  | 57.03  | 19.94   | 8.66   | 13.18      |
| <b>Untreated ITD</b>  | 58.85  | 16.97   | 10.98  | 11.90      |
| <b>Graphited PTD</b>  | 24.05  | 5.97    | 65.01  | 3.94       |
| <b>Graphited ITD</b>  | 19.78  | 5.63    | 69.86  | 3.68       |
| <b>Group A PTD</b>    | 20.78  | 12.63   | 58.01  | 7.52       |
| <b>Group A ITD</b>    | 25.53  | 8.08    | 60.92  | 4.50       |
| <b>Group B Bubble</b> | 42.76  | 32.32   | 14.75  | 9.71       |
| <b>Group B PTD</b>    | 45.22  | 31.98   | 10.04  | 12.21      |
| <b>Group B ITD</b>    | 52.52  | 18.06   | 16.39  | 12.35      |
| <b>Group C Bubble</b> | 58.78  | 15.71   | 12.86  | 11.30      |
| <b>Group C PTD</b>    | 59.99  | 20.32   | 8.22   | 10.77      |
| <b>Group C ITD</b>    | 56.67  | 20.64   | 7.83   | 13.88      |
| <b>Group D Bubble</b> | 45.02  | 33.87   | 8.13   | 12.53      |
| <b>Group D PTD</b>    | 61.94  | 15.66   | 9.31   | 12.13      |
| <b>Group D ITD</b>    | 55.66  | 17.60   | 12.77  | 12.97      |

Table 2: EDS microanalysis detecting the composition of samples as the atomic percentage for the main components (oxygen, carbon, phosphorus, and calcium): minor components are not reported. ITD, intertubular dentine; PTD, peritubular dentine.

### Thermal Evaluation Test (Table 3)

Changes in temperature were measured at 1 mm, 2 mm, and 3 mm of dentine depth at room temperature.

Group B showed temperature rises of 3.2°C at 1 mm, 3.375°C at 2 mm, and 2°C at 3 mm of dentine depth. Group C showed temperature rises of 4.75°C at 1 mm, 4°C at 2 mm, and 2.375°C at 3 mm of dentine depth. Group D showed temperature rises of 13.50°C at 1 mm, 4.875°C at 2 mm, and 5°C at 3 mm of dentine depth.

Temperature variations for groups B, C, and D are reported in Table 3. Statistical analysis revealed a significant difference among the groups ( $p = 0.0026$ ) and particularly for 1-mm and 3-mm thickness; for 1-mm thickness, the difference was significant between groups B and C ( $p = 0.0279$ ), between groups B and D ( $p = 0.0024$ ), and between groups C and D ( $p = 0.0115$ ), whereas for 3-mm thickness, the difference was significant between groups B and C ( $p = 0.0015$ ) and between groups B and D ( $p = 0.0016$ ).

| <b>Temperature variations (°C) (Mean±SD)</b> |                   |                   |                   |
|--|-------------------|-------------------|-------------------|
|  | <b>1-mm depth</b> | <b>2-mm depth</b> | <b>3-mm depth</b> |
| <b>Group B (0.8 W)</b>                       | 3.2±1.034         | 3.375±0.25        | 2.00±0.00         |
| <b>Group C (1.4 W)</b>                       | 4.750±0.289       | 4.00±0.00         | 2.375±0.250       |
| <b>Group D (1.8 W)</b>                       | 13.50±1.000       | 4.875±0.250       | 5.00±0.00         |

**Table 3:** Thermal elevation in groups A, B, and C at different depths, expressed in °C.

## **DISCUSSION**

Dentine hypersensitivity is one of the most common problems experienced by dental patients. It consists of the exposure of dentinal tubules in the cervical region of the teeth: for this reason, its successful treatment requires the complete occluding of the opened dentinal tubules.

Many kinds of treatment have been proposed for reducing or eliminating this disease, including chemical agents (fluoride, potassium nitrate, strontium acetate and chloride, calcium sodium phosphosilicate) that are often included in specific toothpastes and mouthwashes, dentine adhesive sealers (oxalic acid and resins) applied by dentists or dental hygienists, and laser irradiation [18].

While some authors have suggested using photobiomodulation (PBM) [19-20], other studies have proposed treating dentinal hypersensitivity with a combined laser therapy by coupling the advantages of PBM with the use of a high-power diode laser [21].

The success of PBM might be related to a direct effect on the pulp tissue, which is able to reach analgesic and anti-inflammatory results (prompt answer) as well as the formation of tertiary dentine (follow-up) [22], and this might explain the reason for the higher success of PBM when compared with laser fluoride application [23] and also the absence of additional benefits of desensitizing toothpaste and PMB association when compared with laser alone [24].

Regarding the laser utilisation at high power, different wavelengths at different parameters have been investigated by *in vitro*, *ex vivo*, and *in vivo* studies.

Although Maleki-Pour demonstrated, by means of an *in vitro* study, a reduction in the number and diameter of dentinal tubules after irradiation by Nd:YAG laser at 0.25 W and 0.5 W [25] and Farmakis proposed using the same laser at 1 W [26], Abed showed that the application of resin is more effective than the Nd:YAG laser in minimizing the number and diameter of exposed dentinal tubules [27].

To minimise the possible thermal damage induced by laser irradiation, Xiao proposed the use of a water-cooled Nd:YAG laser, demonstrating dentinal tubule occlusion similar to that of the Nd:YAG laser [28]. However, in an original study based on the pulse oximetry system, Birang showed that the

diffusion of heat induced by the Nd:YAG laser into the pulp within the limit of the desensitisation parameters caused no irreversible damages in the dental pulp [29].

The use of erbium lasers has also been proposed by several authors, probably because both Er:YAG and Er,Cr:YSGG are considered “cold lasers” and thus safe for pulp vitality.

Belai compared Er:YAG and CO<sub>2</sub> effects on dentinal tubules and concluded that, even if SEM photomicrographs indicated melted areas around the exposed dentinal tubules and a significantly greater percentage of tubular occlusion in both of the treated groups, the CO<sub>2</sub> laser group showed an evident thermal effect compared with the Er:YAG group [30].

Birang, in an *in vivo* study, compared Er:YAG and Nd:YAG by assessing the patient’s pain using a visual analogue scale and concluded that both lasers had an acceptable therapeutic effect, even if the Nd:YAG laser was more effective than the Er:YAG laser in reducing pain [31].

Because of their low cost, reduced size, and additional advantages, diodes may be considered today the most used dental laser devices. Their effectiveness in the treatment of dentinal hypersensitivity has also been studied.

Pourshahidi compared the diode laser to the Er,Cr:YSGG laser in an *in vivo* study and concluded that the latter was a better choice for the treatment of dentine hypersensitivity in the short term [32].

Saluja, in an SEM analysis, compared the Nd:YAG, CO<sub>2</sub> and diode lasers on exposed human dentinal tubules and concluded that all the three lased groups showed a highly statistically significant result as compared with the nonlased group. The Nd:YAG laser was found to be most effective, followed by the CO<sub>2</sub> laser, and the 810-nm diode laser was found to be least effective. Morphologic changes such as craters, cracks, and charring effect of the dentine were seen most frequently with the use of the CO<sub>2</sub> laser [33].

The choice to use the Nd:YAP laser in this study was made because of the function of its wavelength, particularly its interaction with biological tissues.

In fact, even if this wavelength (1340 nm) is very close to that of Nd:YAG (1064 nm), it is more absorbed by water, which is one of the most common constituents of dentine. This specific absorption may explain the effectiveness of the Nd:YAP laser to occlude the dentinal tubules and also, as the results of our analysis test demonstrated, how the Nd:YAP laser can transform the mineral constituents of the dentinal tubules that melted into amorphous particles in the form of bubbles, without significant changes in the element distribution between irradiated and nonirradiated dentine.

In SEM images, nontreated peritubular dentine showed a smooth collar around the tubules, but after treatment with the Nd:YAP laser, the peritubular dentine was significantly removed. Moreover, laser can cause melting and recrystallisation of the dentine-lased surface, narrowing of the diameter, and occlusion of the dentinal tubules with cracks, melted bubbles, and deeper and wider cracks with higher laser parameters.

According to this *ex vivo* study, the use of Nd:YAP laser irradiation for reduction of tooth sensitivity is safe without causing significant damage to the dental pulp. In the case of 1-mm dentine thickness, the

application time should be less than 5 seconds with a power density of 1.8 W to be sure not to injure the pulp.

According to the study by Zach on the pulp response to externally applied heat, a temperature rise less than 5.5°C generated only minimal intrapulpal changes, and the effect was reversible [20]. The Nd:YAP is safe when used in the pulsed mode, and a resting period is highly necessary to prevent temperature buildup and thermal damage to the surrounding adjacent tissues [17]. The study by Armengol et al [34] showed that the Nd:YAP laser produced a significantly higher temperature rise than the Er:YAG at different dentine thicknesses. They also found that the mechanism of Nd:YAP laser irradiation that decreases tooth hypersensitivity, by its thermal and occlusive effect with different power densities, can cause different changes on the dentine surface. These alterations include melting, recrystallisation, cracking, or fissuring on the dentine-lased surface; narrowing of the diameter of the dentinal tubules; melted bubbles; and dentine desiccation after laser irradiation.

This *ex vivo* study indicates that the temperature response to the Nd:YAP laser with a power density of 1.8 W at 1 mm of dentine thickness is too high, and *in vivo* conditions are not compatible with the vitality of the pulp.

## **CONCLUSION**

This *ex vivo* study, based on temperature recording, SEM observation, and EDS analysis, demonstrated that the Nd:YAP laser at power of 1.4 W can be used in the treatment of dentine hypersensitivity. Moreover, because of its safety in terms of temperature elevation and, consequently, the pulp integrity, it may be successfully used in clinical practice without side effects.

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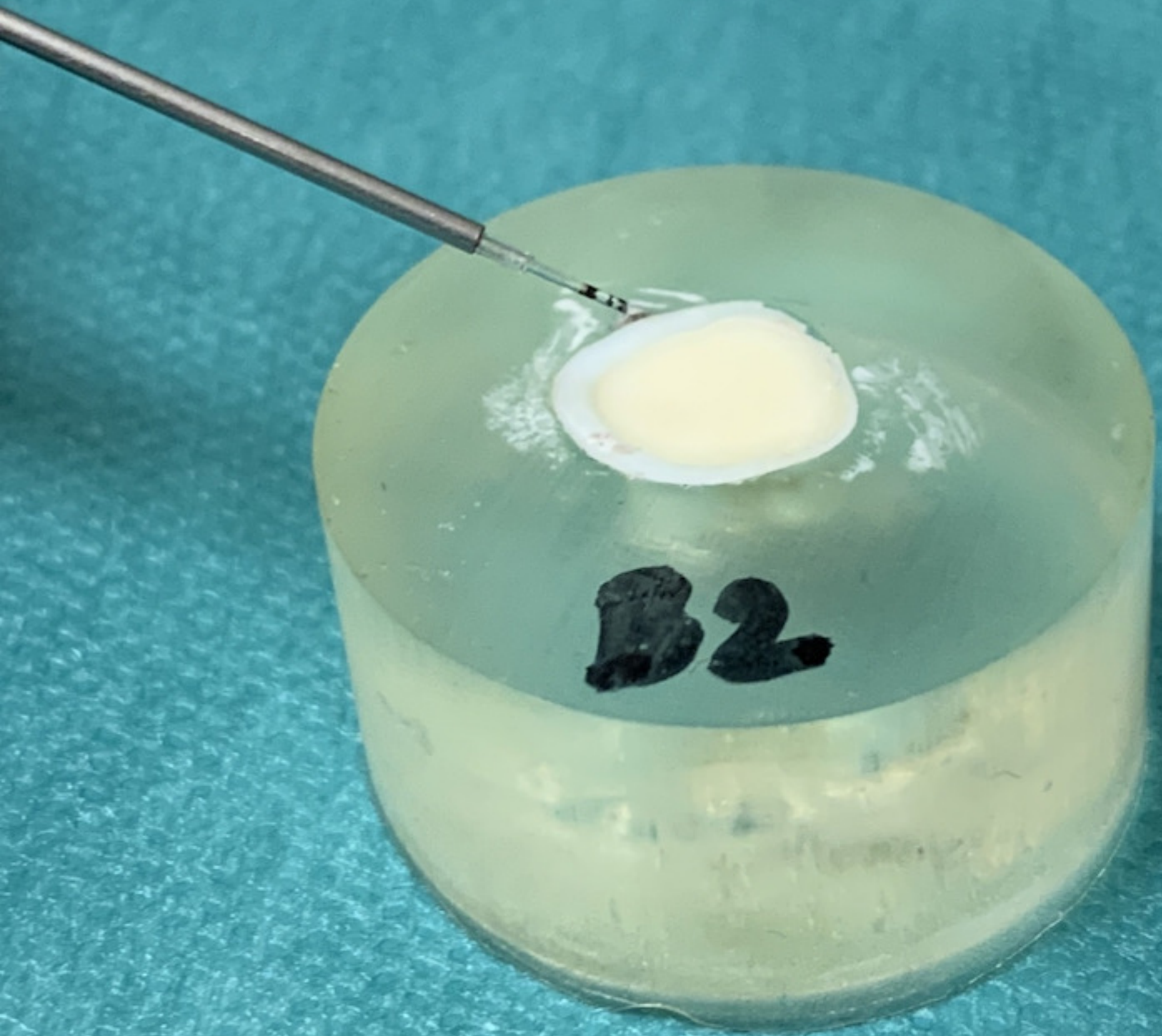
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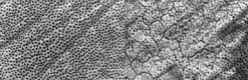
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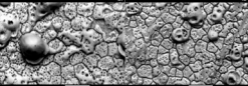


20kV X500

50µm 000000 **a**

20kV X500

50µm 000000 **b**



20kV X500

50µm 000000 **c**

20kV X500

50µm 000000 **d**

## Tubules diameter

