

STEREOMICROSCOPIC ASPECTS OF ROOT CANAL WALLS AFTER CONVENTIONAL LASER ENDODONTICS – A PRELIMINARY STUDY

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Abstract

Bacterial decontamination of root canals can be improved by directly irradiating the dentinal walls with dental lasers or by irradiating/activating the irrigation solutions, the laser having an indirect clinical action. Inside the root canal, laser irradiation produces a thermal effect on the dentine walls and bacteria, which generates unwanted side effects, if the therapeutic protocol is not followed. The aim of this study was to evaluate the root canal walls by stereomicroscopic examination, after their chemo-mechanical instrumentation and conventional laser irradiation. For this study, 35 palatal roots of upper first molars were used. For some of the palatal canals, conventional laser irradiation was performed using a diode laser with a wavelength of 980 nm and two different irradiation protocols. The root canal walls were examined stereomicroscopically at different degrees of magnification. The study demonstrated the effectiveness of the stereomicroscope in examining the walls of prepared and laser irradiated root canals. Areas of morphological alteration were identified and measured on the walls of the root canals for which the irradiation protocol was not applied correctly.

Keywords: stereomicroscope; diode laser; endodontic disinfection; laser assisted endodontics.

1. Introduction

The evolution in the field of physics at the beginning of the 20th century laid the foundations for the laser theory developed by Albert Einstein. The invention of this special form of light was in 1960 and shortly after, researchers began to explore the possible applications of laser technology in medicine and dentistry [1]. In 1965, Dr. Leon Goldman used the ruby laser for the first time, in vivo, on dental tissues [2,3].

Due to the availability of laser technology, researchers' attention focused on understanding the laser-tissue interactions and the effects given by each wavelength, in order

to establish an effective protocol for their clinical indication [4].

In the case of endodontic treatments, the success of the interventions is dependent on the elimination of the intracanal bacterial population and its metabolic products, but also on the patient's general state of health [5]. The results of endodontic treatments have been improved by using the laser technology in all treatment stages. In particular, the bacterial decontamination of root canals improved by using dental lasers to directly irradiate the dentinal walls or to irradiate/activate irrigation solutions, thus having an indirect clinical action [2].

Inside the root canal, laser irradiation

produces a thermal effect on the dentine walls and bacteria, which can generate unwanted side effects, if the therapeutic protocol is not followed. The evaluation of the temperature of dental tissues during laser irradiation of root canals is a subject approached by researchers through numerous investigation methods, such as the application of thermocouples and the finite element method [6-10].

To assess the thermal and decontamination effects of the dental laser in endodontics, studies were carried out using medical imaging methods, especially with scanning electron microscope (SEM) [11-31].

For the same purpose, in literature were reported fewer studies using the stereomicroscope/stereoscope. These researches were done approximately 20 years ago, and the images obtained then do not meet current quality standards, in terms of color and resolution [32-38].

The stereomicroscope was introduced as a medical device in 2005. Over time, this device has been successfully used in ophthalmic and vascular surgeries, but also for surgical education [39].

In research in the field of dental medicine in recent years, the stereomicroscope has been used in particular to examine the external morphology of teeth and their lesions [40-44] and to assess the quality of coronal fillings and those of the root canals using different techniques and materials [45-51]. Given the lack of recent studies in which the stereomicroscopic examination of root canal walls is performed, and the technological advancement, the authors decided to use a stereomicroscope for the objective of the present study.

The aim of this study was to evaluate the root canal walls by stereomicroscopic examination, after their chemo-mechanical instrumentation and conventional laser

irradiation.

2. Materials and Methods

2.1. Sampling and Preparation of Teeth

For this study, 35 upper first molars were used, extracted from 28 patients aged between 30 and 65 years, who presented themselves for dental treatment at the Faculty of Dental Medicine, UMF Craiova. The study was approved by the Ethics Committee of the University of Medicine and Pharmacy, Craiova, Romania (no. 28/24.02.2021).

The inclusion criteria were: (1) patient consent for dental treatment; (2) patient consent to participate in the study and publish the data; (3) the teeth that were to be extracted presented various dental and periodontal diseases for which conservative restorative and/or surgical treatment was contraindicated; (4) the teeth included in the study were upper first molars with intact palatal roots after extractions. The exclusion criteria were: (1) previous endodontic treatment; (2) prosthetic restoration; (3) root fracture or root resorption.

The teeth included in the study were extracted atraumatically, using the conventional technique, disinfected by keeping them in a 10% peroxide solution for 10 minutes, then ultrasonically scaled and brushed. Dental extractions, disinfection, cleaning and storage of the teeth included in the study were performed by 2 dentists, a coordinator and an operator.

2.2. Chemo-Mechanical Instrumentation and Laser Irradiation of Palatal Root Canals

For each tooth extracted and included in this study, chemo-mechanical treatment was initiated according to known techniques [52]. To establish the working length of each canal, a K-file ISO 10 was first inserted inside it until the tip became visible at the level of the apical foramen, and the silicone stopper was placed at

an occlusal land-mark. The working length was determined by reducing the length thus measured by 0.5 mm [53].

All palatal root canals were instrumented using the E-Connect S Endo motor (Eightteeth, Changzhou Sifary Medical Technology, Changzhou, China) and the Reciproc Blue system (VDW GmbH, Munich, Germany). Irrigation of root canals was carried out with sodium hypochlorite 5.25%, sol. NaCl 0.9% and sol. EDTA 17%. Irrigation solutions were ultrasonically activated with Ultra-X Activator (Eightteeth, Changzhou Sifary Medical Technology, Changzhou, China). Root canals were dried with calibrated paper cones. All the procedures described were performed by a single operator, a specialist in Endodontics, using a dental operating microscope (Prima M μ DNT Labomed, Los Angeles, U.S.A.).

Later, the teeth were divided into 3 groups:

1. group A – 15 upper first molars, to which the conventional laser irradiation technique was applied to the palatal root canals at 1 W, continuous wave (CW), 3 irradiation cycles separated by resting periods of 10 seconds;

2. group B – 10 upper first molars, to which the conventional laser irradiation technique of the palatal root canals was

incorrectly applied at 2 W, CW, 3 irradiation cycles, without resting periods between them (10 upper molars);

3. group C – 10 upper first molars, to which the conventional laser irradiation technique was not applied (10 upper molars).

Since the total number of teeth could not be divided equally into 3 groups and the aim of the study was to analyse by stereomicroscopic appearance of the internal root walls after conventional laser irradiation, it was decided to distribute more teeth to group A.

A diode laser with a wavelength of 980 nm (Biolase, USA) and specific endodontic tips (E2-20 EZ Tip, E2-14 EZ Tip) were used to perform the conventional laser irradiation technique of the root canals (Figure 1), as described by specialists [2]. For the teeth included in groups A and B, when the palatal root canals were irradiated, the canals were dry. The endodontic tips were inserted along the entire working length, then withdrawn 1 mm away from the apex. The laser was then activated and the tips retracted in a helical motion at a speed of 2 mm/sec. Irradiation of the root canals with the laser was performed by a single operator, an Endodontics specialist. The distribution of teeth into groups and information about the preparation of palatal root canals were systematized in Table 1.



(a)



(b)



(c)

Figure 1. Sequences of root canal irradiation: (a) the diode laser and the endodontic tips used; (b) positioning the endodontic tip inside the palatal root canal; (c) sequence from palatal root canal irradiation.

No.	Teeth code	Working length of the palatal root canals	Reciproc Blue files used	The group in which the teeth were included
1	M1	20 mm	R40	A
2	M2	19 mm	R40	A
3	M3	20 mm	R40	A
4	M4	19 mm	R40	A
5	M5	21 mm	R40	A
6	M6	20 mm	R50	A
7	M7	22 mm	R40	A
8	M8	21 mm	R40	A
9	M9	22 mm	R25	A
10	M10	20 mm	R25	A
11	M11	22 mm	R40	A
12	M12	21 mm	R40	A
13	M13	18 mm	R40	A
14	M14	19 mm	R40	A
15	M15	20 mm	R25	A
16	M16	20 mm	R40	B
17	M17	20 mm	R50	B
18	M18	19 mm	R40	B
19	M19	21 mm	R40	B
20	M20	22 mm	R40	B
21	M21	19 mm	R40	B
22	M22	20 mm	R25	B
23	M23	20 mm	R40	B
24	M24	21 mm	R40	B
25	M25	19 mm	R40	B
26	M26	19 mm	R40	C
27	M27	20 mm	R40	C
28	M28	19 mm	R25	C
29	M29	22 mm	R40	C
30	M30	20 mm	R40	C
31	M31	21 mm	R40	C
32	M32	20 mm	R40	C
33	M33	19 mm	R50	C
34	M34	20 mm	R40	C
35	M35	21 mm	R40	C

Table 1. Distribution of teeth in groups.

2.3. Preparation of Palatal Roots for Stereomicroscopic Examination

For the stereomicroscopic examination, the palatal roots of the first upper molars included in the study were first standardized. This was obtained by sectioning the palatal roots at a length of 10 mm, measured from the apex with a periodontal probe and marked with a pencil on the external root surface. The

sectioning was done with diamond burs, using an air turbine and water cooling (Figure 2 a,b).

This was followed by breaking the roots into 2 halves, further called "samples". In order to break the roots, 2 longitudinal grooves of less than 1 mm depth were prepared for each root on the external walls, halfway between the buccal and oral surfaces, using diamond burs and air turbine with water cooling [54]. The

breaking of roots was finalized by applying the jaws of a cutting pliers (Dexter) in these grooves and manually tightening the pliers (Figure 2 c). The sectioning and breaking of the roots was done in such manner due to the lack of a precision cutting device. It was considered that the microcracks that can appear using these techniques are not the subject of the study and do not interfere with the examination of possible thermal defects.

The two samples obtained per root were fixed in high consistency silicone (Zetaplus L Intro KIT, Zhermack, Badia Polesine, Italy) (Figure 2 d). The standardization of the palatal roots and the preparation of samples for the stereomicroscopic examination were carried out by a single operator, a dentist trained in Endodontics.

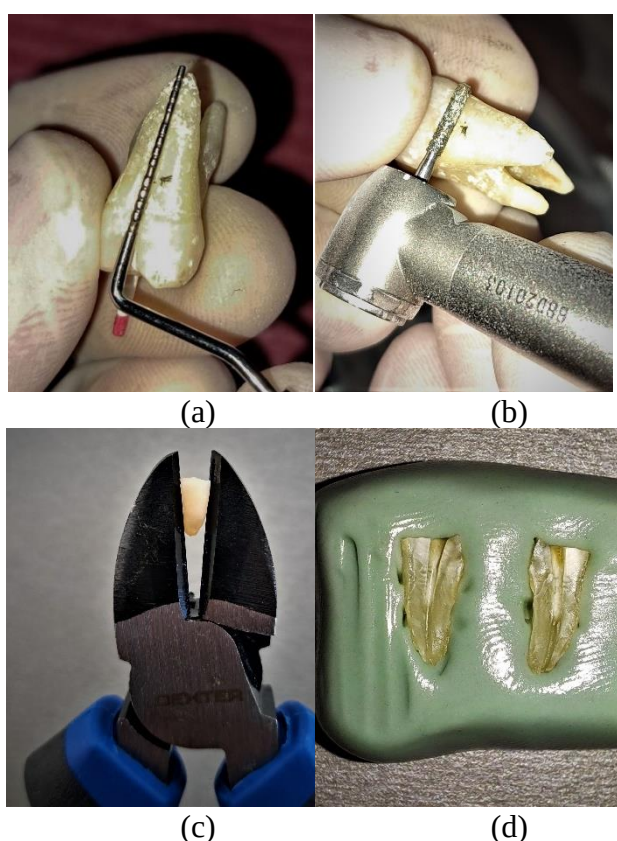


Figure 2. Preparation of a palatal root for stereomicroscopic examination: (a) appreciation of the level where the root will be sectioned; (b) root sectioning; (c) the root during the breaking; (d) the 2 samples obtained from one root.

For this study, the NIKON SMZ 745T stereomicroscope (Nikon, Japan) (Figure 3) was used at a maximum magnification of 75x [55]. The device was used to examine the walls of the palatal root canals. Using the NIS-A-AMEAS software, 2D images of the examined samples were acquired. The images were

stored as TIFF, JPG and PNG files. Through the successive acquisition of 2D images, recomposition was achieved in order to obtain a 3D image of the samples, using the NIS-A-AEDF software.

Various measurements of the elements of interest were made on the 2D and 3D images

obtained, using the Image J program (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, LOCI, University of Wisconsin) [56].

The stereomicroscopic examination was performed by a specialist of Engineering and

Management of Technological Systems. The interpretation and analysis of the obtained images was carried out by 2 specialists in the field of endodontics.

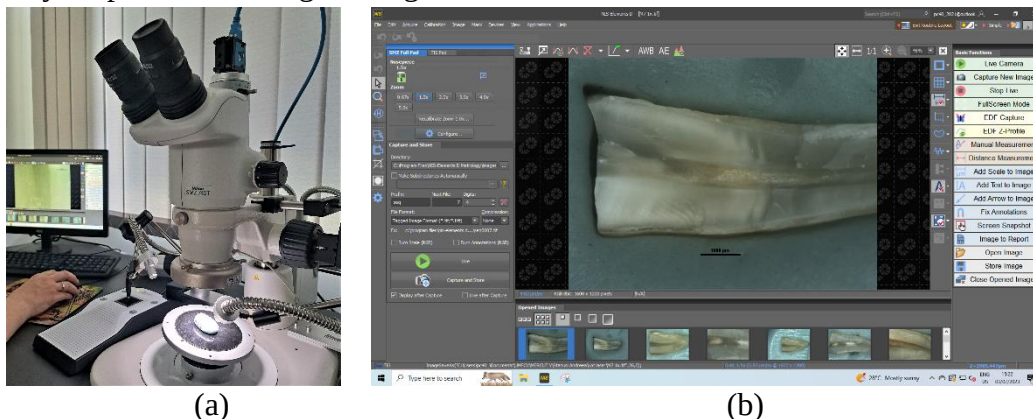


Figure 3. Stereomicroscopic examination of samples: (a) positioning the samples; (b) NIS-A-AMEAS software interface.

3. Results

In total, 35 palatal roots of upper first molars were prepared for stereomicroscopic examination. For each palatal root, 2 samples were obtained after breaking, further named "tooth code-a", respectively, "tooth code-b". In total, 70 samples were examined with the NIKON SMZ 745T stereomicroscope.

During the stereomicroscopic examination, several aspects of the root canal

walls were appreciated: the presence/absence of pulp content, the presence/absence of dentinal detritus, the presence/absence of areas of morphological alteration.

3.1. Stereomicroscopic Examination of Samples from Group A

For the samples from group A, the stereomicroscopic examination showed the absence of pulp content and dentinal detritus (Figure 4).

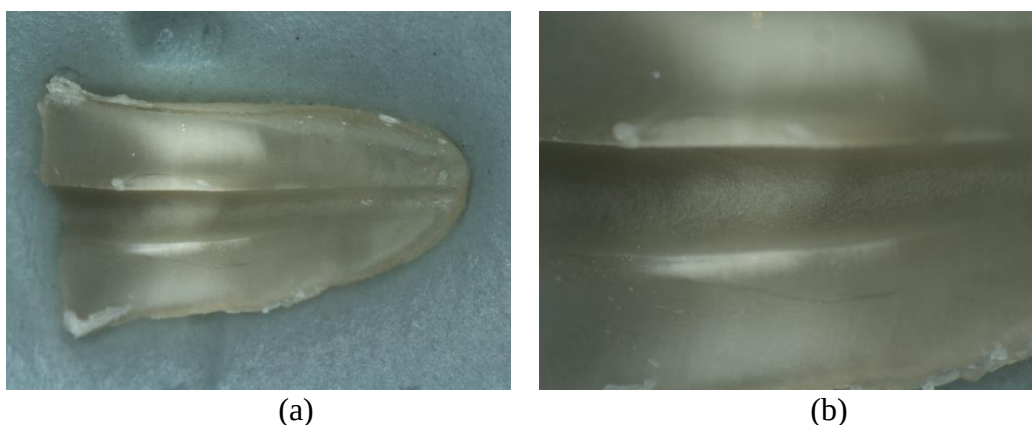


Figure 4. Stereomicroscopic examination of sample M5-a: (a) 15x magnification; (b) 30x magnification.

In few samples, areas of morphological alteration of the root canal walls were identified. These areas were characterized by the existence of two parallel lines, whitish in color and were preferentially located in the apical third of the samples, being interrupted in

some places by areas of normal appearance (Figure 5).

For each morphological alteration area thus identified, the distance between the 2 lines was measured, using the Image J program. The measured distances were approximately 200 μm (Figure 6).

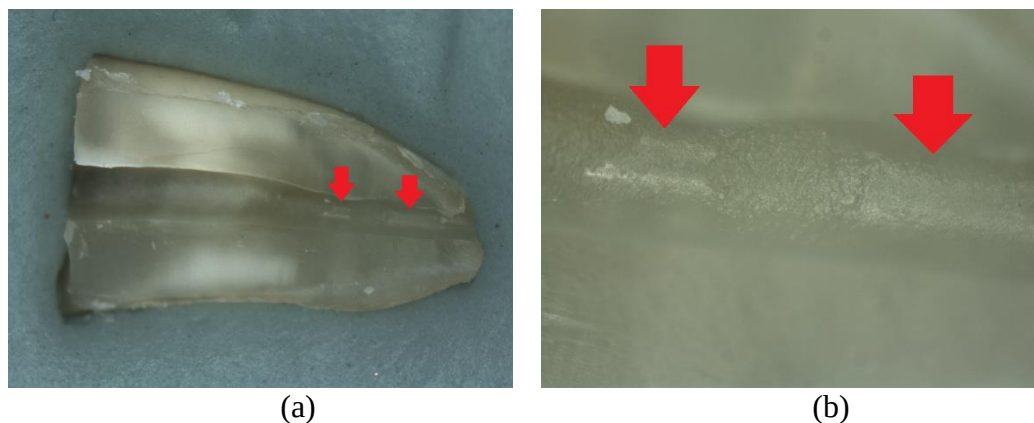


Figure 5. Stereomicroscopic examination of sample M9-b - the red arrows indicate the areas of morphological alteration: (a) 15x magnification; (b) 60x magnification.

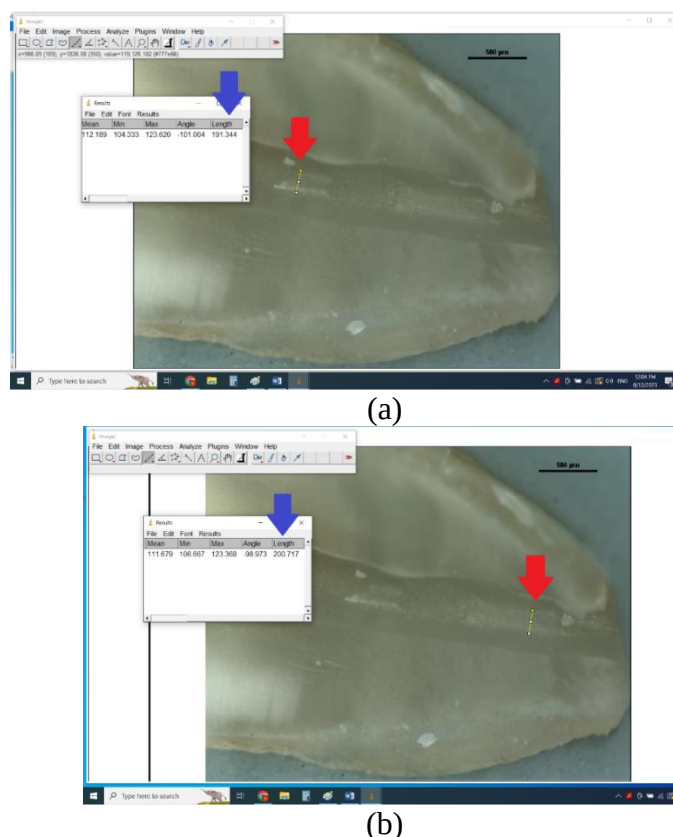


Figure 6. Samples from group A - measurement of areas of morphological alteration in Image J - the blue arrows indicate the measured value; the red arrows indicate the measured distance: (a), (b) measuring the distance between the 2 lines in the apical third.

3.2. Stereomicroscopic Examination of Samples from Group B

For the samples from group B, the stereomicroscopic examination revealed the absence of pulp content and the absence of dentinal detritus. In most of the samples examined, the existence of areas of morphological alteration was observed. These alterations were presented as areas of irregular

shape, whitish/brown in color, without a preferential arrangement and interspersed with areas with a normal appearance (Figure 7).

For each area of morphological alteration thus identified, the affected surface was measured using the Image J program (Figure 8). Each measured area did not exceed $100.000 \mu\text{m}^2$ (0.1 mm^2).

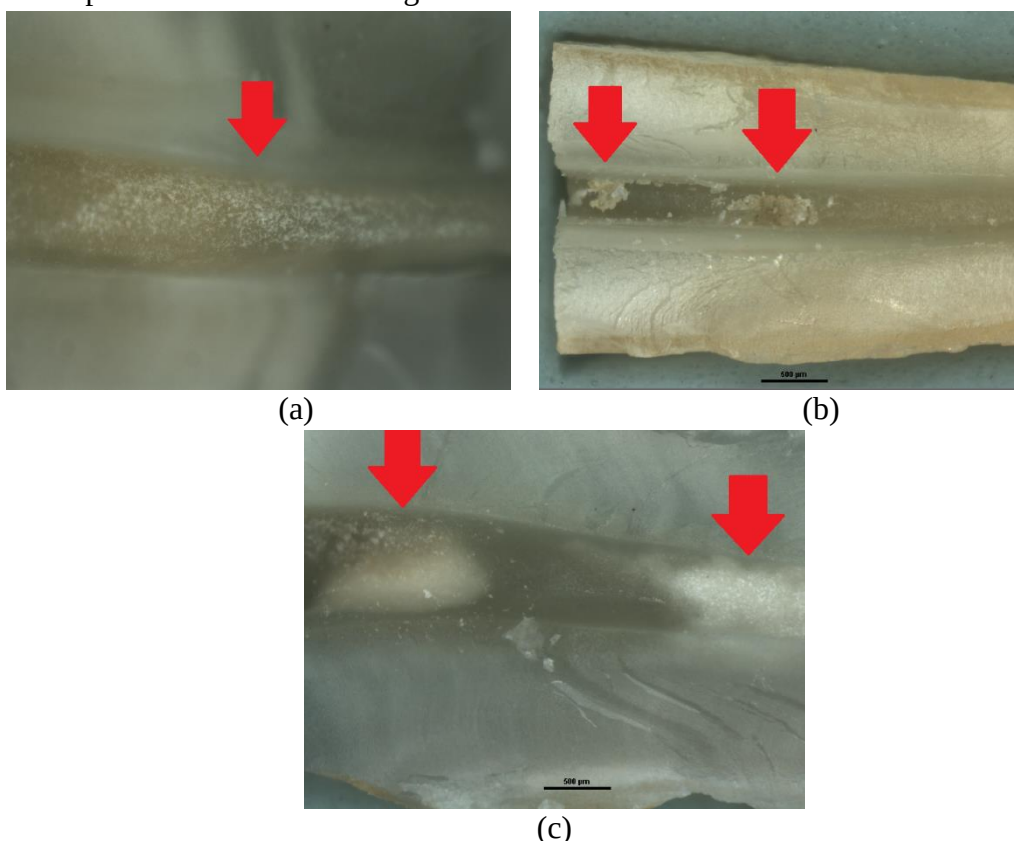
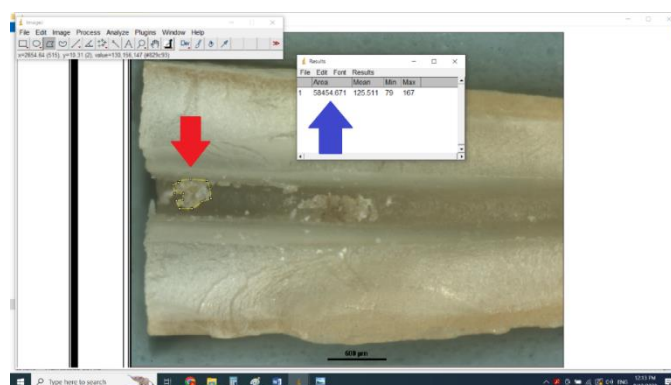
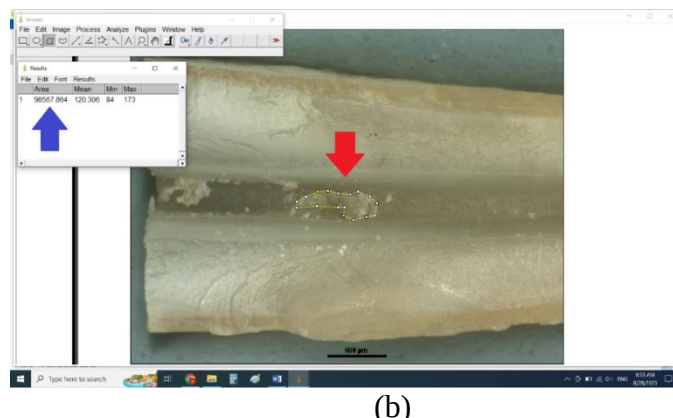


Figure 7. Stereomicroscopic examination of some samples from group B - the red arrows indicate the areas of morphological alteration: (a) sample M16th degree of magnification 45x; (b) sample M17th degree of magnification 30x; (c) sample M25-b magnification 30x.



(a)

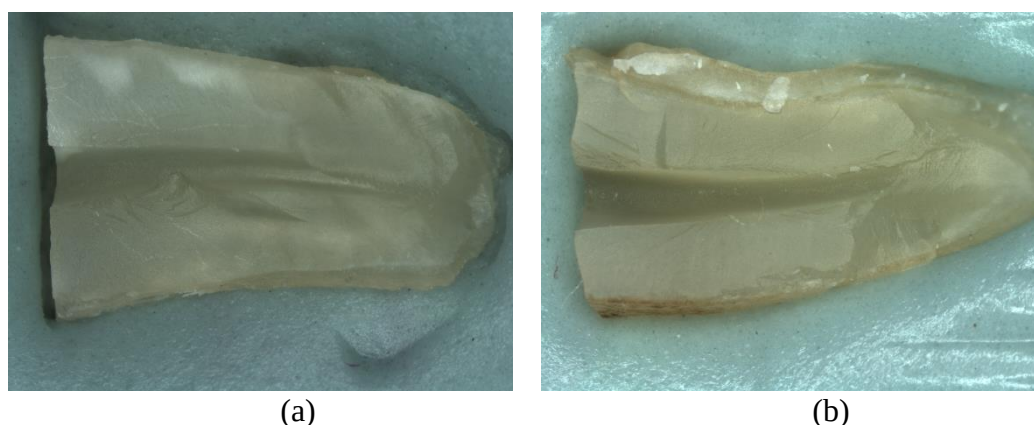


(b)

Figure 8. Samples from group B - measurement of areas of morphological alteration in Image J – the blue arrows indicate the measured value; the red arrows indicate the measured area: (a) measuring the area of morphological alteration in the coronal third; (b) measuring the area of morphological alteration in the middle third.

3.3. Stereomicroscopic Examination of Samples from Group C

For the samples from group C, the stereomicroscopic examination revealed the absence of pulp content, the absence of dentinal detritus and the absence of areas of morphological alteration (Figure 9).



(a)

(b)

Figure 9. Stereomicroscopic examination of samples from group C: (a) sample M28-a degree of magnification 15x; (b) sample M33-b magnification 15x.

4. Discussions

The diode laser was initially introduced in endodontics for the treatment of dentin hypersensitivity [57,58] and for the bio-modulation of the inflammatory response during vital pulp therapy [59,60]. Today, diode lasers represent an alternative to Nd:YAG lasers in terms of root canal decontamination, being preferred by practitioners due to their design, price and ease of handling and use [2,61,62,63,64].

Regarding the mode of action of the

laser light for the decontamination of the root canal, two mechanisms have been suggested. The first mechanism was the one through which the laser light is absorbed by the bacteria, with damage to the cell membrane, osmotic alteration and cell death [65,66,67]. The second mechanism suggests that the laser light is strongly absorbed in the dentinal substrate on which the bacteria adhere, the resulting heat causing a local increase in temperature, high enough to lead to the death of the attached microorganisms [65,68,69].

Although the bactericidal effect and the local increase in temperature during laser decontamination of root canals has been intensively analysed in specialized studies, there are still concerns regarding the unwanted side effects, in particular those caused by the increased temperature and how they can influence the success of the endodontic treatment [68, 70]. Few studies have investigated the morphological changes that appear on the internal root wall [68,71,72,73].

The heat that develops in the root tissues causes the appearance of areas of morphological alteration. Mainly collagen alteration and partial closure of the dentinal tubules occur through a fusion process of the organic and inorganic dental structure [2,68]. This decrease in the permeability of the root dentin compromises the adhesion of some sealers [70] and, implicitly, the achievement of a sealed canal obturation that ensures the success of the endodontic treatment.

In addition, when the terminal portion of the tip or fiber comes into contact with the dental wall during the laser emission, it can produce thermal damage called "hot spots" in the specialized literature [2,64]. In specialized literature, there are very few images of these areas of morphological alteration [2].

Studies using medical imaging methods are available to support practitioners concerned with this topic. Among the medical devices used to obtain tissue images, the digital stereomicroscope stands out for its advantages. A digital stereomicroscope has high degrees of magnification, some devices can be used at degrees of magnification up to 300x, depending on the eyepiece and auxiliary objective [55]. The use of the stereomicroscope and specialized software allows the creation of 2D images, 3D reconstructions and various measurements for the analysis of the examined samples.

The aim of the presented study was the stereomicroscopic examination of the root canals that were chemo-mechanically instrumented and decontaminated with a diode laser, in order to identify the effects of laser irradiation on the dentinal walls.

The study was carried out on the palatal roots of the upper first molars, whose volume facilitated the obtaining of samples for stereomicroscopic examination. Palatal canals were prepared using a reciprocating one-file shaping system, with a large taper, to facilitate the insertion and extraction of the laser tip. In the case of the root canals for which it was decided to apply laser decontamination, the conventional irradiation technique was carried out following the indications of the specialists (group A) and, respectively, at a higher power, without offering a break between the irradiation cycles (group B). Thus, it was possible to appreciate the appearance of the root canal walls and the importance of following the operative protocol.

In most of the palatal canals in group A, no areas of morphological alteration were identified. For some samples examined, the existence of some morphological changes in the apical third was observed, in the form of parallel lines interspersed with unaffected areas. Their location in the apical third of the root canals was noted, a fact that corresponds to the observations made by other researchers regarding the preferential damage to this portion of the root canal. The apical region of the root canal is more prone to thermal damage because at this level, the terminal portion of the tip passes very close to the walls of the root canal due to the existence of anatomical constrictions [74]. Regarding the geometry of these morphological alterations, measurements were made and a width of the respective zones of maximum 200 µm was determined, a size that corresponds to the diameter of the tips

used. It was noted that these morphological alterations were not arranged continuously, but were interrupted by unaffected areas. We consider that the morphological alterations identified in some samples from group A were determined by the direct contact of the endodontic tip with the walls of the root canals in its apical third. We appreciate that their reduced size cannot influence the future canal sealing.

In the case of palatal canals from group B, areas of morphological alteration were identified in most of the samples. These morphological alterations had irregular shapes and sizes up to 0.1 mm² per area examined. It was observed, again, that the areas of morphological alteration did not have a continuous disposition, but were interrupted by unaffected areas. We consider that the morphological alterations identified in the samples from group B were determined by the increase of the local temperature beyond the limits accepted by the dental tissues, as a result of the use of the dental laser at a much higher power and without ensuring the resting period between the irradiation cycles that would allow the local temperature to drop. We appreciate that these areas of morphological alteration can influence, by their size, the sealing of the future canal obturation.

The presented study draws attention to the need to comply with the operating protocol for laser decontamination of root canals. The user of the dental laser must know the thermal effects that develop in the dental and periodontal tissues when using such a device. The increase in temperature causes changes in the tissue's structure that can influence the success of the treatment.

The novelty of the present study results from the identification of areas of morphological alteration through the stereomicroscopic examination of the walls of

the root canals. Also, high-quality stereomicroscopic images were obtained that draw the attention of practitioners to the need to understand the laser-dental hard tissue interaction and respect the operative protocol. To our knowledge, there are no studies in the specialized literature in the last 20 years that use a stereomicroscope to examine the walls of the root canals after their conventional laser irradiation.

The weak points of this study are represented by the small number of teeth examined and the absence of similar studies with which obtained results could be compared.

The present study is a preliminary research and will be continued with the optical coherence tomography examination of the samples, due to the need to correlate the surface alteration identified, with thermal damage of radicular dentine such as tubular occlusion that can be responsible for reduction of canal sealer penetration depth.

5. Conclusions

The present study highlighted the effects of conventional laser irradiation of root canals through the stereomicroscopic examination of their walls.

No areas of morphological alteration were identified on the walls of the root canals for which the irradiation protocol was correctly applied. The study drew attention to the need to comply with the operating protocol for conventional root canal irradiation in order to achieve therapeutic success.

The areas of morphological alteration at the level of the root canal walls were identified and measured for which the conventional laser irradiation protocol was not correctly applied. Correlations were made between the identified morphological alterations and their cause.

The study highlighted the effectiveness of the stereomicroscope in examining the walls of the root canals and in identifying areas of morphological alteration.

The performed stereomicroscopic examination resulted in superior quality images of the areas of morphological alteration by laser irradiation of the root canals.

Further investigation will be performed to correlate the surface images obtained through this stereomicroscopic examination of the root canal walls, with internal thermal alteration of the dentine.

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Institutional Review Board Statement: The study was approved by the Ethics Committee of University of Medicine and Pharmacy Craiova (nr 28/24.02.2021).

Informed Consent Statement: Informed consent was obtained from the legal guardian of the subjects involved in the study.

Data Availability Statement: The authors declare that the data from this research are available from the corresponding authors upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

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