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PhD THESIS

**STUDY ON THE INFLUENCE OF LASER RADIATION
IN ORO-DENTAL PATHOLOGY, PREVENTIVE AND
EXPERIMENTAL MEDICINE IN PATIENTS UNDER
FIXED ORTHODONTIC THERAPY**

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INTRODUCTION

In this doctoral thesis, I will address a topic of wide current interest that is based on the concept that sums up the following areas: preventive and non-invasive treatment in oro-dental pathology, the adoption of experimental laser-assisted preventive treatments on human biological materials (teeth human extracts) and experimental "in vivo " / clinical applications in patients with fixed orthodontic appliances.

The use laser-assisted treatments in the the oral pathology , preventive medicine and experiment has an applicability low due to of a insufficient research from this point of view . Due to the positive effects so far on reducing the number of pathogenic oral bacteria observed in laboratory research, the use of therapeutic lasers in clinical practice has been increasingly encouraged. The antibacterial effect of different types of laser radiation has been and is intensively studied [6-10].

The objectives of this research are to determine the effect of predetermined wavelengths (660 nanometers (nm) - Photodynamic Therapy antimicrobial (aPDT); 980 nm and 1064 nm - change in chemical structure of enamel, 2940 nm - debonding) for numerical reduction of oral microbial colonies retained on the enamel surface under the influence of photosensitizers and laser radiation compared to conventional antimicrobial procedure (chlorhexidine solution 2% CHX) ; comparative evaluation of two wavelengths in terms of their effect on the surface morphology and chemical composition of the enamel but also the effect of laser radiation on the pulp tissue and the patient's painful perception during the removal of the brackets . We will follow a detailed evaluation of the results of laser therapy in this regard, integrating several technologies with specificity and applicability in our field of interest, namely: microbiological analysis - quantitative analysis, scanning electron microscopy (SEM), dispersive energy spectroscopy with X-rays (EDX) and Laser Doppler flowmetry (LDF).

The present research is divided into three experimental phases:

I. In the first stage "in vitro" will be selected as study samples - human teeth, freshly extracted, at the level of which metal orthodontic brackets will be bonded. *Streptococcus mutans* suspensions will be inoculated at the level of the samples, following which Antimicrobial Photodynamic Therapy will be applied. The effect of this therapy was evaluated using a wavelength of 660 nm in combination with two types of photosensitizers: methylene blue (MB) and chlorophyll mixture with phycocyanin (CHL-PC), compared to the 2% CHX solution. The effect of laser radiation was evaluated using quantitative microbiological analysis expressed by unit-forming colonies (CFU).

II. In the second "in vitro" experimental stage, the effect of two wavelengths - 980 nm and 1064 nm - on the chemical composition of the enamel in the study areas adjacent to the orthodontic brackets will be followed . In this sense, human biological materials (extracted teeth) were

selected, at the level of which orthodontic brackets were bonded. The effect of laser radiation was assessed using SEM and EDX techniques.

III. The third "in vivo " experimental phase included human subjects wearing sapphire orthodontic brackets, at which laser radiation was applied in order to obtain a non-invasive effect on the dental units, reducing the painful perception of individuals., during the take-off of the fixed orthodontic elements. The effect of laser radiation on the dental pulp will be evaluated using the LDF technique.

The integration in this project of five methods of investigation with high specificity for our field of interest, in order to obtain the most conclusive and error-free results, is a new approach in the detailed research of preventive medical therapy.

OWN CONTRIBUTIONS

I. DECONTAMINATION OF DENTAL PLAQUE AROUND ORTHODONTIC BRACKETS USING ANTIMICROBIAL PHOTODYNAMIC THERAPY: "IN VITRO" STUDY

Streptococcus mutans, the mosy studied pathogenic microorganism of the cavity mouth, it is known as the main etiological agent of initiation the phenomenon of demineralization at the level hard dental structure, culminating thus with the appearance lesion carious. After a long test period and revaluation, many methods use for reduction oral pathogenic microorganisms, it is possible to afirm that antimicrobial therapy photodynamic can be adopted as a bacterial decontamination procedure in the areas Dental Medicine [205].

The aim of this study was to evaluate and compare the effects of two substance photosensitizers, such as methylene blue and a chlorophyll - phycocyanin mixture, on colonies of *S. mutans*, with and without light activation, compared with classical therapy using a 2% CHX solution.

The working protocol included many stages: preparation tooth samples, calibration procedure for the bacterial suspension and inoculation bacterial phase (performed immediate for all evidence except positive control group) and practice experimental treatment represented by antimicrobial photodynamic therapy.

After experimental steps, was made counting of *Streptococcus mutans* colonies, and the results were reported in CFU / mL (colony forming units per milliliter) by the same investigator. Statistical analysis of the results was made using unidirectional analysis of variance (ANOVA) and Tukey-Kramer test for multiple comparisons, and analysis software statistics used was OriginPro 8 for Microsoft.

RESULTS AND DISCUSSIONS

After the applying of both photosensitizers, it has been found that in the group C, value average of the colonies bacterial decreased to 6.33 from the value $7 (10^7 \text{ CFU / mL})$, in time what the in the group D, it decreased to 5.83 from the value initial dose of $7 (10^7 \text{ CFU / mL})$. After combined therapy (photosensitizing and laser activation), was observed that in the group C, mean value of the colonies bacterial decreased to 4.33 from the value $7 (10^7 \text{ CFU / mL})$, in time what the in the group D, it decreased to 3.67 in value initial dose of $7 (10^7 \text{ CFU / mL})$. In the group E, where applied only classic therapy with 2% CHX, a mean value of 5.5 for colonies bacterial was obtained.

The results of our study showed that both interventions, PDT - combined with MB photosensitizer and PDT - combined with CHL-PC photosensitizer induced a decrease in the number of bacterial colonies of *S. mutans*. Thus, the null hypothesis can be rejected. The results are in concordance with other studies in the literature that have shown and a reduction in the number of bacterial colonies after use of MB and PDT [206, 216, 220] or CHL-PC and PDT [218].

The results of our study showed that the mean values for bacterial colonies recorded in the treated group only with photosensitizer of methylene blue were comparable to those obtained in the negative control group. This one mean that application as single therapy only methylene blue had an minimum influence on reduction the number of bacterial colonies from all other groups experimental. According to the statistical results, value parameter "Sig" is 0 for Level 3 and Level 2 in compare pairs, what what the means that there is no difference statistics significant at an ap value = 0.05.

Through hence the null hypothesis that suggests that any the difference significantly between the two types of therapy in the groups experimental can not be obtained, it is rejected.

The protocol used in this study led to statistically significant decreases of bacteria number between study groups as well between control groups and those of study. In the consequence, the null hypotheses were rejected.

CONCLUSIONS

In the limits this study, the results showed a significant reduction of bacterial colonies number when photodynamic therapy was associated with methylene blue or chlorophyll-phycocyanin. With the benefits of antimicrobial photodynamic therapy, establishing a valid protocol surface decontamination against oral bacterial colonies in the practice orthodontics, can represents a real progress in the the growth quality and effectiveness dental treatment.

II. COMPARATIVE ASSESSMENT OF THE INFLUENCE OF Nd: YAG (1064 nm) LASER AND LASER DIODE (980 nm) ON ENAMEL ADJACENT TO ORTHODONTIC BRACKS: AN "IN VITRO" STUDY

The method of preventing demineralizing lesions in the enamel structure continues to be a challenge in daily dental practice. The clinical appearance of white spots lesions is the substrate that underlies the appearance of carious lesions in the presence of an acidic environment.

Currently, the use of laser radiation is considered an additional method of preventing carious lesions in the structure of the enamel.

The purpose of this study is to evaluate and compare the effects of two laser wavelengths on the structure of the enamel adjacent to the orthodontic brackets, as well as to analyze the composition of the chemical elements and the structure of the enamel surface.

The experimental part consisted of two phases: the first clinical phase of sample selection and the second "in vitro" phase, in which the samples were prepared, the experimental procedure and, finally, the analysis of the structural morphology and chemical elements of the enamel.

a. The irradiation conditions with the 980 nm laser diode were as follows: the laser was applied with a power of 0.8 W, an energy density of $5.33 \text{ J} / \text{mm}^2$, and the operating regime was continuous for 30 s. A 300 micrometer (μm) optical fiber was used as the transmission element, oriented perpendicular to the enamel surface. The fiber optic tip was used in contact mode, and irradiation was done manually by the same operator, who scanned the enamel surface in a uniform motion to cover the entire selected area.

b. The irradiation conditions with the Nd: YAG laser were as follows: the laser was used with a fixed wavelength of 1064 nm, power of 0.75 W, pulse energy of 75 mJ, pulse repetition frequency of 10 hertz (Hz), energy density $5 \text{ Joules (J)} / \text{mm}^2$, exposure time of 30 s and water-assisted cooling. The irradiation mode was performed 1 mm away from the enamel surface, with a 300 μm fiber by a brushing motion scanning once in a horizontal direction, the entire tested area, by the same operator to avoid human errors.

SCANNING ELECTRON MICROSCOPY (SEM) AND ENERGY DISPERSED X-RAY SPECTROSCOPY (EDX) ANALYSIS

To analyze the morphology of the enamel surface and the chemical composition of the delimited sections adjacent to the brackets, the samples were characterized by scanning electron microscopy and elemental analysis with EDX X-ray energy dispersive spectroscopy using FEI Quanta FEG 250

equipment (SEM-EDX, Type Quanta 250 FEG, Model Nr. 1027641, FEI, Brno, 62700, Czech Republic) at the Research Institute for Renewable Energies, Polytechnic University of Timisoara, Romania.

The surface morphology was analyzed for the negative control group - Section A and, before and after the experimental procedure in the case of Sections B and C, at a magnification of x1000.

With the X-ray microanalyzer (EDX), the quantitative number of chemical elements in the studied sections was recorded. The elements evaluated in this study were calcium (Ca), phosphorus (P), oxygen (O) and carbon (C), and their content is expressed as a weight percentage (wt %). The weight percentages of Ca and P are considered to be very important in terms of enamel microhardness, as shown by several previous studies [264 - 266].

A statistical analysis of the obtained data was performed, using unidirectional analysis of variation (ANOVA). A paired t-test was performed to see if there was a significant difference between the working conditions before and after the experimental procedure and the independent t-test to determine the difference between the effects of the two lasers applied.

RESULTS AND DISCUSSIONS

It can be seen that an increase in Ca was obtained after both irradiation treatments (980 nm diode laser and Nd: YAG laser). For P, an increase was obtained only after Nd: YAG laser irradiation. After diode irradiation, the percentage by weight of P decreased for most samples. In addition, the weight percentage of O, C, Na and Cl was lower after laser irradiation compared to the initial assessment.

Statistically significant differences were observed for all chemical elements evaluated, except P, for which no statistically significant differences were recorded after application of the 980 nm diode laser. In addition, no statistically significant differences were observed for the mean Na% and Cl% after both irradiation treatments.

Based on the results of the independent t-test, the hypothesis that there was a difference between the 980 nm diode laser (used for Section B) and the Nd: YAG laser (used for Section C), regarding the modification of Ca and P, was accepted. The results refer to a statistically significant difference in these results at $p < 0.05$.

Regarding the surface morphology evaluated before and after the 980 nm diode laser treatment, it can be stated that there were no major effects on the tested enamel, as no cracks or fracture lines were caused. The SEM results of our study are consistent with those obtained by Umana et al. [269] and Nandkumar et al. [287], which demonstrated that 980 nm

and 810 nm diode lasers set at 0.8 W and 1 W, respectively, do not cause damage to the enamel and dentin surface.

Irradiation of the enamel surface with the Nd: YAG laser led to a smooth, flat and almost perfectly homogeneous enamel surface, with several superficial lines, compared to the area before the irradiation. Moreover, El Mansy et al. [250] and Al-Jedani et al. [273] observed, after MEB assessments, smooth areas after Nd: YAG irradiation at 0.8 W. This is consistent with our results after Nd: YAG irradiation at 0.75 W.

CONCLUSIONS

Both wavelengths (980 nm and 1064 nm) used in this paper were found to affect dental hard tissue in relation to surface morphology and chemical composition, as demonstrated by the results of this study. It can be concluded that the most important improvement of the chemical composition of the enamel was obtained after irradiation with Nd: YAG, which leads to increased acid resistance of the enamel.

III. DEBONDING OF MONOCRYSTAL CERAMIC BRACKUETS USING CONVENTIONAL AND LASER-ASSISTED METHOD (2940 nm Er: YAG) - COMPARATIVE EVALUATION OF BOTH TECHNIQUES ON DENTAL HIPERSENSITIVITY AND VASCULAR MICRODINAMICS OF PULP TISSUE

With increased interest of patients for aesthetics, the use of ceramic brackets became more frequently [294]. The main risk associated with use these brackets is represented by resistance sheared in the removal phase, what they may be cause cracks or adulteration of permanent enamel [294 - 296].

The aim of this study was to evaluate dental sensitivity and vascular pulp microdynamics, comparatively, in follow removal the monocrystalline ceramic bracket through conventional method versus Er: YAG laser.

Level changes of flux blood pulp were registered with the help of LDF equipment.

The experimental part comprises several phases:

- Phase I: Selection of Patients Carrying Monocrystalline Ceramic Fixed Orthodontic Appliances (Radiance Plus Ceramic Brackets, American Orthodontics, Sheboygan, WI 53081, USA),
- Phase II: Obtaining Written Informed Consent,

- debonding phase of monocrystalline ceramic brackets according to group: for the study group: laser radiation Er: YAG (LIGHTWALKER AT S, M021-5AF / 1 S, Photon doo , Ljubljana, Slovenia), and for the control group: Conventional forceps (Radiance Plus American Orthodontics , Sheboygan , WI 53081, USA),
- the clinical assessment phase of pain during the procedure by immediately completing the questionnaires made available to patients,
- the phase of evaluating the vascular microdynamics at pulpal level by recording the blood flow.

Changes in pulpal vascular dynamics were recorded using the MoorLab Doppler laser device (Moor Instruments , Axminster , UK). Doppler laser signals were recorded and analyzed using the software provided by the manufacturer (MoorSoft Windows® / MoorLAB , v2.01

Two treatment evaluation and quantification tools were used:

1. Wong -Baker's FACES pain scale for pain assessment [313, 314];
2. Laser Doppler Flowmetry for recording the vascular microdynamics of the dental pulp.

After completing the experimental procedure, the results will be interpreted and statistically analyzed using ANOVA analysis. The Bonferroni Test and the Post Hoc Test for multiple comparisons will be performed. The T-Test was also performed to check for statistically significant differences in blood microdynamics over time. The Mann- Whitney test was performed to interpret the answers in the pain assessment questionnaire .

RESULTS AND DISCUSSIONS

The Bonferroni test shows that it does not exist statistically significant difference for both experimental methods used. Considering the results through Bonferroni test in depending on those three moments experimental were recorded distinction statistically significant between time initial and at 7 days post-debonding, and between time immediate after debonding and at 7 days where sig. = 0.017 and sig = 0.045, respectively.

And after the Post Hoc Test, there are statistically significant differences only depending on the time of the experimental procedure, more precisely between the initial one and the one at 7 days; and between the one immediately post - debonding and the one at 7 days for which Mr. =. 016, respectively Mr. =. 039.

Differences recorded of moment values after 7 days post-debonding and those of the moment initially are significant statistical for conventional method and laser-assisted method.

Following the application of the Mann - Whitney test, the research hypothesis is accepted that supports the existence of statistical significance for the difference of the means between the comparative laser / conventional pain levels.

We obtained statistically significant differences in the present study in terms of the pain caused by the two methods. Thus, during laser-assisted debonding, the patient had the greatest comfort, without pain, compared to conventional debonding, which caused increased discomfort associated with pain in all subjects.

CONCLUSIONS

The results recorded in the three experimental moments are statistically significant, with clear changes in pulpal blood microdynamics before and 7 days after debonding. Also, the conventional method caused major discomfort accompanied by pain compared to laser-assisted debonding which was the most accepted by the patient.

FINAL CONCLUSIONS AND PERSONAL CONTRIBUTIONS

In the study "*DECONTAMINATION OF DENTAL PLATE AROUND ORTHODONTIC BRACKETS USING ANTIMICROBIAL PHOTODYNAMIC THERAPY: "IN VITRO "STUDY*", the results showed a significant reduction of number of bacterial colonies after the applying of photodynamic therapies associated with photosensitizers such as methylene blue or chlorophyll - phycocyanin mixture. The results obtained after the use of both photosensitizers were compared to those obtained through method conventional decontamination, represented by the application of 2% chlorhexidine solution for 30 s for every sample. In the follow interpretation statistics were not obtained significantly statistical differences between group of chlorhexidine and other experimental groups.

With in the view benefits of antimicrobial photodynamic therapy, establishing a valid surface decontamination protocol against bacterial oral colony in the orthodontic practice, can represents a real progress in the growth quality and effectiveness dental treatment. Moreover, the application of photodynamic therapy for bacterial decontamination at lthe evel of other types of brackets, such as monocrystalline or polycrystalline ceramics would to leads to interesting results.

In the the study "*COMPARATIVE ASSESSMENT OF THE INFLUENCE OF Nd: YAG LASERS (1064nm) AND LASER DIODE (980nm) ON ENAMEL ADJACENT TO ORTHODONTIC BRACKETS: " AN*

IN VITRO STUDY " were used two wavelengths (980 nm and 1064 nm) for analyze their effect on enamel adjacent the metallic brackets, by evaluation with the help of scanning electron microscopy (SEM) and spectroscopy X-ray dispersive energy (EDX).

In the follow analyzing SEM images for control group compared to those obtained after practice 980 nm laser diode and Laser Nd: YAG (1064nm), not observed important aspects changed of the enamel surface structure.

The results recorded through spectroscopy X-ray dispersive energy, shows statistically significant difference for important elements of calcium, carbon and oxygen after laser radiation application compared to those obtained prior to the experimental procedure.

For study areas where it was chosen the application of Nd: YAG laser, they registered statistically significant difference for all the four elements studied comparing the obtained prior values to application laser radiation with those obtained after laser activation.

Thus, those two wavelengths use in this study proved to affect dental hard tissue, in relation to the structure enamel chemistry, as demonstrated by the results of this study. It is possible concluded that the may importance chemical composition improvement of the enamel was get after irradiation with Nd:YAG, which leads to growth acid resistance of the enamel, according to the results reported by others author in the special literature.

In the study *"DEBONDING OF MONOCRYSTALLINE CERAMIC BRACKETS (SAPHIR) BY CONVENTIONAL AND LASER-ASSISTED METHOD (2940 nm Er: YAG) - COMPARATIVE EVALUATION OF THE EFFECTS OF THE TWO TECHNIQUES* debonding by quantifying the results recording pulpal blood microdynamics and pain sensitivity.

Following the conventional and laser-assisted debonding with Er: YAG 2940 nm, there were no statistically significant differences compared to the type of method used. However, in the case of laser debonding, it was found that the return to the initial values was more visible even if from a statistical point of view it was considered to be insignificant. The results recorded in the three experimental moments show statistically significant differences, with changes in pulpal blood microdynamics prior to debonding and 7 days after it.

Also, the conventional method caused major discomfort accompanied by pain reaching even values of 6 on a scale from 0 to 10, compared to laser-assisted debonding which was the most accepted by

the patient, recording values of 0 maximum 2. It turns out that the difference in the level of pain expressed by patients is statistically significantly smaller in the case of laser-assisted debonding compared to the conventional method.

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