Characterisation of dentin surfaces processed with KrF excimer laser radiation

Sónia Eugénio\textsuperscript{a}, Manickam Sivakumar\textsuperscript{a}, Rui Vilar\textsuperscript{a,}\textsuperscript{*}, Ana Maria Rego\textsuperscript{b}

\textsuperscript{a}Departamento de Engenharia de Materiais, Instituto Superior Técnico, 1049-001 Lisboa, Portugal
\textsuperscript{b}Departamento de Engenharia Química, Instituto Superior Técnico, 1049-001 Lisboa, Portugal

Received 3 December 2004; accepted 24 March 2005
Available online 8 June 2005

Abstract

In the present work, the surface microtexture and chemical changes induced in human dentin by laser processing with KrF excimer laser radiation using fluences ranging from 0.5 to 20\,J/cm\textsuperscript{2} were studied by SEM, XPS and FTIR. Two distinct behaviours were observed in the evolution of surface topography. In some samples, the laser-treated surface remained flat, independently of the fluence used. It was covered by a layer formed of redeposited ablation particles, which occluded the dentinal apertures. In other samples the surface topography depended on radiation fluence. When the fluence was lower than 1\,J/cm\textsuperscript{2}, preferential removal of intertubular dentin occurs, producing a columnar structure in which the columns are essentially formed of peritubular material. If the fluence exceeded 1\,J/cm\textsuperscript{2} the processed surface was flat and covered with resolidified material. Despite these topographic changes, the dentin was not significantly affected by the laser treatment. The observed behaviour can be explained by differences in the constitution of dentin.

\copyright 2005 Elsevier Ltd. All rights reserved.

Keywords: Dentin; Laser ablation; Surface modification; SEM; XPS; FTIR

1. Introduction

The study of the influence of laser processing on dentin is of fundamental importance to enable the use of lasers in preventive and restorative dentistry. Dentin is a composite biological material, consisting of about 50\,vol\% of a mineral phase in the form of partially carbonated calcium-deficient apatite, 30\,vol\% of organic matter (largely type I collagen) and about 20\,vol\% of water.

Laser ablation of dental tissues has been extensively studied [1–7]. The effects of processing dentin with infrared and ultraviolet lasers were compared by Pearson and McDonald [4]. The authors concluded that, unlike infrared lasers, UV lasers have negligible thermal effects that do not significantly affect pulpal tissue, but present a much lower removal rate than infrared lasers. The surface morphology and intrapulpal temperature rise resulting from treating with a CW CO\textsubscript{2} laser, pulsed Nd:YAG laser (\(\lambda = 1.064\,\mu\text{m}\)) and pulsed ArF laser (pulse duration = 20\,ns) were compared by Turkmen et al. [3] in samples processed for 30\,s at the same parameter settings with each laser. The average internal temperature increase for CO\textsubscript{2}, Nd:YAG and ArF lasers was 37, 28 and 1\,°C, respectively. Fried et al. [1] showed that treating with 100\,\mu\text{s} pulsed laser radiation in the 9–11\,\mu\text{m} wavelength range and at fluences greater than 2\,J/cm\textsuperscript{2} induces a partial transformation of the carbonated apatite in dentin into a purer hydroxyapatite phase, due to loss of water and carbon dioxide. Grain growth and recrystallisation in the heat-affected zone and cracking were observed. The phase transformation of the inorganic phase of dentin due to the thermal effect of CO\textsubscript{2} laser treatment was studied by
Hirota et al. [2]. Metastable forms of calcium phosphate such as α-Ca₃(PO₄)₂, β-Ca₃(PO₄)₂ and z–, β– and γ-Ca₃P₂O₇ were observed in dentin surfaces irradiated during 0.5 s at a power density of 1.4 × 10⁶ W/cm² [2]. Isolated spheres of recrystallised material were observed in surfaces processed with CO₂ and Nd:YAG lasers, while treating with ArF excimer lasers produced little morphological change in the dentin [3]. The evolution of surface topography in dentin processed with UV laser radiation was studied by several authors. Wilder-Smith et al. [5] studied the changes in surface topography due to treating enamel and dentin with ArF excimer laser radiation (λ = 193 nm), and found surface effects ranging from sealing of dental tubules to microroughening for wide ranges of fluence (0.5–10 J/cm²) and number of pulses (50–1800). A similar study carried out by Sanchez et al. [6] showed that processing with 100 ArF laser pulses at a fluence of 1 J/cm² leads to uniform ablation of dentin, without any evidence of melting. On the contrary, at fluences higher than 1.5 J/cm², conical features appear at the surface and extensive melting occurs. The authors concluded that ablation is photo-chemical at low fluences and predominantly thermal in the high fluence range. The formation of the cone-shaped surface topography was explained by preferential ablation of intertubular dentin, compared to peritubular dentin. Since surface roughening may improve adhesion between restorative materials and teeth, and sealing of dental tubules is desirable because it provides a barrier to the penetration of pathological agents, the authors [5,6] considered that ArF lasers are effective tools for the removal and surface treating of hard dental tissues.

The results available suggest that excimer lasers are more attractive than Nd:YAG and CO₂ lasers for dentistry applications because the shorter wavelength and short pulse duration minimise harmful thermal effects. Although much research has been done in this area, the ablation mechanisms and the effect of UV laser processing on the microstructure and constitution of dental tissues is not yet fully understood and detailed knowledge of the optimal processing parameters for each application is far from being achieved.

2. Materials and methods

Discs of dentin with an average diameter of 8.7 mm and approximately 2 mm thick were cut from human molar teeth, parallel to the occlusal plane, using a low-speed diamond saw. One of the faces of the discs was polished with 600 mesh SiC paper under flowing water lubrication. After cutting and surface preparation, the discs were stored in 5% chloramine solution until laser processing was carried out. The laser treatment was performed using a KrF excimer laser (248 nm radiation wavelength) with 30 ns pulse duration. The optical system used produced a uniform-intensity rectangular spot approximately 300 × 700 μm² at the surface of the samples. The samples were irradiated with 0.5, 1, 2, 4, 10 and 20 J/cm² and 10, 50, 100, 500 and 1000 laser pulses, at a pulse frequency of 5 Hz.

The treated samples were characterised by scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectroscopy (FTIR). Prior to SEM observation samples were stored under primary vacuum for 12 h for drying, and then sputter coated with gold. XPS analysis was performed using an XSAM800 (KRATOS) spectrometer equipped with a MgKα source (photon energy equal to 1253.6 eV). FTIR analysis was performed on transmittance mode on small samples of surface material, carefully removed from dentin surfaces before and after laser treatment, using a scalpel and conditioned in a diamond cell. Infrared spectra were recorded in the 4000–700 cm⁻¹ range, with a 4 cm⁻¹ resolution, by accumulating 256 scans.

3. Results

3.1. Scanning electron microscopy

Unprocessed dentin surfaces are covered by a uniform smear layer that occludes the dentinal structure. The threshold fluence for significant ablation to occur is about 0.5 J/cm². Processing with higher fluences removes the smear layer as well as the underlying unaltered material, leading to the formation of well-defined rectangular cavities with vertical walls and relatively sharp edges (Fig. 1a). The laser-treated area is covered by a layer of redeposited material that can be easily removed by ultrasonic cleaning. In some samples the walls of the cavity are relatively flat (Fig. 1b), but more frequently they consist of parallel cylindrical surfaces (Fig. 1c). In both cases they show evidence of extensive melting and liquid flow. When accidentally fractured, the cylinders show a hollow core, which corresponds to a tubule in the dentinal structure (Fig. 1c). They consist of peritubular material, which resisted ablation, while intertubular material was preferentially eroded, exposing the external surfaces of the peritubular dentin. Observation of fracture surfaces perpendicular to the treated surface by SEM (Fig. 1d) does not reveal any layer of structurally modified material, suggesting that the laser treatment does not change the dentin structure significantly.

Two distinct types of behaviour were observed concerning the surface topography resulting from the laser treatment (Fig. 2 and 4). In some samples, the processed surface remains relatively flat, independently of the fluence used and covered by a layer of redeposited particles, which hides the structure of underlying dentin (Fig. 2a–c). The diameter of the redeposited particles presents a bimodal distribution with a population of particles with diameters in the nanometer range and
another population with diameters in the micrometer range (Fig. 3). The shape of larger diameter particles may be spherical (resolidified droplets) or irregular and angular. In the samples processed with larger fluences, this layer seems to have formed at higher temperatures because individual particles have coalesced to form a low roughness continuous film (Fig. 2c). In other samples, the surface morphology depends on the radiation fluence. When these samples are treated with fluences higher than 1 J/cm² (Fig. 4b and c), the treated surfaces are relatively flat and partially covered by a very thin layer of resolidified material. When fluence is
lower than or equal to 1 J/cm², preferential removal of intertubular dentin occurs, leading to the formation of cone-like or columnar structures, in which the cones are formed by peritubular material (Fig. 4a). In regions where the intertubular distances are smaller than the average column diameter, columns formed around adjacent tubules tend to coalesce. Hollow globules of resolidified peritubular material are often observed on the treated surface, frequently near the openings of tubules (Fig. 5). Some of these globules close the tubules, while others were burst open by gas expanding from the tubule.

3.2. X-ray photoelectron spectroscopy

XPS spectra of treated and untreated surfaces are presented in Fig. 6. The peaks observed correspond to the elements calcium, phosphorus, oxygen, carbon and nitrogen. Figs. 7a and 7b show expanded views of the carbon peak at 284.6 eV before and after laser treatment, respectively. The carbon peak in the spectrum of the untreated sample can be deconvoluted into two peaks: a peak corresponding to a binding energy of 284.6 eV, which is due to surface contamination by hydrocarbons [8], and a smaller amplitude peak, located at 287.5 eV, which can be attributed to the organic compounds present in dentin, in particular type I collagen [8]. This second peak is absent from the
spectrum of laser-treated dentin, showing that organic matter is removed from the most external layer of the processed surface. This conclusion is corroborated by the evolution of the nitrogen peak at 399.7 eV, also associated with type I collagen [8], presented in Fig. 8 for treated and untreated samples. Before laser treatment, this peak is prominent, showing that collagen exists in the smear layer. On the contrary, the peak is absent in the laser-treated sample, meaning that collagen was removed from the surface by the laser treatment. Peaks related to the mineral phase of dentin, namely Ca(2p) and P(2p), are not affected by laser processing, showing that the chemical composition of this phase is not significantly modified.

3.3. Infrared spectroscopy

The infrared spectra of treated and untreated dentin (Fig. 9) are similar and typical of dentin samples prepared by standard mechanical cutting and polishing techniques [9]. The phosphate and carbonate absorption bands in these spectra are associated with the mineral phase of dentin, a calcium deficient carbonated apatite [10]. The peaks at about 1040 and 958 cm\(^{-1}\) correspond to the \(v_3\) antisymmetric PO stretching mode and the \(v_1\) symmetric stretching mode of the PO\(_4^{3-}\) group, respectively [11]. The absorption bands at 1410 and 1450 are attributed to the \(v_3\) mode of CO\(_3^{2-}\) substituted in B-type anionic sites (PO\(_4^{3-}\) sites) and in A-type anionic sites (OH\(^-\) sites), respectively [12]. The band at 870 cm\(^{-1}\) is due to \(v_2\) mode CO\(_3^{2-}\) [12]. The amide bands present in the spectra between 1700 and 1200 cm\(^{-1}\) correspond to collagen, the main organic component of dentin [10]. The bands at 1640, 1540 and 1250 cm\(^{-1}\) can be identified as amide I (C–O bond) [13], amide II (C–N stretching and N–H deformation modes) [14] and amide III peaks of collagen, respectively [12]. The band at 3320 cm\(^{-1}\) can be attributed to the hydroxyl group (OH) [16] and amide A (N–H stretching mode) of collagen [14,15]. The small shoulder appearing at about 3090 cm\(^{-1}\) is due to the overtone of amide II of collagen [15], while the peaks at 2950 and 2850 cm\(^{-1}\) correspond to C–H bonds of organic compounds, in general [16]. The position and relative amplitude of the IR absorption peaks are not significantly affected by laser treatment, confirming that the treatment does not significantly affect dentin within the depth observed by...
the sampling process used to obtain the infrared spectroscopy specimens (10–50 μm).

4. Discussion

Processing of dentin with KrF excimer laser radiation leads to the formation of several types of surface topography, depending on the composition and structure of the samples and on the processing parameters. Prior to the laser treatment, the dentinal structure is covered by a poorly adherent smear layer about 2–3 μm thick, composed of a mixture of partially denatured collagen and mineral particles [17] and formed as a result of the sample preparation process. The laser treatment eliminates the smear layer, in agreement with the results of Moss et al. [7].

Two extreme types of behaviour were observed in what concerns the evolution of the surface topography. The relationship between the cones and dentin microstructure and consideration of the properties of intertubular and intertubular dentin leads to the conclusion that the development of a columnar topography for fluences lower than 1 J/cm² is due to differential ablation, with intertubular dentin being preferentially removed. Intertubular dentin is composed of a network of collagen fibres with dispersed nanocrystals of apatite. Although apatite is the predominant phase (about 70 wt%), the continuous phase (matrix) is collagen. By contrast, peritubular dentin consists essentially of mineral phase with embedded collagen fibres [17]. The absorption coefficient of collagen for 248 nm wavelength radiation (α = 900 cm⁻¹) [17] is much higher than the absorption coefficient of hydroxyapatite for the same wavelength (α = 120 cm⁻¹). The resulting optical penetration depths are 11 and 83 μm for collagen and hydroxyapatite, respectively, much larger than the average ablation depth per pulse. This means that the incident radiation will penetrate the hydroxyapatite/collagen composite up to a considerable depth, and within this depth it will be preferentially absorbed by collagen. In the fluence range in which the formation of the columnar topography is observed (below 1 J/cm²), the intensity of radiation will be higher than the ablation threshold of collagen (0.02–0.03 J/cm²) and lower than or similar to the ablation threshold of apatite (0.5–0.7 J/cm²). Consequently, the collagen which agglutinates the apatite crystals in the dentin structure is ablated. The destruction of the collagen agglutinant will lead to the disruption of the dentin matrix and ultimately to the ejection of the hydroxyapatite particles, even if the fluence is lower than the threshold of hydroxyapatite. We may, then, conclude that in this fluence range the ablation behaviour of intertubular dentin is essentially controlled by collagen. On the contrary, in peritubular dentin apatite is the continuous phase. As a result its ablation behaviour is controlled by the ablation of apatite, and since these low fluences are below or only slightly above the ablation threshold of apatite (0.5–0.7 J/cm²), the ablation rate of peritubular dentin will be negligible or very small. Due to this difference in ablation rates, after laser treatment, hollow cylinders of peritubular dentin stand up above the peritubular dentin surface, just presenting some evidence of melting. The molten depth layer is thin and small because heat penetration in this relatively low heat diffusivity phase during the short laser pulse duration is quite small. The flatness of surfaces processed with fluences higher than 1 J/cm² shows that in this fluence range the ablation rates of intertubular and peritubular dentin are similar, so that both types of dentin are uniformly removed. Taking into consideration the above analysis, the behaviours observed in ablation of dentin are certainly explained by variations in the organic matter and mineral content of dentin from tooth to tooth and from location to location within the same tooth. Samples with a higher mineral content (more mineralised or with a higher percentage of peritubular dentin) should present a fluence-independent topography, while samples with a
lower mineral content show surface topography, which depends on fluence.

The processed surface is covered by nanometer-sized particles, called debris, which are large clusters formed in the ablation plume as a result of the aggregation of atoms, molecules and smaller clusters [18] or directly ejected from the target [19] and spherical or irregularly shaped micrometer-sized particles. The spherical particulates are formed by the resolidification of liquid droplets ejected as a result of hydrodynamic instabilities [20]. In dentin, these droplets consist mainly of molten hydroxyapatite. They are frequently hollow due to the release of water vapour and vaporised P2O5 within the molten phase, due to hydroxyapatite decomposition or from volume contraction of the spherical particles during solidification [21]. Irregularly shaped particulates result from the exfoliation of the heated surface layer, due to the extremely high thermal stress gradient created by the laser pulse [22]. Tubules are sealed by globules formed of resolidified peritubular dentin. Some tubules are closed by a spherical cap formed by a burst of gas through the liquid. This gas may be due to the vaporisation of dentinal fluids and/or decomposition of organic matter or hydroxyapatite. Sealing of dentinal tubules is thought to be beneficial for treating dentin hypersensitivity and to provide a barrier to bacterial penetration through the tubular structure. In contrast to the conclusions of Wilder-Smith et al. [5], the proportion of dentinal tubules that are sealed by the laser treatment varies from sample to sample, without a clear relation being found between the proportion of sealed tubules and the fluence or number of pulses.

XPS and FTIR analysis showed that laser processing with KrF excimer laser radiation does not induce any chemical or structural changes in the hydroxyapatite present in dentin. The peaks assigned to the mineral phase remain unaltered, showing that dentin is not altered by treating with KrF excimer laser radiation. This is certainly due to the fact that the heating and cooling rates induced in the material by the laser pulse are so high (in the range 10^3–10^6 k/s) that chemical reactions involving solid state diffusion are completely inhibited. Melting of the lateral walls of the lesions is explained by the fact that material in these walls is exposed to the extremely hot ablation plume during the complete plume lifetime, which may reach a few microseconds instead of a few tens of nanoseconds for the pulse duration.

Regarding the effect of the laser treatment on collagen, XPS and FTIR give contradictory results. This is due to the fact that the depth of material analysed by the two techniques was completely different. XPS is a surface analysis technique and is only sensitive to the first atomic layers of the material. By contrast, FTIR analysis was performed on specimens obtained by scratching the surface with a scalpel, so the depth of sampling is in the 10–50 μm range. The present results indicate that the laser treatment removes collagen from the outermost layers of the processed surface but leaves the organic matter content of dentin basically unchanged. These results suggest that KrF laser effectively ablates dentin, with negligible thermal damage to the remaining tissue.

The ablation of dentin by excimer laser radiation has been attributed to structure breakdown due to direct disruption of chemical bonds by high-energy UV laser photons [4,7]. The ablation mechanism was thus considered to be photochemical. Sanchez et al. [6] suggested that ablation of dentin is photochemical at below 2 J/cm^2 and thermal above this threshold. The present results suggest that, due to the composite nature of this biological material, both photochemical and photothermal mechanisms coexist in the ablation of dentin, independently of fluence. Collagen is a complex polymer with covalent molecular bonding such as C–H, C=O, C=N, N=O. The energy of these is 4.30, 5.15, 3.04 and 2.4 eV, respectively. Since the energy of 248 nm wavelength photons (5 eV) is greater than the energy of most of these bonds, collagen ablation is essentially photochemical, due to direct breaking of covalent bonds [23]. As a result, a large number of molecular fragments will form over the irradiated spot, leading to a rapid increase in free volume and pressure, and particle ejection at very high velocities [23]. Explosive vaporisation of water has also been suggested as the ablation mechanism for collagen [24], but this mechanism is unlikely for 248 nm radiation because the absorption coefficient of water at this wavelength is too low (α = 0.017 cm^-1) [25]. However, the absorption coefficient of collagen is relatively high (α = 900 cm^-1), confirming that collagen effectively absorbs photons at this wavelength. Given its electronic structure, the ablation mechanism of hydroxyapatite should be completely different. Hydroxyapatite is a ceramic with ionic bonding and a Kohn–Sham band gap of 5.4 eV [26]. This gap is larger than the photon energy at 248 nm (5 eV), so photoexcited interband transitions are prohibited. Since the probability of multiphoton excitation is low at the fluences used in the present work, laser radiation will be predominantly absorbed by electrons associated with defects and converted into heat, leading to melting, vaporisation and, for high fluences, thermal ablation of hydroxyapatite. This is confirmed by the low absorption coefficient of hydroxyapatite at 248 nm (α = 120 cm^-1) [25]). In these conditions, due to the short interaction time (~30 ns) and the extremely sharp temperature gradients in the heat-affected zone during the laser pulse, thermal ablation occurs with negligible heat effects on the remaining material. Only collagen is eliminated from the first atomic layers of the substrate surface.
5. Conclusions

(a) Laser treatment of dentin removes the smear layer and produces well-defined cavities, enabling controlled removal of dentin.

(b) The evolution of surface topography due to laser processing varies from sample to sample. In some samples, the surface topography remains flat for all fluences used (in the range 0.5–20 J/cm²). In other samples, a cone-like surface topography develops when fluence is lower than or equal to 1 J/cm², while the surface remains flat for higher fluences.

(c) Collagen has a much higher ablation rate than apatite at 248 nm. The preferential removal of intertubular dentin at fluences below 1 J/cm² observed in some samples is explained by its higher collagen content, compared to peritubular dentin. When intertubular dentin is not excessively mineralised, its ablation behaviour is controlled by the ablation of collagen, which is the continuous phase, leading to a higher ablation rate than peritubular dentin. Flat surfaces result when the ablation rates for peritubular and intertubular dentin are similar, because both constituents are highly mineralised.

(d) The thermal effects resulting from processing with excimer laser radiation are negligible.

References


