

Comparative assessment of morphological alterations in the dentin surface by sodium fluoride, 980 nm diode laser, and their combined application for use in the treatment of dentin hypersensitivity: An *in vitro* scanning electron microscopy study

ABSTRACT

Context: Lasers have been used as one of the most promising new modalities for dentin hypersensitivity. A synergistic action of lasers in association with desensitizing agents can favor the permanence of the desensitizer for a longer time than when they are used alone.

Aims: The present study was aimed to evaluate the morphological characteristics of dentin surface by scanning electron microscopy after fluoride application and irradiation with 980 nm diode laser and their combined application.

Materials and Methods: Sixty extracted human premolars were collected, prepared, and randomized into three groups. Group A involved tooth treated with sodium fluoride (NaF); Group B involved tooth irradiated by 980 nm GaAlAs (diode) laser; and Group C consisted of tooth sections irradiated by a diode laser with prior application of NaF. The morphological alterations were evaluated and compared using a scanning electron microscope.

Statistical Analysis: The Kruskal-Wallis test was used for multiple group comparisons, and the *post hoc* Dunn's multiple comparison test was used for intergroup comparisons.

Results: Group C, the fluoride-laser treated group, showed the lowest diameter of dentinal tubules ($0.077 \pm 0.021 \mu\text{m}$) and had significantly the lowest number of open dentine tubules. Dunn's multiple comparison tests revealed that Group C had statistically significant tubule diameter reduction and had the lowest number of open tubules compared to both Group A and Group B ($P < 0.0001$ and $P = 0.0007$, respectively). In addition, Group C had significantly greater intertubular distance when compared to both Group A and Group B ($P < 0.001$).

Conclusion: The 980 nm diode laser presents a promising new potential for treating dentin hypersensitivity, both alone and in conjunction with desensitizing agents. However, clinical trials are required to use it in regular clinical practice.

Keywords: Dentin hypersensitivity, diode laser, scanning electron microscopy, sodium fluoride

INTRODUCTION

Dentin hypersensitivity is characterized by short, sharp pain arising from exposed dentin in response to thermal, evaporative, tactile, osmotic, or chemical stimuli and which cannot be ascribed to any other form of dental defect or pathology.^[1] It affects the buccal surfaces of teeth toward the cervical aspect and is extremely common in premolars and canines.^[2] Occlusal disharmony, gingival recession owing to periodontal disease or periodontal therapy, trauma from toothbrushing, and abfraction lesions are the most common causes of dentin exposure.^[3]

**SHWETA TANWAR^{1,2}, AMIT KUMAR³,
RAJESH CHETIWAL³**

¹Department of Periodontics, PDM Dental College and Research Institute, Bahadurgarh, Haryana, India, ²Scientist-C, Indian Council of Medical Research, New Delhi, India, ³Department of Medicine, ESIC-PGIMSR, New Delhi, India

Address for correspondence: Dr. Shweta Tanwar, Indian Council of Medical Research, New Delhi, India. E-mail: drshweta_90@yahoo.com

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Treatment options for desensitizing hypersensitive teeth are desensitization of the nerve, coverage of the dentinal tubules, or endodontic treatment. Topical desensitizing agents such as sodium fluoride gel (NaF) are the most common forms of conventional therapies. The insoluble calcium fluoride crystals are precipitated within the tubules causing mechanical occlusion. However, due to the stresses of the oral environment, its action decreases with time. Various other agents used to relieve pain have relied upon the coagulating effects or occluding properties of strontium chloride, stannous fluoride, potassium oxalate, calcium hydroxide, glutaraldehyde, bonding agents, and resins.

In addition, several kinds of lasers have been used to treat dentinal hypersensitivities, such as He-Ne laser, GaAlAs laser (gallium/aluminum/arsenide), Nd: YAG, and CO₂ lasers. Combining laser irradiation with chemical agents such as NaF can significantly enhance the treatment efficacy. The GaAlAs 980 nm diode laser is a high-energy laser, and several studies have reported its use in endodontic treatment and root canal disinfection. However, the effect of 980 nm diode laser in causing structural alterations in dentin surface has not been studied well. Considering these facts, this *in vitro* study was undertaken to investigate the morphological changes in dentinal tubules by scanning electron microscopy (SEM) after NaF application, after irradiation with 980 nm diode laser, and after their combined application.

MATERIALS AND METHODS

The study protocol was approved by the institutional ethics committee (vide No. PDMDCRI/2013/309). Extracted human premolars were collected, prepared, and randomized into three groups. Grossly decayed tooth with root caries, restored tooth, and fractured tooth were excluded from the study.

Preparation of specimen

The extracted teeth were stored in saline solution to avoid dehydration. The tooth was cleaned to remove debris and calculus from the root surface by doing root planing. Each tooth was sectioned vertically, and then, the anatomical crown was removed using a diamond saw. Furthermore, the roots were amputated at the apical (lower) end of the root trunk. The remaining middle portion was prepared as a sample. The cementum from the root was removed using plain tungsten carbide bur. Specimen of size 5 mm × 5 mm was prepared from each tooth using disc cutting. The smear layer was removed by immersing the specimen in 17% ethylenediaminetetraacetic acid (EDTA) for 5 min and then sodium hypochlorite for another 5 min. All specimens were then washed with distilled water and randomized into three groups. Each group included 20 specimens.

- Group A: Specimens were painted using an applicator brush with a thin film of 5% NaF varnish

- Group B: Specimens were irradiated by 980 nm GaAlAs (diode) laser with continuous-wave emission in noncontact mode at 1 W for 10 s using 200 μ m fiberoptic tip
- Group C: Specimens were first painted by a thin film of 5% NaF varnish using an applicator brush and then irradiated by 980 nm GaAlAs (diode) laser with continuous-wave emission in noncontact mode at 1W for 10 s using 200 μ m fiberoptic tip.

All the specimens were subjected to dehydration process irrespective of their groups by a series of graded ethanol solutions 25% (20 min), 50% (20 min), 75% (20 min), 95% (30 min), and 100% (60 min). The specimens were then mounted on an aluminum stub and luted to the surface with the help of adhesive tape. Sputter coating of each specimen was done with an ultra-thin film of gold about 20 nanometers in thickness. The specimens were examined in a ZEISS EVO18 scanning electron microscope to assess the morphologic characteristics of the dentin surface. The specimens were viewed under $\times 2000$ and $\times 5000$ in SEM analysis. The intertubular distance and tubule diameter were measured using ImageJ software.

Image display and recording

The output signals from the secondary electron detector were amplified and then transferred to the display unit, thus forming an SEM image. The image recorded was stored in a digital format (electronic file).

Statistical analysis

The statistical analysis was performed using GraphPad Prism V6 software (GraphPad Software Inc, San Diego, CA, USA). The Kruskal–Wallis test was used for multiple-group comparisons, and *post hoc* Dunn's multiple comparison test was utilized for intergroup comparisons.

RESULTS

Table 1 shows the comparison of diameters of dentinal tubules, number of open tubules, and intertubular distance in different study groups. Group C, the fluoride-laser treated group, showed the statistically significant lowest diameter ($0.077 \pm 0.021 \mu\text{m}$) with $P < 0.0001$. In addition, Group C had the significantly lowest number of open dentine tubules as compared to Group A and Group B ($P < 0.0001$). On comparing the intertubular distance in different groups, Group C was found to have significantly the greatest intertubular distance between the dentine tubules ($P < 0.0001$).

Figure 1a and b show the SEM photomicrographs of dentin surface treated with NaF (Magnification $\times 2000$ and $\times 5000$, respectively), revealing partially occluded dentinal tubules

in all specimens. SEM images of Group B are depicted in Figure 2a and b, which also reveal partially occluded dentinal tubules in all specimens. Group C specimens showed 20% complete occlusion of dentinal tubules, and the remaining 80% were partially occluded [Figure 3a and b].

On intergroup comparison of dentinal tubules diameter using Dunn's multiple comparison test [Table 2], the mean rank difference between Group A and Group B was 20.00, between Group A and Group C was 40.00, and between Group B and Group C was 20.00. When comparison was made between Group A and Group B, Group B had a significantly lower dentinal tubule diameter ($P = 0.0009$). In contrast, when compared to Group A and Group B, Group C had statistically significant tubule diameter reduction ($P < 0.0001$ and $P = 0.0009$, respectively).

Table 3 shows the intergroup comparison of a number of open tubules. When a comparison was made between Group A and Group B, there were a significantly lower number of open

tubules in Group B ($P = 0.0013$). Group C had the lowest number of open tubules as compared to both Group A and Group B ($P < 0.0001$ and $P = 0.0007$, respectively), which was highly significant.

The intergroup comparison of intertubular distance is done in Table 4. The mean rank difference between Group A and Group B was -8.350 , between Group A and Group C was -34.02 , and between Group B and Group C was -25.67 . In comparison between Group A and Group B, the difference in intertubular distance was statistically insignificant ($P = 0.3913$), whereas Group C had greater intertubular distance when compared to both Group A and Group B, and the difference was highly significant ($P < 0.0001$ and $P < 0.0001$, respectively).

Table 5 shows the comparison of smear layer, melting areas, and microcracks in different groups under SEM photomicrograph. Group A specimens showed the presence of smear layer in 15% of specimens, whereas Group B and

Table 1: Comparison of dentinal tubules diameter, number of open tubules, and intertubular distance in different groups

Variable	Group A	Group B	Group C	Kruskal-Wallis	P
Diameter of dentinal tubules (μm)	0.709 ± 0.120	0.218 ± 0.033	0.077 ± 0.021	52.46	<0.0001
Number of open tubules	23.3 ± 2.319	18 ± 1.685	5 ± 2.937	51.79	<0.0001
Intertubular distance (μm)	8.535 ± 1.052	9.32 ± 0.991	12.67 ± 1.315	41.27	<0.0001

Values are presented as mean \pm SD. SD: Standard deviation

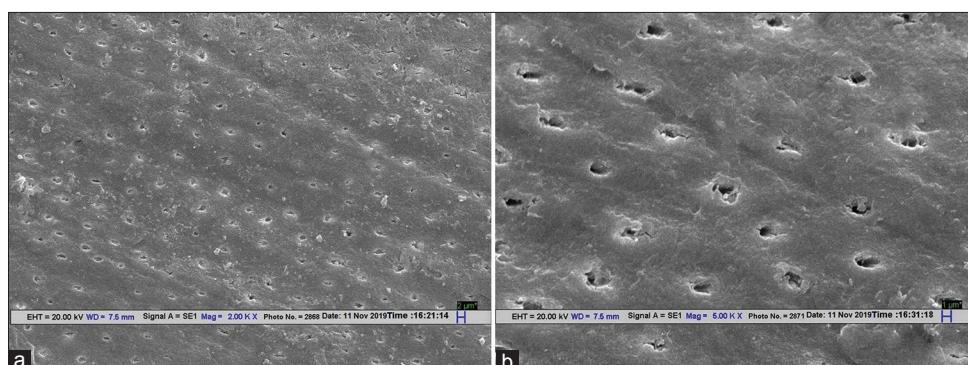


Figure 1: Scanning electron microscopy photomicrograph of dentin surface treated with NaF at $\times 2000$ (a) and $\times 5000$ (b)

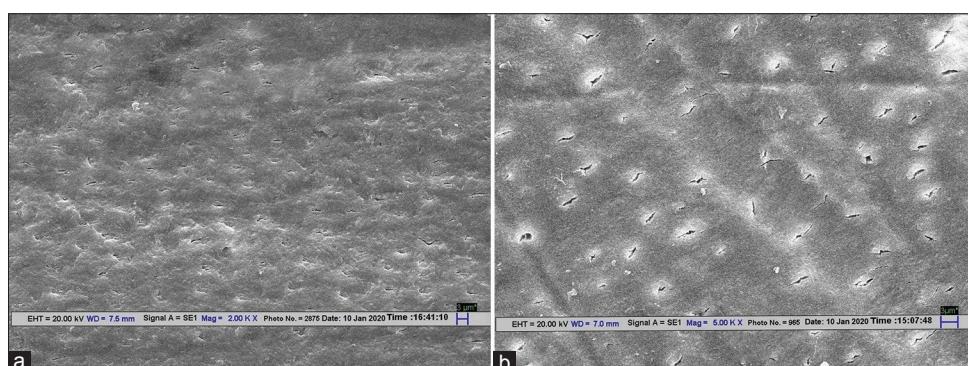


Figure 2: Scanning electron microscopy photomicrograph of dentin surface treated with 980 nm diode laser at $\times 2000$ (a) and $\times 5000$ (b)

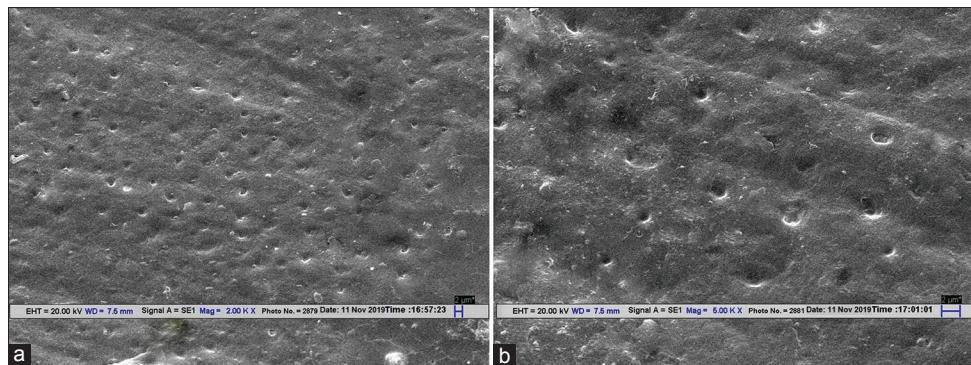


Figure 3: Scanning electron microscopy photomicrograph of dentin surface treated with combination of NaF and 980 nm diode laser at $\times 2000$ (a) and $\times 5000$ (b)

Group C showed the presence of smear layer in one specimen each. In comparing melting areas, Group A showed a complete absence of melting areas on the dentin specimen, whereas all Group B specimens showed melting areas on the dentin specimen (100%). In Group C, 90% of the dentin specimens showed the presence of melting areas. Microcracks were absent in all the specimens of Group A, whereas Group B and Group C showed the presence of microcracks on 50% and 30% of dentin specimens, respectively.

DISCUSSION

Dentin hypersensitivity is a common oral health problem globally and is an important issue to be addressed, both from a diagnostic and treatment perspective.^[4] Treatment to relieve dentin hypersensitivity demands effective and robust dentin occlusion for instant and lasting action. Lasers are the new innovation in the treatment of dentin hypersensitivity. The mechanism of action of lasers in treating dentin hypersensitivity is not very clear. The proposed mechanisms of action of lasers include the occlusion of dentinal tubules, altering the neural transmission in tubules, and coagulation of the proteins inside the dentinal tubules, thereby blocking fluid movement.^[5-7]

The present *in vitro* study was designed to evaluate the efficacy of 5% NaF, 980 nm diode laser, and their combination on the morphological characteristics of dentin surface. Dentin hypersensitivity affects premolars more than other teeth, and the buccal surface is more frequently involved than other surfaces, as described by Porto *et al.* and Madruga *et al.*^[8,9] The same observations have been made by Cummins and Chu *et al.*, the reason of which can be attributed to the diameter of dentin tubules being more at the buccal surface, which gradually tapers off toward the pulp side.^[3,10] For the same reason, the buccal surface of extracted human premolars was prepared as a specimen to look for the efficacy of various treatment modalities.

In the present study, the anatomical crown and the apical end of the tooth were amputated to derive the specimen

Table 2: Intergroup comparison of diameters of dentinal tubules

Dunn'smultiple comparison test	Mean rank 1	Mean rank 2	Mean rank difference	P
Group A versus Group B	50.50	30.50	20.00	0.0009
Group A versus Group C	50.50	10.50	40.00	<0.0001
Group B versus Group C	30.50	10.50	20.00	0.0009

from the cervical third of the tooth. This was done to specifically include the cement-enamel junction (CEJ) in the dentin specimen as this is the most prone area to develop hypersensitivity. The CEJ is an important anatomical structure of the tooth susceptible to various pathological changes such as root surface caries and cervical erosion, resorption, and abrasion. The un-deminerlized dentin specimens under SEM show the presence of a smear layer that looks like an amorphous, irregular, and granular surface. The smear layer is composed of dentin, pulp tissue remnants, odontoblastic processes, and sometimes bacteria. It acts as a physical barrier that interferes with the adhesion and penetration of sealer into dentinal tubules. The purpose of removing the smear layer is to obtain a clean and smooth surface. Current methods employed to remove smear layer include ultrasonic, laser techniques, and chemicals such as EDTA, sodium hypochlorite, and citric acid. The alternating use of EDTA and sodium hypochlorite has been recommended for efficient removal of the smear layer. Yamada *et al.*, in their study, agreed that the sequential use of EDTA and NaOCl resulted in efficient removal of smear layer and debris.^[11] In the same manner, the chemomechanical preparation of the specimens in the present study to remove the smear layer was done with 17% EDTA and sodium hypochlorite solution.

Treatment of DH involves two principal options, firstly occluding the dentinal tubules to prevent fluid flow and secondly desensitization of the nerve so as to make it less responsive to stimulation. Fluorides have been most widely used as topical desensitizing agents. NaF, stannous fluoride, and sodium monofluorophosphate are some of the effective and frequently used agents for the treatment of DH. Many clinical studies have shown the fluoride toothpaste

Table 3: Intergroup comparison of numbers of open tubules

Dunn's multiple comparison test	Mean rank 1	Mean rank 2	Mean rank difference	P
Group A versus Group B	50.18	30.83	19.35	0.0013
Group A versus Group C	50.18	10.50	39.68	<0.0001
Group B versus Group C	30.83	10.50	20.33	0.0007

Table 4: Intergroup comparison of intertubular distance

Dunn's multiple comparison test	Mean rank 1	Mean rank 2	Mean rank difference	P
Group A versus Group B	16.38	24.73	-8.350	0.3913
Group A versus Group C	16.38	50.40	-34.02	<0.0001
Group B versus Group C	24.73	50.40	-25.67	<0.0001

Table 5: Comparison of smear layer, melting areas, and microcracks in different groups

Variable	Group A (%)	Group B (%)	Group C (%)
Smear layer			
Present	3 (15)	1 (5)	1 (5)
Absent	17 (85)	19 (95)	19 (95)
Melting areas			
Present	0	20 (100)	18 (90)
Absent	20 (100)	0	2 (10)
Microcracks			
Present	0	10 (50)	6 (30)
Absent	20 (100)	10 (50)	14 (70)

and concentrated fluoride solution to be very efficient in managing DH.^[12-14] The possible mechanism for tubule occlusion with the use of fluoride (NaF) may be attributed to the precipitation of calcium fluoride at exposed dentin to create a barrier that consequently decreases DH.^[15] Another mechanism suggested by Hoang Dao *et al.* states that 5% NaF forms a crystalline layer with dentinal tubular plugs, thus significantly reducing dentin permeability.^[16] Similar mechanisms may be ascribed for the tubule occlusion seen in Group A in the present study.

The use of lasers in dentistry has increased dramatically in recent years. The mechanism of action of laser in the treatment of DH is twofold. The low-level lasers act directly on nerve transmission with a depolarization process that prevents the diffusion of pain, whereas the high-power lasers such as diode 980 nm and 808 nm, KTP 532 nm, Nd: YAG 1064 nm, and CO₂ laser cause melting and subsequent crystallization of the inorganic dentine component, thereby coagulating the dentinal tubular fluid.^[17] Diode lasers have shown the best results among the high-power lasers, even in high-grade DH cases. The role of Nd: YAG laser, Er: YAG laser, and 830 nm wavelength diode laser in DH has already been proven.^[18] The applicability of 980 nm diode laser in endodontic treatment and root canal disinfection has been reported by several studies.^[19] However, there are very few

studies on the effect of a 980 nm diode laser on the dentin surface and the subsequent structural alterations. To evaluate the efficacy of 980 nm laser in the management of DH, it was used in the present study. As both 1064 nm Nd: YAG laser and 980 nm diode laser fall in the near-infrared region of the electromagnetic spectrum, their mechanism of action seems to be similar.^[20]

Morphological alterations in dentin irradiated with 980 nm diode laser depend on the energy transmitted to the dentin, which is further related to the output power, frequency, and mode of application. In the present study, the 980 nm laser was used with a power of 1 W for 10 s to evaluate the role of the 980 nm diode laser in sealing dentin tubules. Furthermore, continuous wave mode was employed as it provides an uninterrupted laser beam that aids in scanning the whole dentin surface. Umana *et al.*, in their clinical study, concluded that diode lasers (810 and 980 nm) used at 0.8 and 1 W were both harmless for pulp vitality and effective in the treatment of DH.^[20]

Since dentin hypersensitivity has multifactorial etiology, combining therapy with laser application with tubular occluding agents like fluorides could be more beneficial. The present study showed that a 980 nm wavelength diode laser could effectively seal dentinal tubules. It was found that a higher degree of tubule occlusion was seen in Group B than in Group A. In addition, Group C showed greater tubule occlusion in comparison to both Group A and Group B. Several studies have shown the greater efficacy of laser over fluorides in the treatment of DH. Goharkhay *et al.*, in an *in vitro* study, evaluated the use of KTP, 810 nm diode, and CO₂ lasers to seal dentinal tubules with and without prior application of fluoride. The SEM investigations did not reveal the complete closure of tubules with either KTP or diode laser. However, the CO₂ laser in combination with fluoride resulted in the occlusion of most tubules.^[21] Umberto *et al.* conducted a clinical study for the treatment of DH by a 980 nm diode laser and reported positive results. They suggested that lasers induced superficial melting to occlude the dentinal tubules.^[22] A clinical study by Raichur *et al.* for the treatment of dentin hypersensitivity by 940 nm diode laser, stannous fluoride, and potassium nitrate gels concluded that the diode laser was most efficacious.^[23] In the present study, SEM analysis revealed that a significant reduction in the mean diameter of dentin tubules occurred in Group C in comparison to Group A and Group B. In addition, the mean number of open dentinal tubules was also significantly less in Group C compared to Group A and Group B. A similar reduction in the mean number of open dentinal tubules was reported by Birang *et al.* on irradiation with Nd: YAG laser as compared to that in the control group.^[21] Liu *et al.* also demonstrated a reduction in

the mean number of open dentinal tubules when irradiated with a 980 nm diode laser at different power settings.^[18] On intergroup comparison, Group B (laser alone) showed a significantly greater reduction in the mean diameter of dentinal tubules and the number of open tubules compared to Group A (NaF group). The statistically significant reduction in dentinal tubule in Group B over Group A could be due to the coagulating effect of laser on the organic elements, causing their accumulation and reduction in dentin tubule orifice.^[24]

The combined treatment with NaF and 980 nm diode laser showed the best results in our study. The laser irradiation could have probably burnt the NaF into the dentinal tubules, thereby increasing the sealing effect. Several previous studies have shown the better performance of the combination of NaF and laser compared to either treatment alone in treating dentin hypersensitivity. Studies conducted by Liu and Lan and Moritz *et al.* using semiconductor laser and CO₂ laser, respectively, in combination with fluoride to treat DH demonstrated favorable clinical results with enhanced treatment effectiveness.^[25,26] In an *in vitro* study, Lan *et al.* observed that treatment with Nd: YAG laser irradiation and NaF occluded most dentinal tubules.^[27] Kumar and Mehta assessed Nd: YAG laser with and without NaF in the treatment of DH, both clinically and with SEM, and stated that the SEM findings correlated to the clinical findings as the reduction in number of patent tubules was associated with improvement in treatment efficacy.^[28]

SEM findings also demonstrated that the dentin structure had changed due to the thermal effects caused by the laser energy, as was shown by the presence of melting areas in Group B and Group C. Similar effects were observed by Faria *et al.* in an *in vitro* study which revealed that the application of 980 nm diode laser on the dentin surface resulted in ultrastructural modifications including initial melting.^[29] Overheating caused by laser and subsequent cooling of the dentin surface results in causing such changes. Microcracks were seen in some of the specimens irradiated by laser (50% in Group B and 30% in Group C). Similar findings were reported by Israel *et al.* and Gaspric *et al.* in their studies where micro-cracks have been mentioned as possible side effects following laser irradiation.^[30,31]

CONCLUSION

Both alone and in combination with desensitizing drugs, the 980 nm diode laser has significant new possibilities in the treatment of dentinal hypersensitivity. Since the current study was conducted *in vitro*, the findings must be verified in a clinical setting before being used in clinical practice. As a result, randomized controlled clinical trials are suggested as a way to better detect the desensitization effects of this cutting-edge technology.

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Conflicts of interest

There are no conflicts of interest.

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