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# Morphology of ablation craters generated by ultra-short laser pulses in dentin surfaces: AFM and ESEM evaluation

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ABSTRACT

In this study, the surface morphology and structure of dentin after ablation by ultra-short pulses were evaluated using environmental scanning electron microscopy (ESEM) and atomic force microscopy (AFM). The dentin specimens examined were irradiated by a chirped-pulse-amplification (CPA) Ti:sapphire laser (800 nm) and the optimal conditions for producing various nanostructures were determined. Based on the ESEM results, it was possible to identify an energy density range as the ablation threshold for dentin. The laser-induced damage was characterized over the fluence range 1.3–2.1 J/cm$^2$. The results demonstrate that by selecting suitable parameters one can obtain efficient dentin surface preparation without evidence of thermal damage, i.e., with minimized heat affected zones and reduced collateral damage, the latter being normally characterized by formation of microcracks, grain growth and recrystallization in the heat affected zones.



## 1. Introduction

Different approaches for painless, fast and localized laser treatment of dental hard tissue have been the object of continuing research [1,2]. The human tooth structure comprises several layers, namely, enamel, dentin and pulp. The dentin is the major constituent of teeth and consists of 70% $Ca_{10}(PO_4)_6(OH)_2$, 20% organic matter (collagen fibers) and 10% water. Its internal structure consists of microscopic canals, which are essential for the growth of the tooth. Dentinal hypersensitivity (DH) has been studied for several years. DH is characterized by short sharp pain arising from exposed dentin in response to stimuli – typically thermal, evaporative, tactile, osmotic or chemical. Most commonly, cases of dentine hypersensitivity are associated with erosion or gingival recession. DH is one of the most common complications that affect patients after periodontal therapy and is reduced when the dentinal tubules are occluded. Several attempts have been performed previously to obtain closure of dentinal tubules using Nd:YAG laser treatment [3] by inducing melting and resolidification of the dentin. Studies of dentine ablation with Er:YAG and Ho:YAG lasers have indicated several weaknesses in the surface preparation process expressed in heat accumulation, fissuring and slow material removal rates. The attempts to apply these lasers to certain procedures in dentistry, especially for caries therapy in order to replace conventional drilling, have not been fully successful. Due to the high absorption of Er:YAG radiation by water molecules in the dentin tissue, the preparation process results in microexplosions breaking the hydroxyapatite structure and leading to the appearance of microcracks. The latter could become initial points for the development of new caries lesions. Modifications of these systems (Er, Cr:YSGG) have been introduced in the dental practice in combination with water spray in order to improve the surface preparation by cooling the ablated region and increasing the ablation rate [4–6]. In recent years, new ultra-short laser techniques for modification and structuring of dental hard tissue have emerged as a promising tool and an alternative to Er:YAG lasers [7–9] due to the advantages expressed in negligible heat and shock wave impact. The interaction of high-intensity ultra-short laser pulses with materials is characterized by fewer thermal side effects and a lower ablation threshold ($F_{th}$). Studies of the surface topography evolution in dentin processed by femtosecond lasers revealed surface effects, such as sealing of dentinal tubules, microdrilling of human enamel and dentin (for caries therapy) and microroughening for a wide laser fluence interval (0.5–10 J/cm$^2$) and number of pulses ($N$) ranging from 100 to 5000, leading to enhanced structuring quality in comparison with CW and ns lasers. Depending on the dental treatment procedure to be performed, one could utilize different types of laser-induced physical effects to obtain maximum benefits. For example, the effect of melting is used for sealing dentinal tubules in order to reduce dentin hypersensitivity and pathogenic agents' penetration. This procedure has so far been carried out by $CO_2$, Ho:YAG, Er:YAG and Nd:YLF lasers; however, the thermal

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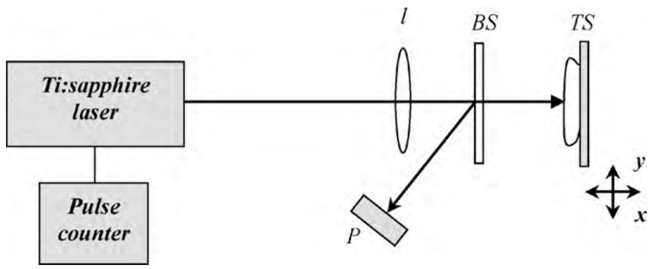


**Fig. 1.** Schematic diagram of the experimental setup where l is a focusing lens, BS is a 90:10% beamsplitter, P is a beam profilometer and TS is *xy*-translation stage on which are mounted the dentine disks.

side-effect associated with the use of these conventional lasers, namely, the appearance of a heat affected zone in the order of micrometers, results in rough surface structuring with deep cracks. For example, isolated spheres of recrystallized material have been observed in surfaces processed by $CO_2$ and Nd:YAG lasers [10]. The present study was motivated by the possibility to improve the control over the precision of the procedure for DH treatment and dental tissue microstructuring by using fs lasers.

The review of the literature revealed that the application of Ti-sapphire laser pulses with duration <50 fs for ablation of dental tissue, followed by examination with ESEM and AFM, has been rarely attempted. Few studies have been performed to compare the use of different number of fs laser pulses [11] and energies and their effect on the hard dental tissue. The purpose of the work reported here was to obtain new data and understanding on the morphology of the craters created after ultra-short laser ablation of dentin. Different structural modifications in terms of multiple ablative layers, thermal stress cracking and exfoliation sputtering were observed after the interaction of ultra-short laser pulses possessing different parameters with the material. Moreover a well-defined topography pattern was observed at and around the ablated crater rim. The surface was examined for the presence of collateral damage. The ablation threshold fluence of dentine was evaluated. The influence of varying different parameters, such as the number of laser pulses and laser fluence, was analyzed and the respective efficiencies were compared.

## 2. Materials and methods

### 2.1. Sample preparation

Freshly extracted human molars were used. The teeth samples were sliced transversally in 1-mm-thick disks using diamond-bladed saw and polished by 1200-grit SiC paper. After the cut, the dentine disks were stored in distilled water until examination. The laser used was a CPA Ti:sapphire (Femtopower-Compact pro) emitting at 800 nm central wavelength, with pulse duration of 25 fs, repetition rate of 1 KHz and average output power of 800 mW. The experiments were performed in air with the laser beam focused on the dentin disks to a focal spot with diameter of 30 $\mu$m by means of a lens with focal length of 8 cm. Fig. 1 shows a schematic diagram of the experimental setup used.

An electromechanical pulse shutter allowed the selection of the desired number of laser pulses. Since the spatial beam profile is an important condition for the quality of the processed area, the beam shape quality after the focal point was monitored by a beam profilometer in order to guarantee a Gaussian distribution. The beam diagnostics was carried out by a CMOS camera (LaserCam-HR, Coherent).

### 2.2. ESEM and AFM

The irradiated dentine disks were examined using environmental scanning microscopy (XL30 ESEM-Philips operating in a wet mode at 1 Torr pressure and 20 kV electron beam) and atomic force microscopy (molecular probe ASYLUM Research MFP3D). The ESEM technology permits imaging of hydrated organic structures with no prior specimen preparation, thus avoiding the dehydration artifacts and artifacts induced during the preparation steps [12], and provides information from the surface of a bulk biological material in its “natural” state. The AFM was operated in air in a contact mode using Veeco tips. For the analysis, a surface area of approximately 1 mm × 1 mm was irradiated uniformly. The samples were positioned in the focal plane and moved with respect to a fixed laser-beam position using a translation stage after a given number of shots. The data were analyzed with respect to the tooth surface morphology and integrity produced by laser ultra-short ablation.

## 3. Results and discussion

The ESEM and AFM data will be discussed separately.

### 3.1. Environmental scanning electron microscopy (ESEM)

Laser irradiation induces different effects in the processed material depending on the laser parameters applied. The first lasers used in dentistry (ruby, $CO_2$ lasers) with pulse durations in the microsecond range generated considerable heat in the region of the pulp chamber during the ablation process, because, during the pulse, heat diffusion plays an important role in the interaction mechanism. Niemz et al. demonstrated that the application of a femtosecond Yb:KYW amplifier system providing 900 fs at 1030 nm allows successful removal of dental decay or caries and overcomes the limitations, such as thermal side effects, associated with conventional systems (Er:YAG lasers, Ho:YAG lasers, excimer lasers). The work provided promising results in view of applying fs pulses in dentistry by introducing a minimally invasive concept for treatment [13,14]. However, there is a lack of studies dealing with the influence of the pulse duration and with the effect of varying the number of pulses on the quality achievable in dental processing. The results described here prove that femtosecond laser pulses are in fact minimally invasive in the treatment of carious lesions. By reducing the amount of collateral damage the ablation of a “healthy” tooth substance is reduced to a minimum. Effective results in ablation of hard dental tissue by ultra-short laser pulses were obtained at several sets of laser parameters. We performed a systematic study of the dependence on and relation between the laser fluence and the number of laser pulses in what concerns the effects on the dental tissue. Combining AFM analyses with ESEM morphological studies, we examined the processed surface on a nano-scale and demonstrated that pulses with durations <30 fs are an efficient tool for precise, crack-free processing of dental tissue. Furthermore, we evaluated the ablation threshold fluence of dentine. The ESEM micrographs of ultra-short-laser-pulse ablated dentine surface revealed an improvement in the surface processing quality. Fig. 2a illustrates the surface modification of dentine ablated by $N = 500$ pulses with $F = 1.36$ J/cm$^2$.

At sub-ablative fluences, flat surfaces similar to non-irradiated dentin were produced (Fig. 2a). As the laser intensity was increased further, we could define the ablation threshold, namely, the fluence at which a smooth pattern without ablation or cracks was observed (Fig. 2b).

As the laser energy was raised above 1.5 J/cm$^2$, we observed the development of a crater (Fig. 3a).

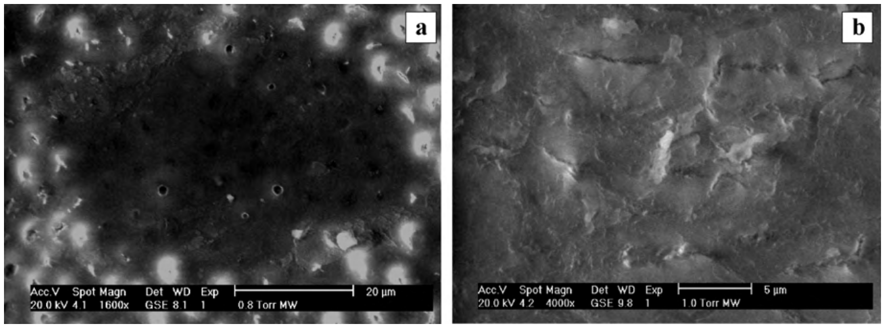


**Fig. 2.** ESEM image of femtosecond Ti:sapphire ($\lambda$ = 800 nm, 30 fs, 1 kHz repetition rate) laser ablation of dentine pulses in air: (a) showing the surface modification of dentine at modest laser energy $F = 1.36\,J/cm^2$ and $N = 500$ pulses, 1600×; (b) $F = 1.5\,J/cm^2$, $N = 500$, 4000×.

Examination of the craters produced revealed the formation of cracks at the crater surroundings and at the bottom of the crater in samples irradiated at laser fluences above $2\,J/cm^2$ for $N = 500$ (Fig. 3b and c). Summarizing the ESEM results, clean ablation cavities were created at fluences in the range 1.5–2 $J/cm^2$ for $N = 500$. The cavity walls were extremely steep and clean. Similar results have been previously observed by other researchers for femtosecond laser ablation with $F \approx 1\,J/cm^2$ [15].

Our results did not indicate any thermal alteration for a number of pulses of less than 600 (Fig. 4a and b).

The crater surroundings were clean without melting of the dentin. Dentin irradiation by a larger number ($N > 1000$) of accumulated laser pulses resulted in a more pronounced surface modification than for 1000 pulses.

As the number of pulses was raised (Fig. 4c–f), the presence of cracks at the bottom of the ablated crater was observed, heat is generated in a volumetric region in depth. After a certain number of pulses, a sufficient amount of heat per pulse is deposited in a cumulative manner leading to an increase in the local temperature and initiating a process of thermal ablation followed by formation of cracks at the cavity bottom. The advantages of plasma-mediated ablation consist in the very precise cutting of tissues and the absence of undesired thermal side effects. Most materials have an electron-phonon coupling time of between a picosecond and a nanosecond, with the typical heat diffusion times falling between a nanosecond and a microsecond. The thermal relaxation time for biological material is in the order of 1 $\mu$s. Since thermal diffusion is too slow to dissipate the laser energy during this plasma lifetime, the thermal energy is confined to the zone of the plasma and no thermal interaction occurs in the adjacent tissue regions. In the case of a single laser pulse, the size of the side effects (shock waves, crack formations) depends mainly on the total amount of energy deposited in the material. However, application of repetitive pulses can lead to cumulative effects that can be controlled. At a higher magnification (8000×), cracks are clearly observed at the bottom of the crater (Fig. 4d and f). We estimated that the least mean number of pulses that still promoted cavity formation was $N = 600$ at $F > 1.5\,J/cm^2$. The successive pulse energy deposition and heat conversion should take place within a short time interval with respect to the heat diffusion times. The fs technique allows for a high processing speed in comparison to irradiation with longer laser pulses. In summary, the ESEM results demonstrate that, having chosen suitable ultra-short laser parameters, one can achieve very effective processing of hard dental tissue without any collateral damage.

### 3.2. *Atomic force microscopy (AFM) of irradiated dentin surfaces*

Little information is available on the interaction of high energy ultra-short laser pulses to record micro- and nano-scale surface structures of irradiated dentin.

The data reported here extend the existing information [16] about ultra-short laser pulse dentin processing. Morphological studies of surface modifications of this material have been recently carried out by using the ESEM and SEM techniques. These could be used to compare the morphology of the ablated craters with the new data obtained by AFM. The advantages of the AFM technique [17] are that it permits operation in air, compared to electron microscopy, where the samples are examined under vacuum conditions causing drying of the specimens and structural alterations. Our aim was to characterize the dentin surface microstructure around the crater boundary on a nano-scale in order to enhance the understanding of laser ablation processing with ultra-short laser pulses. Fig. 5a and b presents a typical micrograph of a dentine surface before laser exposure. One can see a dentin surface with opened tubules without a smear layer (thin layer with small crystalline characteristics formed due to the specimen preparation method). Using AFM, we followed the surface morphology evolution after laser irradiation. Thus, a well-defined topography around the ablated crater rim was observed (Fig. 6a and b). Furthermore, the formation was seen of a thin layer with granules distributed around the crater surroundings sealing the open dentinal tubules (Fig. 7a and b). When comparing Fig. 5a and b (unexposed dentin) to

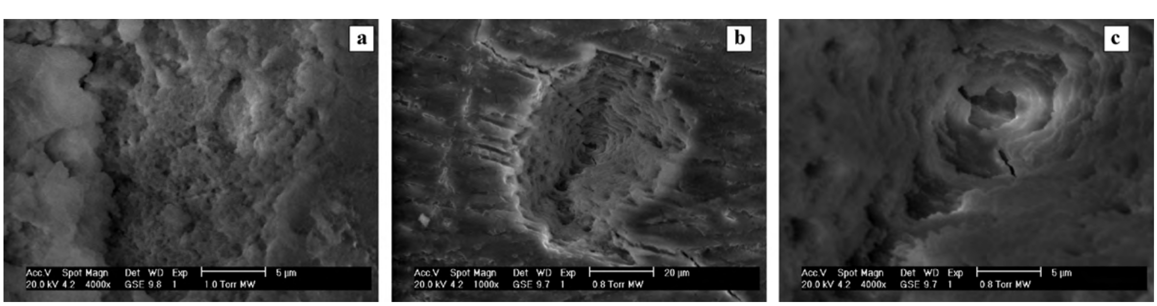


**Fig. 3.** ESEM image of femtosecond Ti:sapphire ($\lambda$ = 800 nm, 30 fs, 1 kHz repetition rate $N = 500$ pulses) laser ablated crater on dentine in air at different laser energies: (a) showing the surface modification of dentine at $F = 1.6\,J/cm^2$, 4000×; (b) $F = 2.1\,J/cm^2$,1000×; (c) $F = 2.1\,J/cm^2$, 4000×.

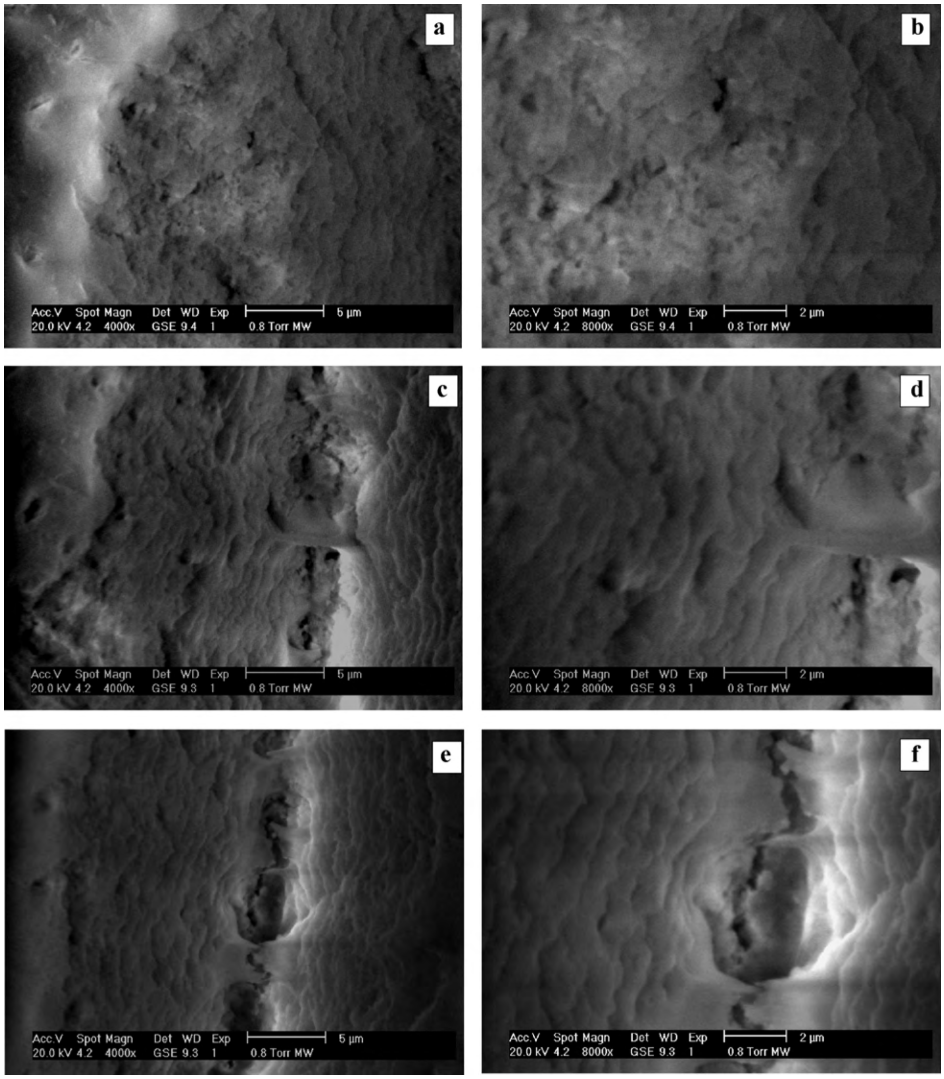


**Fig. 4.** ESEM micrographs of dentin surfaces irradiated with 1.55 J/cm$^2$ in air with different number of pulses: (a) $N = 600$ pulses, 4000×, (b) $N = 600$ pulses, 8000, (c) $N = 5000$, 4000×, (d) $N = 5000$, 8000×, (e) $N = 10{,}000$ pulses, 4000×, (f) $N = 10{,}000$ pulses, 8000×.

Figs. 6a, 7a and 6b, 7b, one can distinguish the difference expressed in an overlayer of resolidified dentin material which occludes the dentin tubules. The examination of the irradiated area was performed very closely to the crater rim, where the formation is visible of a bump-like structure created after the propagation of a shock wave (Fig. 7b).

A relevant common novel feature observed by the AFM technique was the formation of a surface nano-layer around the irradiated crater boundary. This layer most probably appears due to the high peak-intensity causing multiphoton ionization leading to the generation of a shock wave and instantaneous local melting of the material.

The interaction of an ultra-short laser pulse with a tissue is a plasma-mediated ablation. In this process, the extremely high intensity of the ultra-short pulses first leads to multiphoton absorption in the material. This results in ionization of some atoms and molecules thereby providing initial carriers. The free electrons and ions absorb energy from the electromagnetic field of the laser

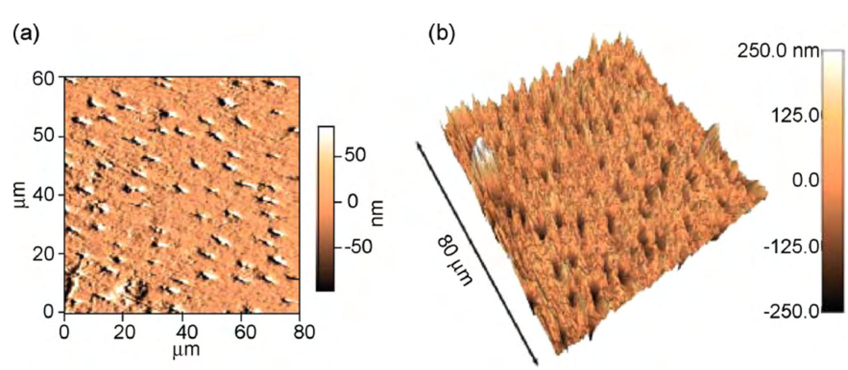


**Fig. 5.** AFM plots showing the flat dentin surface with opened dentinal tubules before laser exposure (scan range 80 µm × 80 µm), (a) AFM lateral trace image (b) 3D image.

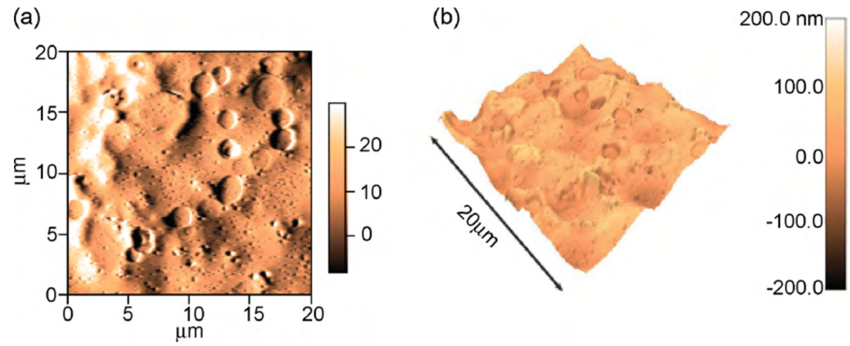


**Fig. 6.** AFM images showing the alteration of dentin surface and the formation of a thin layer around the periphery of the irradiated area at 800 nm, (a) AFM lateral trace image of exposed dentin, $N$ = 5000, $F$ = 1.55 J/cm$^2$ scan range 20 μm × 20 μm, (b) 3D AFM image of exposed dentin, $N$ = 5000, $F$ = 1.55 J/cm$^2$.

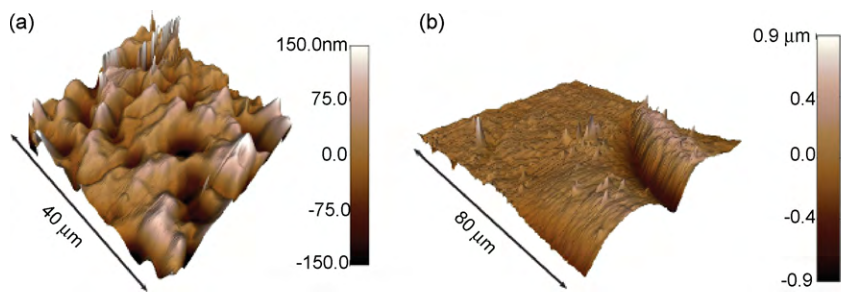


**Fig. 7.** 3D AFM images showing the alteration of dentin surface at $N$ = 5000, $F$ = 1.55 J/cm$^2$ obtained for two different scan ranges at areas around the crater boundary (a) formation of a thin layer of resolidified material, scan range 40 μm × 40 μm, (b) formation of bump-like structure created after shock wave propagation scan range 80 μm × 80 μm.

radiation by inverse bremsstrahlung resulting in their acceleration. The subsequent avalanche-like multiplication of free carriers finally leads to laser-induced optical breakdown (LIOB), rather than heat transfer, and to the generation of microplasma [18]. In addition, owing to the expansion of the heated plasma, a high-pressure transient wave propagates radially from the LIOB center into the surrounding environment. This shock wave also contributes to the ablation by disruption. Thus, the advantages of plasma-mediated ablation consist in the very precise cutting of tissues and the absence of undesired thermal side effects, since the plasma conversion is confined within the laser-beam focus. The formation of a surface nano-layer as revealed by AFM observations indicates that in the sub-ablative energy range ($F$ = 1.55 J/cm$^2$) chemical or structural changes can be induced which could improve the dentin resistance and strength [19].

As our study demonstrated, AFM is a technique suitable for examining the irradiated dentin morphology. However, a disadvantage of the AFM approach is the fact that we could obtain images of the processed sample surface from the area around the crater boundary only; to examine the area inside the crater was not possible since the cantilever tip started to vibrate due to the large depth of the craters created and the image was distorted. Therefore, the AFM technique has also some limitations related to the requirement for flatness of the surface investigated so that, in order to obtain useful imaging of changes in dentin after laser irradiation, samples exposed to sub-ablative fluences inducing slight surface modification should only be examined.

## 4. Conclusions

The surface morphology of dentin irradiated by consecutive laser pulses ($N$ = 100–10 000) with energies in the range 1.3–2.16 J/cm$^2$, $\tau$ = 30 fs and $\lambda$ = 800 nm was investigated by ESEM and AFM. It was shown that the interaction of fs laser pulses with dentin results in excellent cavity quality. Laser energy densities of $F$ = 1.36 J/cm$^2$ at 800 nm were found to be sufficient for inducing slight surface modifications of the samples. Irradiation by lower energies ($F$ = 1.3–1.6 J/cm$^2$) with the number of consecutive pulses being <5000 led to development of a characteristic molten morphological region (modified ablation layer) on the nanometer scale. A cumulative effect due to the repetitive illumination by an increasing number of pulses ($N$) occurred in the energy range 1.6–2 J/cm$^2$. These findings can be applied to choosing the optimal irradiation conditions (avoiding collateral damage) in order to achieve sealing of the dentinal tubules necessary for the prevention and treatment of an early-stage carious lesions and to make the tissue more resistant to development of new caries.

In summary, the present paper indicates that femtosecond lasers could be an alternative to other lasers for caries prevention therapy.

Comparing femtosecond laser dentistry with previous results of applying lasers to dentistry (Er:YAG laser) in what concerns ultra-short laser processing reveals clear evidence of improved processing quality and absence of collateral thermal or mechanical damage. Cracks formation and laser-induced pressure transients, which limit the precision of the treatment procedure when using ns and ps pulses, are reduced dramatically when fs pulses are applied.

Our latest results on the application of femtosecond laser processing of dental hard tissue presented here make evident the great potential of introducing this technology to the medical practice. However, a femtosecond laser is more complex than a continuous-wave laser and the scientific sector is still the biggest market for femtosecond lasers, notwithstanding the fact that other sectors, such as diagnostics (imaging and spectroscopy), are of increasing importance in this respect. Nevertheless, a femtosecond laser is currently an expensive piece of equipment which restricts the application of ultra-short-pulse lasers into the wide medical practice.

Conventional femtosecond oscillators are relatively simple systems typically operating at a repetition rate around 100 MHz. The output energy of such systems, however, is limited to a few nJ (up to 10 nJ for sub-50-fs pulses) and is in general too low for material processing applications. As the maximum energy that can be achieved directly from an oscillator is constrained by the available pump power and the increased nonlinearities in the laser medium, further energy increase can be provided by coherent amplification of the femtosecond pulses. Amplifier systems are generally used for micro-machining applications. Because of the low repetition rate of such systems only low processing speeds can be achieved. A reliable system working at a MHz repetition rate would allow one to increase the processing speed, thus making the whole process less susceptible to intensity drifts.

Any future research should explore the possibility to use a pulse-shaping technique. This technique could provide an alternative in improving the control over the optical damage and the quality of the cavity produced. Temporal shaping of femtosecond laser pulses is used for optimization of laser material processing [20]. Indeed, phase and amplitude shaping allows sophisticated temporal profiles to be created, including double pulses and “tailored” spectral profiles. The temporal tailoring process is based on dynamically altering the spectral phase information of an incident bandwidth-limited laser pulse which is spatially dispersed and reformed in a zero-dispersion stretcher. The use of LC-modulators allows phase/amplitude modulation thus introducing different optical paths to the spatially separated spectral components and, in turn, tailoring the pulses to desired temporal shapes by keeping constant the energy in the pulse, which is delivered at different rates. A large amount of information can be found in the literature on the effects of the laser wavelength, pulse duration, energy and repetition rate on the ablation process of wide range of materials, but still very little is known about the influence of the temporal profile of the laser pulse. The use of double or triple pulses with adequate separation has been proven to reduce the amount of material that overlaps at the rims, leading to cleaner borders of the structure [21]. Moreover, adaptive temporal pulse shaping based on real-time pulse tailoring can open up possibilities for controlling the ablation process. We believe that using tailored pulse shapes could represent a novel approach to controlling laser processing of dentin.

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