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The Alternative Treatment of Apical Cists with Diode Laser

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Abstract: ***In our study we tested the efficacy of disinfection with diode laser Nd : YAG root canals of single-rooted teeth and lesion models of different sizes (6, 10 and 14 mm), in which we put the teeth and made an in vitro model. Teeth and models we re-infected with S. mitis as one of the most common microbes for re-infection, till the growth of the biofilm. The growth of the biofilm was confirmed microbiologically on agar plates. Viability of microorganisms in the samples after laser treatment and compared to samples without treatment, were analyzed with a fluorescence method by flow Cytometry. Therapy with diode laser Nd : YAG showed a significant increase in the percentage of dead cells compared to the control group. At the same time we have found a significant difference between the different dimensions of periapical lesions. The smallest dimension lesions of 6 mm showed the highest number of dead cells.***

Keywords: Periapical lesions, diode laser Nd : YAG, root canal disinfection

1. Introduction

In our study we used 68 teeth :one-rooted premolars extracted for orthodontic reasons, approved by Ethic Comittee in University Dental Clinic, Prishtine and 68 artificial models from methyl methacrylate. All the extracted teeth were placed in fiziologic solution 0.9%. The crowns of the teeth were cut with Isomet 1000 (Buehler GmbH, Germany) in order to increase the length of the root with 15 mm. In the study only the teeth with circular entrance of the canal were used. We measured the length of the treated canal with Kerr#10 (Mailler Instruments SA, Switzerland) through apex locator Apex. NRG BLUE (Medic NRG, Israel) which was 0,5mm shorter than the length of the specimen. The root canal was prepared with Protaper instruments (Maillefer Instruments, Switzerland) #35 (F3) irrigating with NaOCL 2,5%.

2. Main Text

Artificial models were made by methyl methacrylate with three different sizes of periapical lesions (Fig.5). The model with the largest size of lesion had a diameter of 14mm and depth of 14 mm (n=42), the model with average size of lesion had a diameter of 10 mm and depth of 10 mm (n=40), while small lesions had a diameter of 6 mm and depth of 6 mm (n=40). EDTA 17 % was used to remove the debris.

After the preparation of the canals, teeth and models were sent in the Institute of Microbiology and Imunology of Faculty of Medicine, Ljubjane. In the laboratory of Liquid Citometry teeth were primery sterilized with absolute alcohol, than rised with EDTA 17% to remove debris. Canals were dried with paper points (Dentsply Maillefer).

The sterilization of canals were proved with the incubation of the liquid gained after the rinse and application of PBS(pufferit phosphate) in canals in agar blood, which were incubated for 24 hours in temperature of 37 grade C. In agar no growth of bacterial colony was proved. Specimen prepared in this conditions, were used in our experiment.

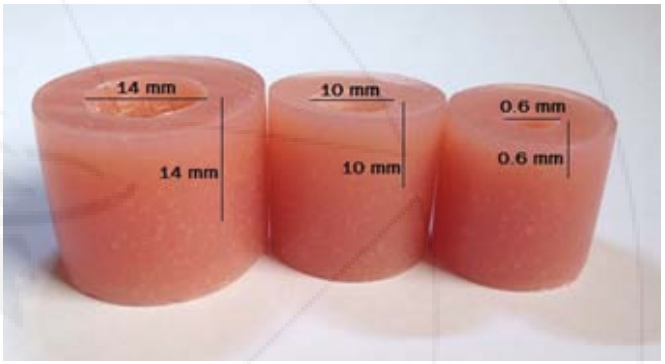

Figure 5: Models of methyl methacrylalate of periapical lesions

Figure 6:.Models with periapical lesions

S. mitis *were prepared in plates with agar blood.* For the identification of different colonies a microscopic sample was prepared according to Gram. The suspensions of bacteria in exact concentrations were incubated in sterilized root canals and in the walls of models with periapical lesions. S. mitis were used for inoculation.

For the inoculation suspensions of 5 Mc Farlanda cultures were used, prepared in TIO. The concentration of bacterias

were measured with Nefelometer. The teeth and the models with periapical lesions first were inoculated with this suspension and then incubated for 7 days in 37 C in aerobic conditions. Inoculation and incubation procedure was as following:

1. First day: 30 µl suspension 5 McFarland were inoculated in root canals and were incubated for 24 hours in 37 degree temperature.
2. Second day: 30 µl suspension 5 McFarland were inoculated in root canals and were incubated for 24 hours in 37 degree temperature.
3. Third day: 30 µl suspension 5 McFarland were inoculated in root canals and were incubated for 24 hours in 37 degree temperature.
4. Forth day: 30 µl liquid tio-gliconat were inoculated in root canals and were incubated for 24 hours in 37 degree temperature.
5. Fifth day: 30 µl liquid tio-gliconat were inoculated in root canals and were incubated for 24 hours in 37 degree temperature.
6. Seventh day: 30 µl liquid tio-gliconat were inoculated in root canals and were incubated for 24 hours in 37 degree temperature.

Figure 7: The evolution of biofilm in root canal and in models with periapical lesions

Root canals and the walls of periapical lesions were rinsed with PBS with pH=8,3 and the colected suspension was placed in agar blood, and were incubated for 24 hours in 37 degree. Next day the growth of bacteria were made in a plate and it was evaluated if there are more than 300 white bacterial colonies.

Aplication of Diode Laser Nd: YAG

For this procedure 68 teeth and 68 models with periapical lesions have been used. For S. mitis 54 teeth were used and 54 models with periapical lesions. The rest, 14 teeth and 14 models were used for control without using laser. After that in root canal and in models with periapical lesions diode laser Nd: YAG (Fotona XD-2,Ljubjane, Slovenia) was used, with special sond with micro-fibers 200 µm and 1,5 W power in different intervals of time of 1, 3 and 5 minutes.

Tabel 2: Parameters of Diode Laser Fotona XD-2

Alication: ENDO	
Power: 1-1.5W	Frekuency: N/A
Duty cycle: CW	Time: 1, 3, 5 min
Fibre: 200 µm	Handpiece: R21-B

After laser radiation the biofilm was irrigated with 2,5 ml PBS and EDTA with pH 8,3. Root canals were rinsed, while the gained suspension was collected and the definite volume of the suspension with microorganisms cells was 2,5 ml. This suspension was further used for analyses in liquid Cyto-metria. Also the suspension gained from the irrigation of the models with periapical lesions was further used for analyses in liquid Cyto-metria.

Viability Analise with liquid cytometri 500 µl suspension of cells from each tooth with infected root canal and each model woth periapical lesions were used for analyses in citometer. The Cell Viability Kit with Liquid Countaing Beads (BD Biosciences) was used to define the viability.The use was made under the instructions of the producer. 500 µl suspension was placed in an epruvet for analyses in citometer. 5 µl colour TO and 5 µl colour PI were added,then 50 µl BD Liquid Counting Beads was added and mixed well. Then were incubated for 10 minutes in dark and in room temperature. After the incubation, specimens were analysed in liquid citometer model BD FACS Canto II.

Controlling procedures.

Controlling procedures were made in two ways: with growth in blood agar and with the analyses in liquid citometer. The controlling group (positive one) were 14 infected teeth with S. mitis and 14 models with periapical lesions without using diode laser Nd: YAG. We had 6 models with periapical lesions with diameter 14mm, 4 models with diameter 10 mm and 4 models with diameter 6mm. For S. mitis 3 models from the first group and 2 models from the second and third group were used.

In liquid cytometer analyze 14 teeth with infected root canals and 14 models with periapical lesions were used. Teeth inoculated with microorganisms were rinsed with 2,5 ml PBS and 17% EDTA. From 2,5 ml gained suspension 500 µl were taken for the viability analyze and 100 µl for inoculation in blood agar.

For positive control, for the growth of microorganisms in agar the collected suspension from the irrigation of the models with periapical lesions was taken. For the controlling procedures 100 µl suspension of rinsed cells, which are placed in agar and are mixed in such a way to realize a better division of cels-liquid re-suspension of rinsed cells. Then the whole mass is placed in the plate and finally is incubated for 24 hours in 37 degrees in aerobic conditions. Next day the colonies of CFU of S. mitis were counted.

3. Results

In this study the efficency of antimicrobial termal therapy with diode laser Nd: YAG toward S. mitis was demonstrated.

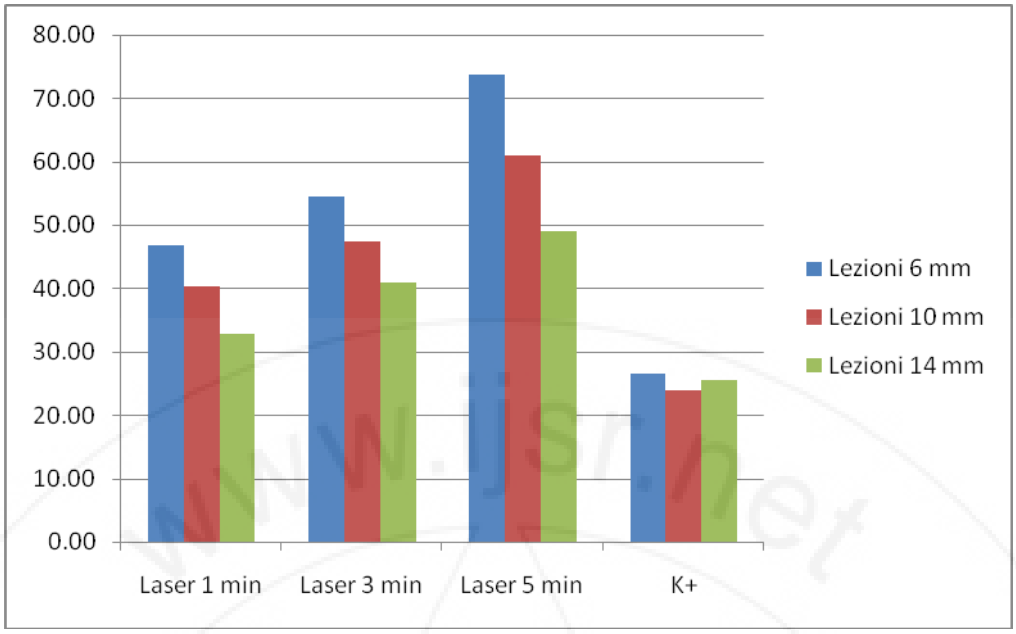

Diagram 1. The percentage of dead cells of S. mitis after laser application in models with periapical lesions with different sizes.

From the statistical results, the highest percentage of dead cells of S.mitis after diode laser Nd:YAG application for 5 minutes is in the periapical lesions with diammeter of 6mm, 82,98%; while the lowest percentage is in the periapical lesions with diammeter 14 mm and the time of application of laser is 1 minute, 32,56 %.
The percentage of dead cells of S.mitis is higher after diode laser application Nd:YAG for 5 minutes in periapical lesions with diammeter 6mm.

- S. mitis:

Periapical lesion with diammeter 6 mm
1: > 300 CFU
2: > 300 CFU

Periapical lesion with diammeter 10 mm
1: > 300 CFU
2: > 300 CFU

Periapical lesion with diammeter 14 mm
1: > 300 CFU
2: > 300 CFU
3: > 300 CFU

The data were analyzed with ANOVA with test Post-Hokc, IBM SPSS Statistics 20 (IBM, NEW YORK, USA) program. The significant scale is $p<0,05$.

Through ANOVA test, a significant difference was noticed between the time of radiation with laser ($p<0.001$, $F = 680,552$); between the dimensions of periapical lesions ($p <0.001$, $F = 219,755$) and between the time of radiation with laser and the dimensions of periapical lesions ($p <0.001$ dhe $F = 25,733$). The combination of all variables did not have any co-relation ($p = 0.222$ dhe $F = 1,402$).

From the statistical results, the highest percentage of dead cells of E.faecalis after diode laser Nd:YAG application for 5 minutes is 73,65%.

Based on statistical results, we have demonstrated that the largest the periapical lesion, the lowest is the percentage of dead bacterial cells of S.Mitis. P-values are the same ($p<0,001$).

4. Conclusion

In this study we intented to prove the antimicrobial effect of diode laser with heat producing toward S.mitis in root canals and in periapical lesions. The conclusion of this study are:

- The ex vivo treatment with diode laser Nd:YAG was efficient in sterilizing root canals and periapical lesions compared to control group.
- The applicaion of diode laser Nd:YAG in a longer time has demonstrated an improved sterilization of root canals and periapical lesions.
- The radiation of 5 minutes showed an average of 70% in destroying the microorganisms, the radiation of 3 minutes showed an average of 50% in destroying the microorganisms, the radiation of 1 minute showed an average of 30% in destroying the microorganisms.
- The radiation with diode laser Nd: YAG for 1 minute does not affect the sterilization of root canals and periapical lesions.
- The effect of diode laser Nd:YAG toward S.mitis is almost inefficent in large periapical lesions, and this method can be used only like an additional agent in the standartization of sterilization or in combination with Photodynamic therapy.
- Diode laser Nd:YAG with length of wave 810 nm can be used for the different procedures in the daily dental practice, icluding a variety of procedures in soft tissues like surgery in soft tissues, periodontal treatments, also like an efficent method in implantology and endodonti.

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