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Oral Sciences

Comparative evaluation of periodontal radicular therapies - conventional and er:yag laser - based on rat connective tissue response

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Abstract - The Er:YAG laser has been proposed as an alternative or adjunctive approach for radicular mechanical therapy. However, further information is needed on the use of laser radiation on radicular surfaces. The aim of this study was to evaluate the alterations occurring on radicular surfaces irradiated with the Er:YAG laser, using the rat subcutaneous tissue response resulting from human dental root specimens implanted at 7, 14, and 28 days of healing. The treatment groups were: group 1- Er:YAG laser with 60 mJ, 10 pps, for 15 s; group 2- Er:YAG laser with 100 mJ, 10 pps, for 15 s; group 3- scaling and root planning followed by surface demineralization with citric acid and tetracycline for 3 min; and group 4- scaling and root planning. The results of histological and morphometric analyses showed a higher number of inflammatory cells in group 4, for the period of 7 days, in comparison to all the other groups. In groups 1, 2, and 3, some areas showed fiber adherence in the favorable angulation to attachment, denoting more biocompatibility than that in group 4. The protocols used in this study presented favorable results, suggesting that the Er:YAG laser may represent an alternative approach in periodontal radicular therapy.

Keywords - Lasers, periodontics, dental cementum

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Introduction

Lasers have recently been developed for use in Dentistry due to their excellent ability for ablation of hard tissues without causing local thermal damage, as reported in several studies (1,2).

Because they present many advantageous features, such as ablation or vaporization, homeostasis and the sterilization effect, laser treatment with Er:YAG has been proposed as an alternative or adjunctive approach to conventional treatment in radicular mechanical therapy (3-8). However, the effect of Er:YAG laser radiation on radicular surfaces, as well as tissue response to this kind of treatment, is still not well known.

Due to the scarcity of reports in the literature documenting the actual effect of Er:YAG laser radiation on the treatment of radicular surfaces, this study aimed at evaluating the effect of Er:YAG treatment on roots of periodontally involved extracted human teeth, using as a parameter the conventional method of scaling, root planning, and conditioning with acid solution. The response from the implantation of radicular fragments, submitted to different treatments, into rat subcutaneous connective tissue was also assessed.

Materials and Methods

Fifteen periodontally involved human teeth were extracted and used for the preparation of radicular fragments measuring 4 mm long, 2 mm wide, and 2 mm thick.

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Pulpal surface was marked with a spherical diamond bur #1016, for clinical and histological identification (Fig.1).

After the fragments were prepared, they were stored in flasks containing sterile saline solution and kept inside a refrigerator. The fragments were then submitted to different treatments. A total of 60 fragments were studied.

Er:YAG laser equipment (KEY LASER 2, Kavo), with a 2.94 ***m***m wavelength, pulsed mode, and a delivery system with optical fibers, was used. A quartz tip #2056 (0.5 x 1.8 mm), developed for use in Periodontics, was positioned at a 45º angle to the radicular surface. Radiation with Er:YAG laser was performed under water irrigation and powerful suction to collect debris. Sample manipulation during the laser application was performed with the use of gloves, masks, and special protection glasses in a closed environment with a warning on the door. Safety regulations were strictly adhered to during utilization of the laser equipment (9).

Fifteen fragments were used for each treatment group. The experimental groups were as follows:

Group 1 - The surface of the entire fragment was irradiated with the Er:YAG laser, with 60 mJ, 10 pps for 15 s. The fragments were then irrigated during 30 s with saline solution using a sterile disposable syringe.

Group 2 - The surface of the entire fragment was irradiated with the Er:YAG laser, with 100 mJ and 10 pps for 15 s. Fragments were then irrigated as in group 1.

Group 3 - Scaling and root planning were performed with 20 strokes with the aid of a new and sharp curette (Gracey #5-6, Hu-Friedy), following the application of citric acid (50%) with tetracycline gel (Decalcific-Biotechnol, Bauru-SP) pH 1.0 for 3 min. Acid application was performed with a sterile cotton roll. Fragments were then irrigated as in group 1.

Group 4 - Scaling and root planing were performed with 20 strokes, using a sharp new curette (Gracey #5-6, Hu-Friedy). Fragments were then irrigated as in group 1.

To allow for the execution of the above mentioned procedures, all the fragments were fixed with the aid of a hemostatic instrument.

The fragments submitted to all the treatments were implanted into subcutaneous connective tissue of 15 adult male Wistar rats (*Rathus norvegicus – albinus rodentia mammalia*). Animals were 2 months old and were anesthetized with an intraperitoneal injection of ketamine chloridrate (Dopalen) and xylazine chloridrate (Anasedan) (0.1 + 0.1 mL/body weight). A tricotomy as well as antisepsis were then performed with ethanol 70% (v/v). Incisions were performed with a #15 (Bard-Parker) razor blade, followed by divulsion of aponeurosis to allow for the insertion of fragments into the subcutaneous connective tissue. A fragment of each treatment group was implanted into each rat, so that four fragments were placed in the dorsal region of each animal. Fragments were placed a reasonable distance apart to avoid interference in local tissue responses.

The incisions were then sutured with silk suture 4.0 mounted in a curved needle (Ethicon). Five animals were sacrificed at different time healing times after the fragment implantation (7, 14, and 28 days).

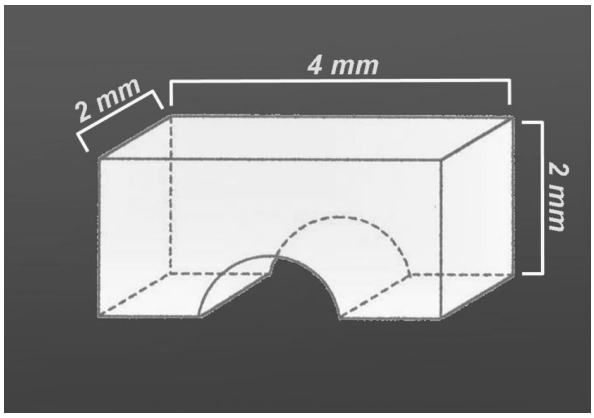

Figure 1. Schematic drawing of the prepared specimens.

The material collected was processed by histological techniques. The slides were examined in a light microscope (Olympus CH-2) at x4, x10, and x40 magnification for the histological analysis, and immersion at x100 magnification for the morphometric analysis.

Histological analysis consisted of a subjective examination of collagen fibers formed along the fragments. Fiber disposition and direction and connective tissue density were observed. In morphometric analysis (stereological), the density of the cellular volume and structures in the proximity of each fragment as well, as the number of cell types per $mm^{2.}$ were assessed by means of a grade II Zeis' integration reticulum. For each group, 20 selected microscopic fields were analyzed. Grade II Zeis' integration reticulum was superposed (10).

Since fibroblast´s cytoplasm and collagen fibers present the same coloration by hematoxylin and eosin, both components were considered together for purposes of density volume, including the dots located on the fibroblast´s nucleus. For cell number determination, the number of profiles of cellular nuclei seen in the microscopic field delimited by the area considered in the reticulum was considered. All the morphometric data were statistically confronted. Analysis of variance (ANOVA) was performed, taking into consideration the 4 variables (treatment groups) for the healing periods of 7, 14 and 28 days. The values of each group were also assessed comparing the data of 7 days with 14 and 28 days. When a statistically significant difference was detected, the Tukey test was applied.

Results

At 7 days, it was observed that all groups presented an intense inflammatory infiltrate. However, group 3 differed from the others since it presented collagen fibers with a perpendicular or oblique orientation to radicular surfaces. In the other groups, parallel or undefined fiber orientation was observed (Fig.2).

At 14 days, groups 1, 2, and 3 presented similarities in terms of connective tissue interaction with the surface of radicular fragments, mainly in relation to the orientation of the collagen fibers, which were oblique or perpendicular to radicular surfaces (Fig.3). Only group 4 differed in relation to this tissue interaction, presenting a high number of flat and soft fusiform cells with a fibroblast profile. Fibers disposed in a parallel orientation to the radicular surface were characterized a highly fibrotic tissue. In none of the treated fragments in this group, at this point, was maintenance of connective tissue in contact with radicular surface observed. (Fig.4).

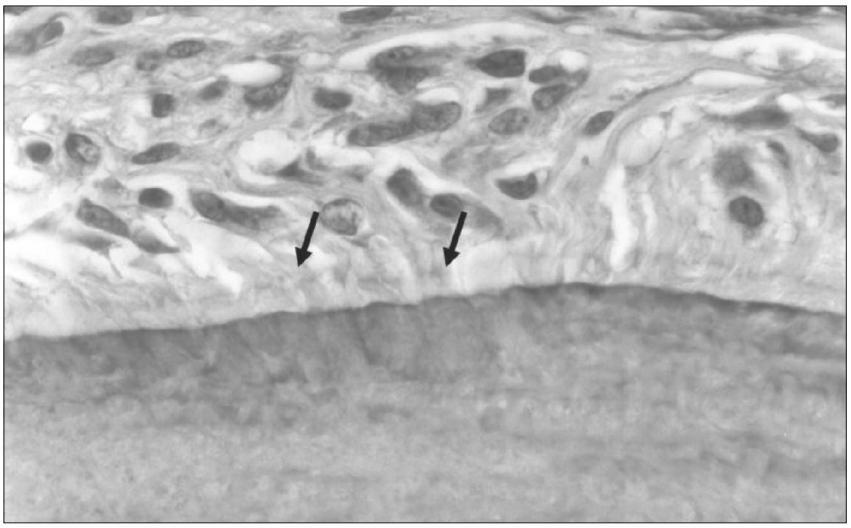

Figure 2. Photomicrograph of the adjacent area of a radicular surface in group 3, 7 days after the implantation, showing collagen fibers formed at an oblique or perpendicular orientation to the treated radicular surface (HE 1.000X). Arrows = collagen fibers.

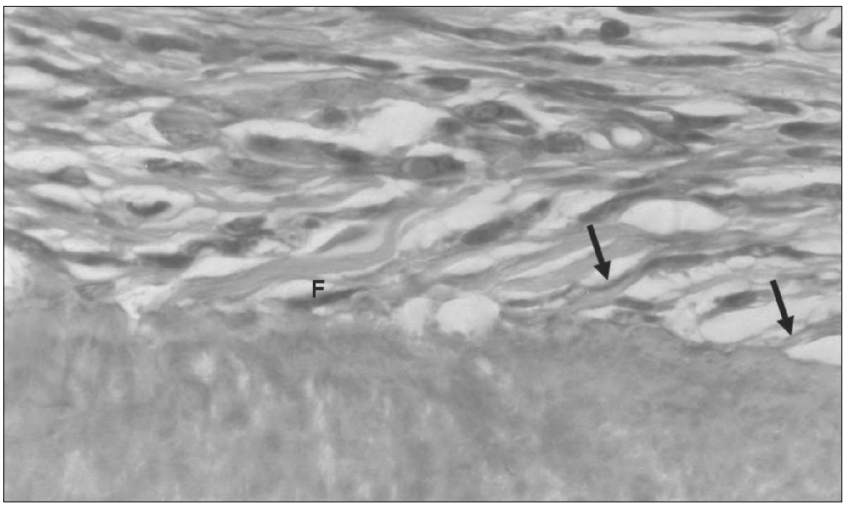

Figure 3. Photomicrograph of the adjacent area of a radicular surface in group 2, 14 days after the implantation. The oblique orientation of the formed collagen fibers in relation to the radicular surface was observed. (HE 1.000X). F = fibroblast, Arrows = collagen fibers.

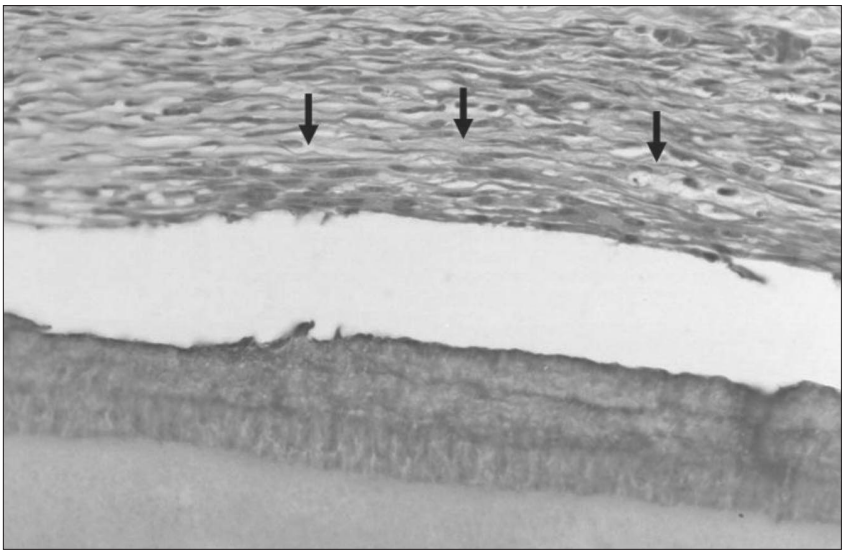

Figure 4. Photomicrograph of the adjacent area of a radicular surface in group 4, 14 days after the implantation. Fibers are disposed in parallel to the surface. Maintenance of connective tissue in contact with the radicular surface was not observed. (HE 400X). Arrows = collagen fibers.

The histological aspect at 28 days, of groups 1, 2, and 3, was highly similar since some fiber adhesion to radicular surfaces was observed in all groups (Fig.5). In group 4, a large fibrosis of connective tissue was detected. Thus, as at 14 days, there was no interaction between connective tissue and the implanted radicular surface. It was noted that the fibroblasts were very soft and juxtaposed, and disposed in a parallel mode to the radicular surface, therefore forming a fibrotic encapsulation.

The results of the morphometric analysis showed statistically significant differences among the different groups only regarding the number of inflammatory cells at 7 days. The number of inflammatory cells - macrophages, neutrophils, lymphocytes, and plasmocytes - per mm^2 is presented in Graph 1.

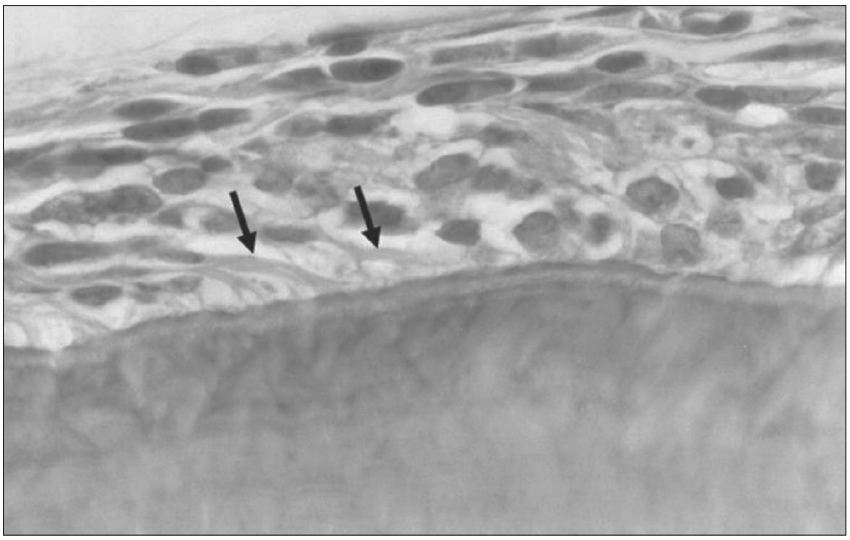

Figure 5. Photomicrograph of the adjacent area of a radicular surface in group 1, 28 days after the implantation. The presence of oblique collagen fibers in contact with the radicular surface with likely adhesion was observed. (HE 1.000X). Arrows = collagen fibers.

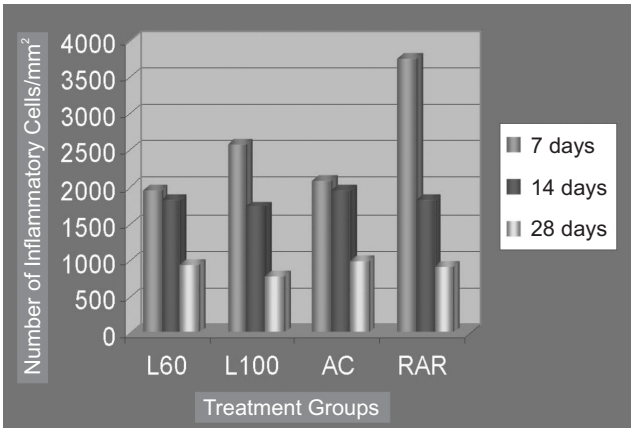

Figure 6. Graph showing the number of inflammatory cells per mm^2 in the different treatment groups, at 7, 14, and 28 days of healing time (L60 = laser 60 mJ; L100 = laser 100 mJ; AC- citric acid; RAR- scaling and root planing).

There was a statistically significant difference in the number of inflammatory cells in group 4 at 7 days as compared to groups 1 and 3. Even though the number of inflammatory cells was higher in group 4, there was not a statistically significant difference in relation to group 2. In group 1, a difference at 28 days was observed as compared to 7 and 14 days. In group 3, a difference was observed at 28 days as compared to 7 and 14 days. In group 4, there was a statistically significant difference among all periods.

Discussion

In the histological analysis, the response of rat subcutaneous connective tissue was assessed, observing the degree of tissue organization in the different treatment groups at different healing times.

Descriptive histological analysis, with the aid of optical microscopy, showed that at 7 days of healing there were better results in groups 1, 2, and 3. Only group 3, which received treatment with citric acid and tetracycline, presented a more defined orientation of newly

formed fibers in relation to the radicular surface of the fragments. The presence of fibroblasts close to the radicular surface was also observed. Group 4 showed parallel orientation of connective fibers in relation to the radicular surface of the fragments, denoting a less favorable response as compared to the other groups in the same healing time.

Along those lines, the works of Polson, Hanes (11,12) and Polson, Proye (13) showed that the effects of acid treatment may promote radicular surfaces that are more receptive to fibroblasts, which will produce fibers and thus form new tissue.

Oliveira (10) also demonstrated favorable results with the use of citric acid, either isolated from or associated with growth factor (PDGF), only in relation to scaling and root planing.

In the present study it was observed that at 7 days of healing there were no statistically significant differences in fibroblast density, inflammatory cells, and blood vessels in the different groups of treatment, which confirmed the findings of Oliveira (10), who compared different treatments, including scaling, root planing, citric acid, and PGDF, also in rat subcutaneous connective tissue.

As for the number of inflammatory cells, it was noted that there was a statistically significant difference at 7 days of healing for group 4 (treated only with scaling and root planing), which had the higher number of inflammatory cells compared to the other groups (1, 2. and 3). The smaller number of inflammatory cells in group 3 (citric acid with tetracycline) as compared to group 4 (scaling and root planing) may be related to the decontaminating effect that citric acid exerts, thus reinforcing some findings in the literature (14-17).

Similarly, a possible decontaminating effect of the radicular surfaces of the fragments treated with Er:YAG laser, promoted by ablation (groups 1 and 2), could justify the significant difference in the number of inflammatory cells found at this healing time, which was significantly smaller than that in group 4, which only received scaling and root planing. There was no a statistically significant difference in the number of inflammatory cells among groups 1, 2, and 3.

This possible decontamination of the radicular surfaces is supported by the research of Ando *et al*. (3), who demonstrated the bactericidal effect of Er:YAG laser on periodontopathic bacteria, and also by Yamagushi *et al*. (18), who observed the capacity for removal of 81.3% of total lypopolisaccharide from radicular surfaces treated with the Er:YAG laser. These results suggest that the Er:YAG laser may be useful for radicular conditioning by promoting decontamination of the surface.

At 14 days of healing, it was observed in groups 1, 2 and 3 that there was close contact between newly formed fibers and the treated radicular surfaces, with the presence of collagen fibers disposed in a perpendicular and/or oblique orientation to the radicular surfaces of the fragments, including the adhesion areas.

However, in group 4, treated only with scaling and root planing, this type of contact with the surface was not observed. It was noted that the fibers adopted a parallel positioning to the surfaces of the fragments, not showing contact with them due to the feature of fibrotic tissue, which is typical of encapsulation. These findings are similar to those obtained by Oliveira (10), who found that the group treated only with scaling and root planing, at 15 days of healing, did not show fiber adhesion to the radicular fragments.

The current study was not concerned with the presence or absence of cement on the radicular surface. The different treatments were applied with the aim of reproducing a clinical situation. The levels of contamination of the radicular surfaces were assessed by the level of inflammation shown by the surrounding soft tissue, as well as by the orientation and occurrence of adhesion of connective fibers.

It is believed that either mechanical treatment followed by application of citric acid (group 3) or laser treatment (groups 1 and 2) were effective in decontaminating the surface of the fragments, which facilitated fibroblast proliferation and fiber adhesion. Along those lines, Feist (19) found that radicular surfaces treated with the Er:YAG favored adhesion and fibroblast proliferation. The number of cells and the speed of proliferation were higher than those observed for surfaces treated with root planing. These results reinforce the possibility of using the Er:YAG laser as an adjunct to conventional treatments.

As was observed at 7 days of healing, there were no statistically significant differences at 14 and 28 days as for fibroblast density,

inflammatory cells, and blood vessels, in the different experimental groups. The number of cells did not present statistically significant differences at this time either.

At 28 days of healing, it was noted that in groups 1, 2, and 3 there was still fiber adhesion with a favorable positioning to attachment, with maintenance of the oblique and/or perpendicular orientation to the surfaces of the radicular fragments.

In group 4, the pattern of fibrotic encapsulation was maintained. These results were also reported by Rubo (20) who used a similar methodology for the implantation of radicular fragments in rat subcutaneous connective tissue. His results showed a characteristic of fibrotic encapsulation for the group treated with 20 strokes of hand curette, at 30 days of healing time.

As for significant differences in cell density and blood vessels and the number of cells observed at the different healing times, within the same treatment group, one can consider that all these findings present normal patterns in evolution of the healing process, such as the increased number of fibroblasts, decreased number of inflammatory cells, as well as the diminushed density of blood vessels, in accordance with the process of tissue maturation.

Conclusions

- The groups treated with the Er:YAG laser (groups 1 and 2) exhibited, in the optical microscopic analysis, results similar to the group treated with citric acid and tetracycline (group 3), thus offering therapeutic advantages as compared to the group treated with scaling and root planing.
- The group treated with scaling and root planing with hand curette (group 4), in the optical microscopy, presented a higher number of inflammatory cells as compared to all the other groups, at 7 days of healing, indicating that the use of alternative methods, such as acidic substances or even the Er:YAG laser, may decontaminate the radicular surfaces.
- The protocols used in this study demonstrated favorable results for the response of rat subcutaneous connective tissue, thus it is possible to conclude, within the parameters evaluated, that the Er:YAG laser is potentially an alternative approach in radicular therapy or an adjunctive method for conventional therapy.

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Resumo

FRANCO, Eric Jacomino, GREGHI, Sebastião Luiz Aguiar, ASSIS, Gerson Francisco de. Avaliação comparativa entre terapias radiculares periodontais, convencional e *laser* Er:YAG, sobre a resposta de tecido conjuntivo subcutâneo de ratos. Oral Sci., mai./ago. 2005, vol.1, no.1, p.35-42.

O uso do laser de Er:YAG têm sido proposto como uma alternativa ou adjunto à terapia mecânica radicular periodontal. Entretanto, a influência da irradiação deste laser nas superfícies radiculares ainda não está totalmente clara. Com o objetivo de avaliar os efeitos do laser de Er:YAG, utilizou-se a resposta tecidual decorrente da implantação de fragmentos radiculares, em tecido conjuntivo subcutâneo de ratos, por períodos de 7, 14 e 28 dias, comparado com terapias convencionais. Os grupos de tratamento foram: grupo 1- laser de Er:YAG com 60mJ, 10pps, por 15 s. ; grupo 2- laser de Er:YAG com 100mJ, 10pps, por 15 s. ; grupo 3- raspagem e alisamento radicular e aplicação de ácido cítrico com tetraciclina, por 3 min. e grupo 4- raspagem e alisamento radicular. Os resultados das análises histológica e morfométrica mostraram maior número de células inflamatórias, no grupo 4, no período de 7 dias, comparado aos demais grupos. Observou-se fibras colágenas aderidas as superfícies radiculares nos grupos 1, 2 e 3, indicando maior biocompatibilidade comparado ao grupo 4, que demonstrou característica de encapsulamento fibroso, observado também nos períodos de 14 e 28 dias. Os protocolos utilizados neste trabalho demonstraram resultados favoráveis, sendo que o laser de Er:YAG pode representar uma alternativa na terapia radicular periodontal.

Palavras-chave: *Lasers*, periodontia, cemento dental.

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