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The evaluation of the effect of carbon dioxide laser radiation on dentine tissue

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Purpose: Tissue constitution and construction determine the scope of the structural changes that develop under laser light. The aim of this study was to analyze the effects of carbon dioxide (CO_2) laser light on the structure and elemental composition of dentine. *Methods*: The evaluation was conducted on samples from extracted teeth. The surface of the dentine was exposed to the radiation from a CTL 1401 CO_2 laser (Centre of Laser Technology, Poland). The radiation and frequency parameters were as follows: group I with 5 W and 1 Hz, group II with 10 W and 1 Hz, group III with 5 W and 5 Hz, and group IV with 10 W and 5 Hz. The altered dentine structure was macroscopically and microscopically evaluated using a Nova NanoSEM 200 Scanning Electron Microscope (FEI Europe) with integrated microanalysis X-ray system for elemental analysis in points. *Results*: There were significant differences between groups in the macro- and microstructure of laser defects. *Conclusions*: CO_2 laser radiation causes irreversible, destructive changes in dentine. The structural dentine lesions developed under the influence of the CO_2 laser radiation may hinder proper adhesion of bonding systems with the damaged tissue. Laser defects in the structure should be treated like defects of noncarious origin requiring preparation and filling with composite materials in accordance with the procedures.

Key words: *microstructure, scanning electron microscope (SEM), dentine, carbon dioxide laser*

1. Introduction

Hard tooth tissues differ significantly in terms of their particular principal components. Enamel, which is the hardest tissue in the human body, is by weight 95% minerals and 5% organic substances and water. Dentine contains smaller amounts of inorganic substance than does enamel, at 70% by weight. Unlike enamel, dentine has a higher level of organic compounds (20%) and water (10%). Cementum, the least hard of these tissues, is 65% minerals, 23% organic substances, and 12% water [3]. The diverse elemental composition, the percentage of organic and inorganic substances, and the quantity of tissue water all determine the range of damage caused to these tissues by laser radiation. In the case of CO_2 lasers, which have affinity for water and hydroxyapatite, the quantity of water and inorganic substances is of special significance [1], [11], [13]; incident laser light is thus absorbed to the greatest extent by these components. The constitution and construction of the tissue thus determine the scope of structural changes that develop under laser light.

In the oral cavity, CO_2 lasers are used to perform operations on soft tissues [2]. This treatment can lead to interactions between laser radiation and the teeth. Dentine, which is often exposed in the cervical region of the tooth, may be damaged by CO_2 laser light. This tissue can be affected by laser irradiation, even when it has passed through the enamel and the enamel–dentine junction [5]. CO_2 lasers are also used as a method of dentinal hypersensitivity treatment [15]. It is for these reasons that we undertook this study aimed at analyzing the effect of CO_2 laser light on the structure and elemental composition of dentine – the principal component of the stroma of the tooth.

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2. Materials and method

The investigation was carried out on 40 human molar teeth that had been extracted for periodontal or orthodontal reasons, or because of disrupted eruption. Informed consent was obtained from all the individual participants included in the study. All the procedures performed in this study were in accordance with the 1964 Helsinki declaration. The Bioethical Committee of the Jagiellonian University approved the experiment (KBET/16/B/2013, 28 February 2013). The teeth used for the tests all had the proper structure devoid of visible damage of a carious or noncarious nature. Prior to the structure being examined, the teeth were stored in a solution of physiological saline at 4 °C. Following cleaning in ultrasonic cleaner for 5 minutes, the dentinal samples were collected. Using a round-end tapered diamond ISO 198-021 handpiece turbine, the entire layer of enamel was removed from the buccal and glossal or palatal surfaces of the selected tooth. The samples were prepared under water-spray cooling. From the central part of the prepared area of the tooth, one of the resected 4×4×2 mm samples with an outer layer of dentine was selected. The isolated surface of the dentine was exposed to radiation from the CTL 1401 CO_2 laser at a wavelength of 10,600 nm (Centre of Laser Technology, Warsaw, 1995). In order to ensure comparable conditions during the application of the laser beam, the handle of the laser was immobilized with heavy body c-silicone impression material (Zetaplus, Zhermack, Italy), 2 mm from the sample. The tests were carried out in four groups with diversified laser parameters. The radiation and frequency parameters were as follows: group I with 5 W and 1 Hz, group II with 10 W and 1 Hz, group III with 5 W and 5 Hz, and group IV with 10 W and 5 Hz. The time of exposure to the CO_2 laser radiation was 3 seconds. 20 tests of the dentine surface were conducted in each experimental group. The distribution of samples into particular groups was performed randomly.

The altered structure of the dentine was evaluated macroscopically and microscopically. The macroscopic evaluation was based on a visual analysis of the samples performed by one person. Attention was paid to the diversity of color within the damage and the visible structural changes within the defects. Measurements of the depth and transverse dimension of the defects were also made. Measurements in both dimensions were made gradually. In the first stage, digital photographs were taken of the defect (digital camera Nikon D40X, photo lens AF-S VR Micro-Nikkor 105 mm) and the pattern. The digital correction was then processed using computer software (Adobe Photoshop CS2 version 9.0). Using digital zoom, the size of the damaged area was calculated to an accuracy of 10 μm. First, the transverse dimension of the defect was measured. The samples were then cut in the sagittal plane with a diamond bur using water cooling, in order to measure the depth of the defect in a similar way. In line with other studies of various authors that examined the changes in the structure of the enamel and cementum caused by CO_2 laser radiation, the microchanges in the dentine surface were examined at the Laboratory of Scanning Electron Microscopy and X-ray Microanalysis at the University of Science and Technology in Kraków [17], [18]. The tests were conducted using a Nova NanoSEM 200 (FEI, Europe) – an ultra-high resolution field-emission gun Schottky-type Scanning Electron Microscope – with an energy-dispersive X-ray spectroscopy system (EDS, EDAX) for elemental analysis in points. The Nova NanoSEM can examine the microstructure of conductive and nonconductive materials, as well as of those susceptible to contamination. It can also work as a system for detecting secondary electrons and backscattered electrons [16]. The connection with the X-ray dispersion analyzer allows quantitative elemental and oxide analysis, analysis of the distribution of elements in microareas, and linear analysis. In testing the microstructure of the dentine surface, an accelerating voltage of 15 kV and a low vacuum secondary electron detector were employed. In accordance with the repeated testing scheme for analyzing the enamel and cementum areas, two pictures at 350× were taken of each sample, as were pictures of the details, such as the dentinal tubules (at 1000×, 2000×, and 4000×), during the evaluation of the structural lesions in the dentine [17], [18]. In order to better visualize all the zones of the defect, the images at 350× are presented here, although at this magnification some small anatomical elements are not clearly visible. The analysis of the structures' elemental composition was conducted at selected points.

Table 1. Descriptive statistical analysis of defect dimensions

Test group	Dentine defect	Average [mm]	Minimum [mm]	Maximum [mm]	Std. dev. [mm]
Group 1	Transverse	0.81	0.76	0.87	0.037
	Depth	0.17	0.14	0.19	0.016
Group 2	Transverse	0.91	0.84	0.97	0.035
	Depth	0.23	0.18	0.26	0.027
Group 3	Transverse	1.02	0.93	1.11	0.052
	Depth	0.25	0.21	0.27	0.019
Group 4	Transverse	1.25	1.14	1.36	0.056
	Depth	0.27	0.23	0.317	0.026

The results were analyzed using the Statistica software. At the first stage, the results on the accuracy of defect dimensions (depth and transverse dimension) were subjected to analysis using descriptive statistics. The results were expressed on a ratio scale and the minimal and maximal values, the average values, and the standard deviations were determined (Table 1).

The distributions of the variables were also tested and presented in numerical and graphic forms. The tests included the normal distribution of variables for premolars and molars in each group with the null hypothesis H0: that the deviations were normally distributed. The value of the level of significance was taken to be $\alpha = 0.05$. The outcome of the Shapiro –Wilk test was compared with the given level of significance α (if p was less than α, H0 was rejected and the alternate hypothesis H1 confirmed; if p was greater than α, there are no grounds for rejecting hypothesis H0). In each case, the results of the Shapiro –Wilk tests were greater than 0.05. Thus, the hypothesis H0 could not be rejected and the distributions had normal distribution.

Table 2. Descriptive statistical analysis of mass percentages

Test group	Element	Average [wt. %]	Minimum [wt. %]	Maximum [wt. %]	Std. dev. [wt. %]
Group 1	C	2.32	1.27	3.23	0.66
	O	41.91	36.35	48.17	3.64
	Na	2.53	1.14	3.76	0.86
	Mg	1.42	0.55	2.03	0.48
	P	18.66	16.16	20.44	1.15
	Cl	1.24	0.52	2.02	0.49
	Ca	35.83	28.57	43.66	4.61
Group 2	C	2.27	1.32	3.37	0.69
	O	42.81	36.24	47.81	3.75
	Na	2.52	1.12	4.01	0.97
	Mg	1.34	0.56	2.18	0.54
	P	18.13	16.11	20.52	1.48
	Cl	1.30	0.57	2.04	0.46
	Ca	35.01	28.08	43.38	5.05
Group 3	C	2.38	1.35	3.46	0.73
	O	42.44	37.23	47.68	3.41
	Na	2.42	1.11	3.84	0.84
	Mg	1.18	0.52	2.14	0.53
	P	18.27	16.42	20.28	1.35
	Cl	1.24	0.58	1.84	0.37
	Ca	35.42	28.90	43.43	4.04
Group 4	C	2.56	1.44	3.40	0.55
	O	42.41	37.49	47.56	3.17
	Na	2.31	1.03	3.75	0.86
	Mg	1.27	0.57	2.09	0.46
	P	18.57	16.19	20.54	1.37
	Cl	1.24	0.56	1.91	0.42
	Ca	36.86	29.01	43.04	3.83

The next statistical procedure was based on measurements of defect dimensions, depending on research groups with one-way analysis of variance (ANOVA). To conduct the analysis, the following criteria were established: homogeneity of variance, normality of the distribution, numerical equality of the groups (the null hypothesis H0 was that there are no differences between the groups using different impression materials. The alternative hypothesis H1 stated that there is a difference between the groups). The outcome of the test is $p < 0.05$. The hypothesis H0 was rejected and alternative hypothesis H1 was accepted. For this reason, it was recommended to conduct post-hoc tests. The Least Significant Difference Test (LSD) and Tukey's procedure were applied, with both giving similar results. Statistically significant differences ($p < 0.05$) were seen between all groups for both dimensions.

The results of the elemental analysis were tested using descriptive statistics, selecting the minimal and maximal values, the average value and the standard deviation (Table 2). The variable distributions were analyzed using the Shapiro–Wilk test and proved to be not normally distributed. In the next stage, we analyzed the mass percentages (wt. %) in each group using the Mann–Whitney test, showing no statistically significant differences between the research groups for any elements.

3. Results

Visual analysis of the dentine surface allowed macroscopic peculiarities of the samples to be distinguished. The damage was round or oval in shape, with an average length (transverse dimension) of the defects ascending from group I to group IV: 0.81 mm in group I, 0.91 mm in group II, 1.02 mm in group III, and 1.25 mm in group IV. A similar relation was observed for the depth of the dentine defect: 0.17 mm in group I, 0.23 mm in group II, 0.25 mm in group III, and 0.27 mm in group IV. Statistically significant differences were found for the depth (Fig. 1) and transverse dimension (Fig. 2). Macroscopic evaluation of the structure of the damaged dentine in group I showed the presence of thin black widely separated areolae surrounding a lighter center. No refraction of the surface was observed. Around the circular changes, small areas of yellowish discoloration were present on the dentine surface. In group II, there was also a round defect with a light center and black areola. Unlike in group I, the yellowish discoloration of the dentine surface outside the areola was greater, and a small refraction of the central part of the defect structure was seen. The defects in the dentine structure in groups III and IV were characterized by a black areola and, unlike groups I and II, a chalky-white center to the defects which looked like ash, with a significant refraction of the defect surface. In group IV, the surface defects formed a typical crater. In both groups, areas of yellowish dentine outside the defects typically also appeared.

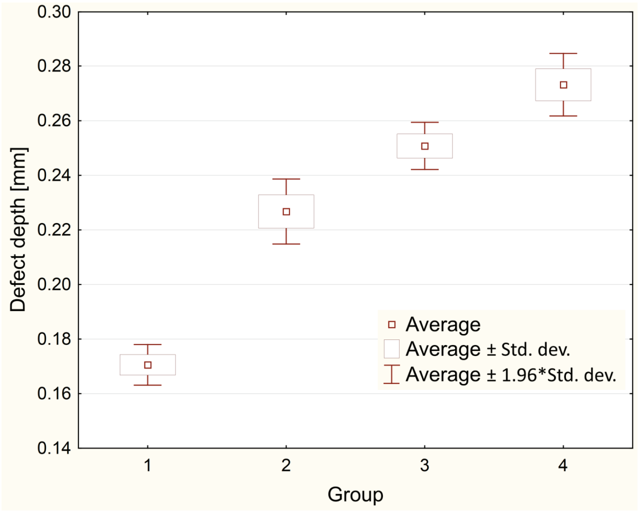

Fig. 1. Box and whisker plots illustrating the distribution of defect depths

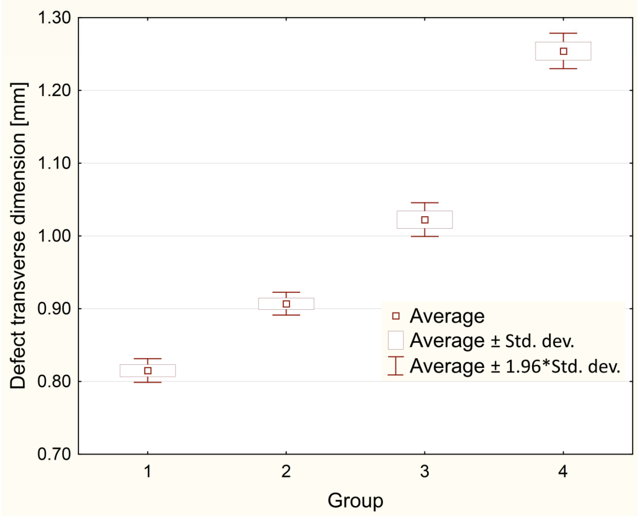

Fig. 2. Box and whisker plots illustrating the distribution of defect transverse dimensions

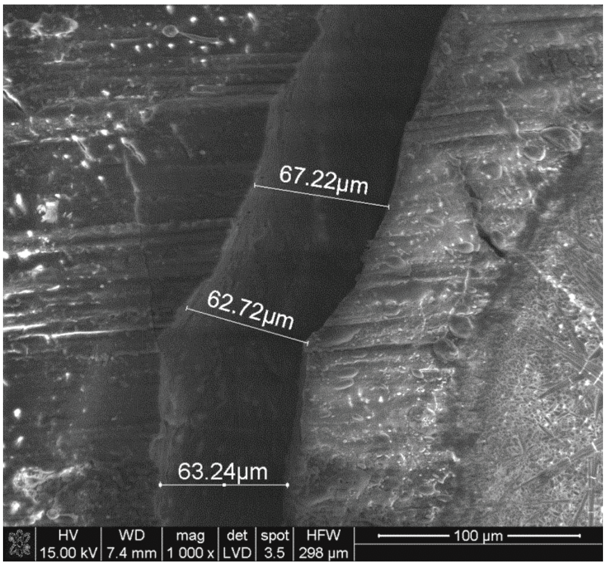

Fig. 3. SEM surface morphology of dentine exposed to CO_2 laser radiation (group 1); magnification 1000×

Fig. 4. SEM surface morphology of dentine exposed to CO_2 laser radiation (group 1); magnification 350×; (a) carbonization zone; (b) fusion zone; (c) partially opened dentinal tubule zone; (d) crater zone

In analyzing the scanning electron microscope images, a few differences could be seen among the groups. In group I, it was visible that the area treated with the laser was separated from the surrounding parts of the sample by a wide fissure with a width of up to 67.22 µm (Fig. 3). Usually, there was only a small crack running through the center of the damaged area, remaining within the diameter of the defect. Partially opened dentinal tubule orifices were present in the separated center, and areas of crystallization of the dentine structure occurred on the almost flat surface of the lesion (Fig. 4). It could be seen from the microscopic analysis that group II showed visible concavities and a refraction of the dentine surface in the middle of the defect, with a fissure of 8.22 µm in width. A fragment with opened dentinal tubule orifices was found in the central part of the defect, while these were not noticeable on the circumference (Fig. 5). The area exposed to radiation was also separated from the other part of the sample by a surrounding fissure. Within group III, an area of the crater containing the center of the lesion and partially opened dentinal tubule orifices could be seen. Structureless tissue masses that did not reveal dentinal tubules occurred more rarely. The fissures separating the damaged area from the other parts of the dentine were most visible externally, reaching sizes of up to 82.46 µm (Fig. 6). The lesions in group IV were characterized by the presence of deep craters located in the center of the defect, together with a fracture running through the center whose size was 36.22 µm at the widest point. In the immediate vicinity of the crater, there was an area of partially opened dentinal tubules. Outside of the area, as in group III, there was a zone of fusion and circumferential fissures (Fig. 7). In general, the damage to the dentine caused by the activity of the CO_2 laser includes the zone of the crater, which is least apparent in group I; the zone of partially opened dentinal tubules; then the zone of fusion and the zone of carbonization with circumferential fissures.

The examination of the elemental composition at the chosen points did not reveal any specific statistically significant differences between the zones (Fig. 8). The highest level of carbon was observed in the most eccentric zone – that of carbonization. However, the

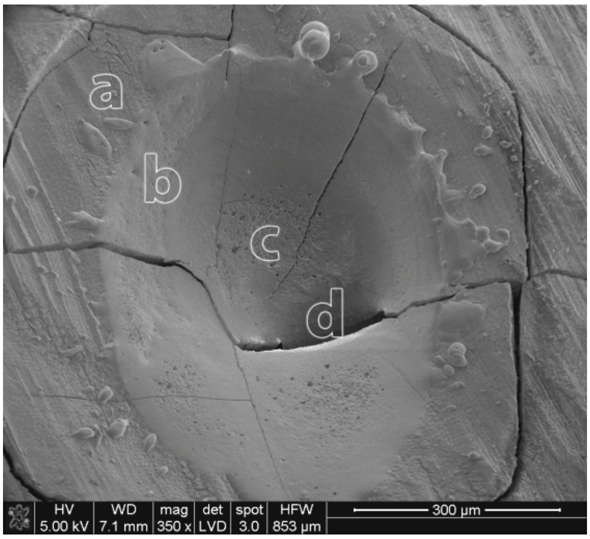

Fig. 5. SEM surface morphology of dentine exposed to CO_2 laser radiation (group 2); magnification 350×; (a) carbonization zone; (b) fusion zone; (c) partially opened dentinal tubule zone; (d) crater zone

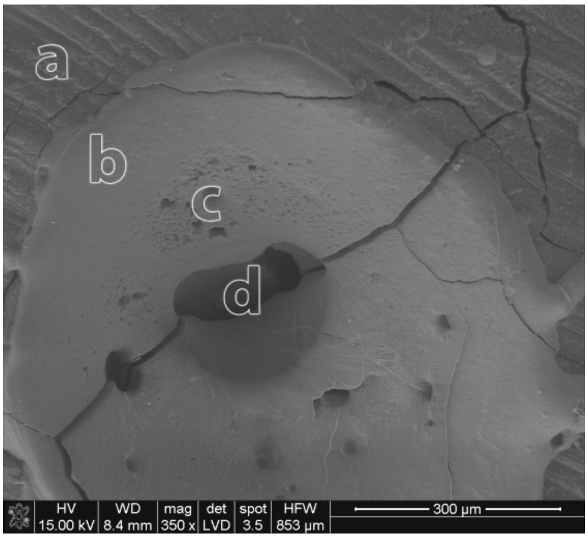

Fig. 7. SEM surface morphology of dentine exposed to CO_2 laser radiation (group 4); magnification 350×; (a) carbonization zone; (b) fusion zone; (c) partially opened dentinal tubule zone; (d) crater zone

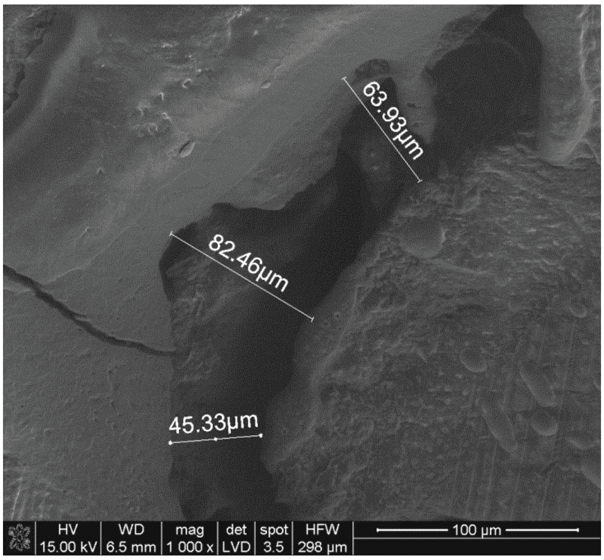

Fig. 6. SEM surface morphology of dentine exposed to CO_2 laser radiation (group 3); magnification 1000×

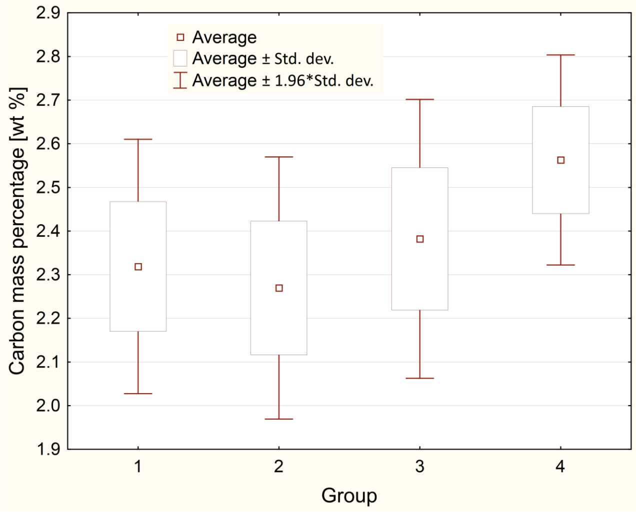

Fig. 8. Box and whisker plot illustrating the distribution of carbon mass percentages

differences among the particular groups were not significant from the statistical point of view.

4. Discussion

Microscopic examination of the effect of the CO_2 laser on the dentin revealed the presence of craters, fissures, fusions, recrystallization, and carbonization of the structure. These results are consistent with the reports of other authors. Nomelini et al. [12] evaluated the influence of various parameters of the CO_2 laser on dentine. The application of the 2 W beam did not cause any visible melting of the structure. Radiation at 4 W caused cracks and fissures, as well as the melting and recrystallization of some areas. The 6 W beam, as with Nomelini, induced sizeable fissures, areas of melting (similar to volcanic lava) of merged, smooth structure, invisible dentinal crystals, and closed dentinal tubules.

As our research suggests, the deformations of the dentine structure resulting from the laser beam are more similar to the defects of the enamel, rather than to those of the cementum – both of which were also exposed to the laser as part of the testing methodology [17], [18]. The development of craters in the center of the area tested, the fusions, and the presence of the external area of carbonization are common both to the enamel and dentine damage. Similarly, the nature of the structural lesions in the observed tissues is probably due to the high mineral levels of enamel and dentine, as compared to the quantity of inorganic minerals in the root cementum. Much greater damage to the cementum was caused during the application of the same laser parameters, and this shows the delicate nature of the tissue and the caution that is required during clinical procedures, whether using laser radiation or direct contact.

The sequence of appearance of the zones in the dentine damage is connected with the pathomechanism of the laser beam. The incidence of greatest energy intensity in the middle of concentrically arranged zones activates the development of the crater through the evaporation of tissue. Thus, small pores can be observed at the bottom, and in the immediate vicinity there is an area of only partially closed dentinal tubules. When the tissue is deprived of water, the location contracts, and fissures and cracks occur in the area of the crater. In the place where less energy is incident, the tissue melts, leading to a considerable percentage of the closed dentinal tubules. According to Kim et al. [9], under the influence of laser radiation, the dentinal tubules are obliterated first; next, due to the melting of the dentine layer and the covering, the tubule orifices and structureless layer of the tissue mass proceed to melt. In the experiments of Kim et al., 77% of the dentinal tubules were closed by a 3 W CO_2 laser beam. This high percentage was reached because of the low power employed, which causes minor melting of the surface layer of the dentine without producing any considerable impact on the structure deeper in the tooth. Laser radiation has been applied to dentine, as mentioned above, in the treatment of dentine hypersensitivity, which has become a difficult clinical problem [10], [20]. Romano et al. [15] analyzed the use of calcium hydroxide and CO_2 laser light in an in vitro model of the treatment of dentinal hypersensitivity, observing the percentage of closed dentinal tubules following the therapy. The results of the tests showed better outcomes in the group where both calcium hydroxide and the CO_2 laser were used than in the group with only the laser activity. In the group with synergistic laser activity and calcium hydroxide, the percentage of closed tubules was over 50%. Birang et al. [4] indicated that the application of laser radiation to treat dentinal hypersensitivity beings about a change in the structure of the dentine surface and a decrease in the transmission of stimuli within the nerve endings of the pulp by direct action on the nerve. However, research by Romano et al. has demonstrated that the application of a CO_2 laser at 0.5 W is safe for pulp, as it does not cause an increase in temperature greater than 5 °C [15]. In the experiments of Zach et al. [23], it was confirmed that no histological lesions occur in the tooth pulp during an increase in temperature of less than 5 °C. Preiskorn et al. [14] have presented studies of heat transfer phenomena in human teeth, modeled without and with the pulp.

Apart from the use of lasers in the treatment of dentinal hypersensitivity, the impact of the CO_2 laser on the antierosive dentine potential needs to be considered, as the results to date have not been promising. Wegehaupt et al. [21] indicated that the greatest antierosive potential of dentine occurs after the synergistic application of cerium chloride and aminofluoride without laser radiation. In that study, no positive modulating effect of laser radiation on dentine was seen. Experiments carried out by Steiner-Oliveira et al. [19] concerning the same issue showed only minor protection of dentine against erosive action.

In a different experiment, susceptibility of dentine to demineralization after the use of CO_2 laser light was considered. Tests by Esteves–Oliveira [7] demonstrated a decrease in the susceptibility of dentine to demineralization following synergistic laser activity and the supplementation of the tissue with fluorine. The use of the laser alone had no effect on the solubility of dentine in the artificial model of caries. The analysis of the effect of laser radiation on enamel showed increased tissue resistance to acids. This phenomenon has been confirmed experimentally by Diaz-Monroy et al. [6], who characterized changes in elemental composition indicated in the laser-treated enamel. When comparing the effect of laser radiation on enamel, which produced an increased resistance to caries, to the small increase observed in the caries resistance of dentine, the mechanism of the microexposure of water molecules under the laser radiation should be mentioned. Unlike enamel, the greater content of water in dentine causes tissue contraction following laser radiation. Dehydration leads to the disintegration of the organic mold, which takes the place of the lost phase of mineral dentine. Thus, the resistance of the tissue to demineralization does not increase. From the clinical point of view, the structure of dentine following the use of CO_2 laser radiation does not meet the requirements necessary to ensure the proper adhesion of tissue with systems combining composite materials. The presence of defects in the form of fissures, cracks, craters, and areas of fusion make it impossible to find an appropriate combination of dentine with the composite material. Furthermore, serious tissue dehydration in the area of the possible tooth–material combination hinders the correct adhesion of the systems combining with the dentine. It is well known that proper combination requires the dentine not being dried out. However, in the case of tissue that has undergone laser radiation, dehydration occurs immediately before etching and applying the bonding system. However, the most important issue concerning adhesion between dentine and bonding resin is to create a special layer between them to guarantee proper adhesion. In order to obtain such a junction, the infiltration of resin into the etched intertubular dentine should occur to form a hybrid layer. However, the resin should penetrate into the open dentinal tubules creating resin tags. In the altered dentine structure resulting from the application of the CO_2 laser, the superficial layer of dentine will not form either a hybrid layer or a micromechanical junction with the use of resin tags. It is thus advisable to use a mechanical preparation of dentine that has been affected by CO_2 laser radiation of the same power as that applied in the experiment. The procedure of filling in the defect should not differ greatly from the generally accepted principles [8].

The situation is different in the case of composite–enamel junction, where the retention is obtained by micromechanical connection [22].

5. Conclusions

The CO_2 laser radiation causes irreversible, destructive changes in dentine.

The structural dentine lesions that develop under the influence of CO_2 laser radiation may hinder the proper adhesion of bonding systems with the damaged tissue.

Laser defects of structure should be treated like defects of noncarious origin, requiring preparation and filling with composite materials in accordance with general procedures.

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