Efficacy of the Er, Cr: YSGG laser in the Laser Assisted Endodontic Treatment

» Blind Randomized Clinical Trial «

Orientador:
Prof. Doutor Manuel Fontes de Carvalho

Co-Orientador:
Prof. Doutor Norbert Gutknecht
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Faculdade de Medicina Dentária, Universidade do Porto: Scientific Committee

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TO MY PARENTS,

Maria do Rosário Antunes Rodrigues Martins

Miguel André Duarte Martins
To all my friends and colleagues...

...who have supported and encouraged such personal achievement.
Muito para além do seu conteúdo científico, esta tese tem um enorme conteúdo emocional pois foram muitas as pessoas que contribuíram com a sua sabedoria mas, sobretudo, com a sua forte amizade.

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Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

TITLE

Efficacy of the Er,Cr:YSGG laser in the Laser Assisted Endodontic Treatment

Blind Randomized Clinical Trial
ABSTRACT

Introduction: Clinical studies conducted to explore the safety and efficacy of new therapies is considered an important focus in endodontic research. The primary objective of this randomized clinical study was to compare radiographic evidences of periapical healing after root canal therapy assisted by the Er,Cr:YSGG laser Radial Firing Tips (RFT) versus the concomitant use of 3% sodium hypochlorite and interim calcium hydroxide paste in necrotic teeth with chronic apical periodontitis. We hypothesized to find similar outcomes in both groups in order to assess the predictability of this new laser assisted endodontic protocol.

Methods: 36 and 43 (anterior and premolar) teeth were randomly assigned for the 6 and 12-month analyses. In group 1 teeth were manually prepared and irrigated with 3% sodium hypochlorite and calcium hydroxide inter-appointment dressing was applied; in group 2 teeth were manually prepared with saline solution and irradiated with Er,Cr:YSGG laser using the RFT2 (140µs, 37.5mJ, 20Hz) and the RFT3 (140µs, 62.5mJ, 20Hz) at the first and second appointment respectively, four times each, moving at 2mm.s⁻¹ from apical to coronal. The primary outcome measure was change in apical bone density either at 6 and 12 months of follow up, using the periapical index (PAI) for blind radiographic assessment. Results: At the 6-month assessment 29 patients were subjected to statistical analysis (12 in group 1 and 17 in group 2) while at the 12-month assessment 30 teeth were analyzed (12 in group 1 and 18 in group 2). There was one treatment failure after 6 months and two failures after 12 months, all in group 1; treatment failures were not included for analyses. In the 6 and 12-month outcomes, both groups exhibited a statistically significant decrease in mean PAI score. However, no differences were found between groups. Conclusion: The present findings suggest that this laser assisted protocol might achieve equally predictable and effective outcomes, while overcoming potential hazards and limitations associated to conventional therapies.

Key Words: Chronic apical periodontitis, Er,Cr:YSGG laser, radial firing tip, sodium hypochlorite, calcium hydroxide, periapical index.
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

RESUMO

Introdução: Estudos clínicos que pretendam explorar a segurança e eficácia de novos procedimentos são considerados um foco primordial na investigação endodôntica. O objectivo principal deste estudo clínico randomizado consistiu em analisar evidências radiográficas de cicatrização apical após o tratamento canalar assistido pelo laser de Er,Cr:YSGG (Fibras de Dispersão Radial - RFTs) em comparação com a utilização simultânea de hipoclorito de sódio a 3% e aplicação de hidróxido de cálcio entre consultas em dentes necróticos com periodontite apical crónica. A hipótese consistiu em encontrar resultados similares em ambos os grupos de modo a atestar a predictibilidade deste novo protocolo endodôntico assistido por laser.

Métodos: Para o controlo dos 6 e 12 meses, 36 e 43 dentes (anteriores e pré-molares) foram aleatoriamente distribuídos. No grupo 1, os dentes foram preparados manualmente, irrigados com hipoclorito de sódio a 3% e, entre consultas, foi aplicado um revestimento de hidróxido de cálcio; no grupo 2 os dentes foram manualmente preparados, irrigados com soro fisiológico e irradiados com o laser de Er,Cr:YSGG utilizando a fibra RFT2 (140µs, 37.5mJ, 20Hz) e a fibra RFT3 (140µs, 62.5mJ, 20Hz) na primeira e segunda sessão respectivamente. Cada fibra foi introduzida quatro vezes, à velocidade de 2mm.s⁻¹, desde apical até coronal. Os objectivos principais desta investigação consistiram na avaliação cega das diferenças radiográficas referentes à densidade óssea apical, após 6 e 12 meses, utilizando o índex periapical (PAI).

Resultados: Após 6 meses, 29 dentes foram submetidos a análise estatística (12 no grupo 1 e 17 no grupo 2) enquanto após 12 meses, 30 dentes foram submetidos à mesma análise (12 no grupo 1 e 18 no grupo 2). Registou-se um insucesso aos 6 meses e dois insucessos aos 12 meses, ambos no grupo 1; Os insucessos, porém, não foram submetidos a análise estatística. Na avaliação após 6 e 12 meses, ambos os grupos exibiram uma redução estatisticamente significativa na média de resultados. Contudo, não existiram diferenças significativas entre os grupos. Conclusão: Até à presente data, os resultados sugerem que este protocolo assistido por laser poderá atingir resultados igualmente seguros e previsíveis, ultrapassando potenciais riscos e limitações associados às terapias convencionais.

Palavras-chave: Periodontite apical crónica, Er,Cr:YSGG laser, radial firing tip, hipoclorito de sódio, índice periapical.
JUSTIFICATION

The man of science has learned to believe in justification, not by faith, but by verification.

Thomas H. Huxley (1825-95)
English biologist
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

Miguel Rui A. R. Martins

The primordial objective of any endodontic treatment is to achieve microorganism eradication from the root canal system, obtain a proper canal conformation achieved by mechanical means and finish with an efficient tridimensional obturation.

If every one of these requirements is necessary to achieve the success of the endodontic therapy, the first one can be considered truly the most important. It is unanimous that the effective elimination of all microorganisms inside the root canal it’s the main factor that can determine the success or the failure of the endodontic therapy.

The disinfection or sterilization of all root canal system it’s traditionally done by innumerous antiseptic agents which can act against the microorganisms when introduced and maintained, for a certain while, inside the main canal. In first instance that can be done with rinsing solutions as sodium hypochlorite, and in second instance through the application of semisolid formulas as calcium hydroxide, just as an example. There are also other physical means to achieve canal disinfection – which have being used and studied – such as ultrasounds, electro surgery or even laser assisted therapies.

The aim of this research was to test a new root canal system disinfection method using the Erbium, Chromium doped Yttrium, Scandium, Gallium, Garnet (Er,Cr:YSGG) laser with Radial Firing Tips recently developed for endodontics. With this technology it should became possible to tri-dimensionally disinfect the root canal system, overcoming hazardous effects and limitations commonly associated to chemical solutions such as sodium hypochlorite.

During conventional endodontic treatment, the laser application as disinfection mechanism should be considered not as a substitute but as complement for traditional endodontic procedures. However, due to the inherent costs it should be done a proper evaluation of its risk-benefits along with its polyvalence. Hence, a primary indication for laser adjunctive application can be those cases in which - under some circumstances – chemicals can be potentially hazardous or ineffective (e.g. wide apical constrictions or persistent infections).

Although innumerous publications can demonstrate the bactericidal potential of different wavelengths – diode, CO₂, Nd:YAG and Erbium lasers– in assisting root canal treatments just few are in fact in vivo studies. Though, some controversies remain while choosing the ideal parameters for each laser, resulting in relevant discrepancies in terms of safety and efficacy.

In our case, following several precedent studies performed in vitro which could attest the safety and efficacy of all procedures, we could define the up-to-date ideal parameters and perform a randomized clinical trial. As endodontic researchers, we found interesting and a scientific challenge to compare clinical outcomes obtained by Er,Cr:YSGG - Radial Firing Tips laser assisted endodontic treatment, with those achieved by a conventional endodontic treatment protocol (3% sodium hypochlorite irrigation and calcium hydroxide dressing), in necrotic teeth.
INTRODUCTION

*Every sentence I utter must be understood not as an affirmation, but as a question.*

Niels H. Bohr (1885-1962)
Danish physicist
**Historical context of lasers in Dentistry and the paradigm-shift**

Since Theodore H. Maiman produced the first laser based on a synthetic ruby crystal in 1960, the use of lasers has undergone a technological revolution. Dentistry, for example, has witnessed a paradigm-shift as technological breakthroughs have enabled a wide range of hard and soft tissue procedures with improved patient outcomes, combined with the absence of tissue direct contact, vibrations or pain as well as significant reductions in post-operative symptoms and complications.

Based on a theory originally postulated by Albert Einstein, T. Maiman created a device where a solid-state ruby crystal medium was stimulated (pumped) by a flash lamp as source of energy, leading to the emission of laser light. A year later, Snitzer developed the first neodymium laser (Nd:YAG laser). However, early dental research focused on laser systems was largely ignored.

In fact, experiments done by Stern and Sognnaes in 1964 found that ruby laser was not an effective wavelength for cutting enamel or dentin. In overall, hard tissue applications for these lasers were not promising; hence research focused on soft tissue surgery where argon, carbon dioxide, and Nd:YAG lasers proved to be successful for cutting and coagulation.

The first reported oral surgical application using a CO₂ laser occurred in 1977 by Lenz et al. Nevertheless, it took a decade longer for the first Food and Drug Administration clearance for the CO₂ laser in oral surgery (January 1987). This could be considered the true beginning for the acceptance and viability of using lasers in oral cavity in clinical environment.

From then, innumerous rounds of research into the laser field lead to broader oral applications that have accomplished a definitive dental laser revolution. This technology refinement is actually expanding the scope of procedures that a dentist can offer their patients, shaping an ineluctable standard of preventive, effective and minimally invasive dentistry.

To understand the physical background of lasers is not strictly necessary to be aware of their technical set-up; however it is mandatory to have the knowledge background regarding properties of light generated according to the laser principle. For instance, “laser” is an acronym that completely describes the whole physical process of light generation: *Light Amplification by Stimulated Emission of Radiation*. It is equally important to understand several terms and concepts which can be briefly described.

Lasers are usually named for their “active medium” that is charged with energy inside the laser unit (resonator) to create laser light. Though, laser is a physical phenomenon
traduced by a unique energy transformation where different kinds of energy are transformed into a new kind of optical energy with peculiar properties. This energy form is completely artificial and cannot be found in nature.

It can also be stated that a laser device transforms energy of “low quality” into a kind of energy that has “high quality”. The problem that inhabits in this transformation, which generates a high degree of energy order, is that this causes a decline in entropy, limiting the efficiency of laser light generation itself.

Another key concept is that different wavelengths react with different tissues in different ways, as the biological interaction between laser light and tissue is strongly dependent of the tissues optical properties – absorption coefficients. The absorption of the laser energy can be illustrated by the absorption spectrum for each wavelength in the targeted tissue or tissue components. Apart from wavelength and absorption, reflection, scattering and transmission (degree in which the laser’s energy is able to penetrate into the tissue) are phenomenon’s that also play a crucial role in laser-tissue interaction (Fig. 1).

That is the main reason why the extensive study of enamel, dentin and soft tissues composition is demanded in order to find the most suitable wavelength to work with.

Enamel is considered a highly mineral tissue, composed of 85% mineral, 12% water and 3% organic material, by volume (2). Dentin however, has a significantly higher content of organic material (33%) and water (20%) whereas only 47% is mineral, by volume. The mineral content is composed by carbonated hydroxyapatite crystals organized in enamel rods of approximately 5µm diameter (3). Due to their composition, enamel and dentin demonstrate high absorption coefficients for wavelengths in the mid infrared range, between 2.6-3µm (coincident with Erbium lasers peak emissions). In this spectrum region, scattering is almost negligible and the energy deposition is mainly determined by absorption coefficients and tissue reflectance (4). In this context, at a wavelength of 2.94µm there is a strong absorption in water (800cm⁻¹) while for a wavelength of 2.78µm the peak absorption is coincident with a narrow hydroxyapatite absorption band (400cm⁻¹) (5). The highest absorption peak from dental mineral bands is coincident with the phosphate groups observed at 9.6µm (6500cm⁻¹) for the CO₂ laser (6).

Currently, distinct laser sources – wavelengths - are available each having its own specific properties. The correct understanding of these properties, as light behavior and tissue interactions, is a precondition for using dental lasers appropriately in safe conditions. Thus, the aim for dentists should be to know what kind of laser(s) could be suited for a specific indication in order to achieve the maximum benefit through the implementation of laser technology in their dental practice.
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

**Figure 1:** Absorption coefficients (µA) of several wavelengths (µm) in dental materials. (Illustration gently provided by AALZ Institute, Aachen University – Germany)

Although it can be practical to operate accordingly with the instructions provided by the manufacturer without notions of the real clinical benefits and hazardous potential, to select a wavelength from the various laser systems which are at the dental practitioner’s disposal requires an advanced training concomitant with a good understanding of each wavelength characteristics and its biological interactions.
Demands for New Strategies in Endodontic Procedures

Cleaning and disinfecting the root canal system

Root canal infection occurs frequently concomitant with dental caries. In addition, bacterial penetration and colonization of intact pulp can be due to either dental treatment or trauma.

Although the biological diversity of oral microbiota can enormous in terms of number of species, just approximately 50 strains were reported to be predominant in root canal infections (7, 8). However in this context, it must be understood that the methodologies of microbiological root canal sampling are complex and that the diagnostic accuracy is still poorly known. Still, if microorganisms hiding in biofilms or in untreated parts of the canal system may be hard to sample and detect, remnants of the previous medication might also depress laboratory analysis (9).

As bacterial contamination is considered the primal etiologic factor for the development of pulpal and periapical lesions, to obtain the root canal system free of irritants has been showing to be endodontic therapy primordial goal (10-12).

The idea that an absence of cultivable microbes at the time of obturation will favor healing is consistent with the notion that microorganisms are the primary cause of persistent apical periodontitis. Fabricius et al. (2006) reported results from an extensive experiment conducted on 175 monkey root canals supporting this position (13). Several studies have reported a tendency towards more favorable outcomes in teeth yielding negative cultures before the root canal filling (14-16); Accordingly, other investigators have suggested that the presence of microbes at the time of root filling will adversely affect the outcomes (17-19).

The bactericidal effects of conventional irrigation strategies during and after root canal preparation with solutions such as hydrogen peroxide (H₂O₂) or sodium hypochlorite (NaOCl) has been studied by numerous investigators such as Bystrom et al. in 1985, Smith et al. in 1986, and Orstravik et al. in 1990 (20-22).

NaOCl is used for endodontic applications at concentrations ranging from 0.5% to 5.25% (v/v), and has been showing to be the most reliable endodontic irrigant from several decades until the present. Although it is reported that it can exert its maximum capabilities as an antibacterial agent and solvent of organic substances at a concentration of 5.25% (23), the most effective concentration and temperature for reducing the root canal system bacterial load may vary according to the author and methodology. In fact, the ideal concentration and
temperature of NaOCl in root canal therapy remains as controversy and topic of debate within endodontists (20-22, 24).

Hence, in order to penetrate more readily into non-instrumented areas of the root canal system and increase the agent’s efficiency, recent improvements in the NaOCl chemical structure have been tested. These chemical modifications allow practitioners to lower its surface tension, increasing contact with dentinal walls and possibly enhancing not only its antimicrobial effectiveness as well as its ability to dissolve pulp and organic tissue (25).

Another relevant finding is that mechanical-chemical enlargement with irrigation solutions such as NaOCl is limited to effective bacterial reduction up to a depth of 100µm whereas heavy E. faecalis infection was found 800µm deep into the canal lumen. Additionally, in some of the samples, bacterial propagation into the dentinal tubules has reached up to 1100µm in depth (22, 26-28).

However, sodium hypochlorite use is not risk free since it is unselective and damages human cells, dentine, and/or periodontal tissues, with clinical consequences. Under adverse clinical situations, it may affect branches of the facial nerves or the inferior alveolar nerve, leading to severe sequelae (29, 30).

Although 5.25% NaOCl is thought to be the most effective concentration, it has recently been found to be associated with a significant increase in apically extruded debris after rotary preparation as compared to lower concentrations and chlorhexidine (31).

Nevertheless, the potential replacement of NaOCl by other antimicrobial agents such as chlorhexidine is matter of further discussion (32). Whereas no significant differences were found between the alternative irrigation with 0.12% chlorhexidine solution when compared with 2.5% NaOCl in a clinical trial (33), Vahdaty et al. suggested that chlorhexidine and NaOCl were equally effective antibacterial agents against E. faecalis. In addition, both irrigants were capable of effectively reducing bacterial counts in the superficial 100µm of dentinal tubules but still failed to disinfect up to 50% of dentine samples (27).

Biomechanical instrumentation of the root canal system aim to remove infected root canal dentin and various techniques have been successfully reported and clinically adopted over the years. Moreover, while shaping the canal, either by manual or mechanical preparation, we could enlarge the root canal space in order to facilitate the chemical action and penetration of irrigation solutions. Concomitantly due to the complexity of the root canal system it has been shown to be virtually impossible to completely eliminate debris and sterilize the intricate parts of the root morphology.
During the canal enlargement proceedings, a *smear layer* is mechanically produced, covering the instrumented walls of root canal (34-38).

The *smear layer* is known to be a superficial layer on the surface of the root canal wall approximately 1-2µm thick with a deeper layer trapped in the dentinal tubules up to a depth of 40µm (37). Along with organic and inorganic substances, it also includes microorganisms and necrotic debris (39). Together with the possibility that the smear layer itself may be infected, it can also protect the bacteria harbored in the dentinal tubules by preventing the application and effective infiltration of successful intra-canal disinfection solutions (28, 37, 39).

Fogel *et al.* has consistently reported that the formation of a smear layer reduces the root dentin permeability from 25% to 49% (40). Hence, it is generally accepted that the complete removal of the *smear layer* would be consistent with the elimination of irritants from the root canal system (41). Root canal disinfection will be inefficient in the presence of smear layer as most current intracanal medications not only have limited antibacterial spectrums but also have shown limited ability to diffuse into the dentinal tubules (42).

Regardless of the instrumentation system or technique applied, it has been reported that, with the root canal preparation and enlargement, a substantial removal of pathogens can be achieved (43). However, it has also been clearly demonstrated that more than 35% of the canal surface area remained unchanged/untouched in a study that evaluated four distinct Nickel-Titanium preparation techniques (44).

Though, it has been suggested that newer treatment strategies designed to eliminate microorganisms from the root canal system should be considered. These must include agents that can penetrate the dentinal tubules and destroy the microorganisms beyond the host defense mechanisms, where they cannot be reached by systematically administered antibacterial agents (42). Therefore, other alternative possibilities such ozone treatment (45-47), ultrasonic and laser assisted treatments are being suggested as suitable, alternative methods to achieve endodontic disinfection, possibly overcoming the limitations of commonly used chemical solutions as well as any hazardous effects (48-50).

Depending on the wavelength, lasers have been mostly used in endodontics for either vaporizing the smear layer or eradicating pathogens, not only in the main root canal but especially deep in the root canal tubular network where rinsing solutions could never efficiently reach.

These can be considered the true goals for the adjunctive application of lasers in root canal therapy: the ability of infrared light to interact with water and efficiently remove the smear layer and debris from the root canal walls, together with the ability of light to propagate into the dentinal tubules further than any chemical solution, thus providing deep disinfection.
The goal of laser assisted endodontic treatments (LAET) is thus to provide increased outcomes, namely in cases of persistent infections or per-operative obstacles (e.g. isthmus, recurrent canals, internal resorptions, root canal perforations, or wide apical constrictions) which are often associated to either lower or compromised clinical expectations.

The increase of reported research suggesting the efficacy of such procedures has been proving the potential of LAET as a powerful and versatile method to achieve predictable endodontic outcomes.
Necrotic pulp condition: Bacterial aspects & *E. faecalis* role

Injuries to the pulp may lead to complete tissue breakdowns. The non-vital (or necrotic) pulp is defenseless against microbial invasion and allows indigenous microorganisms to reach the pulp chamber, either via direct exposure, uncovered dentinal tubules or cracks in the enamel or dentine. Lateral canals exposed as a result of progressive marginal periodontitis may also serve as pathways for bacteria to reach the pulp (51).

The specific environment in the root canal, characterized by the degrading pulp tissue and lack of oxygen, results in a microbiota dominated by proteolytic and anaerobic bacteria. Via the apical foramen, microbes and their by-products may reach the periapical tissue promoting an inflammatory response. This response induces reabsorption of the surrounding bone, which is often visible by x-ray assessment as localized periapical radiolucency. Such inflammatory reaction may progress and stimulate epithelial cells in the periodontal membrane to proliferate and form a periapical cyst (52, 53).

The first observation of these microorganisms was carried out by Anthony van Leeuwenhoek. In 1683, with a homemade microscope he was enabled to make the first drawings of dental plaque bacteria. However, it took about 200 years before root canal microorganisms received further scientific investigation, by the “father” of oral microbiology, Willoughby D. Miller (1953-1907).

A classical study in germ-free and conventional rats in 1965 demonstrated the essential role of microorganisms in the pathogenesis of periapical lesions (10).

Even before Kakehashi’s experiments, Miller described in 1890 the clinical effects of gangrenous tooth-pulps as centers of infections varying from hardly perceptible periapical inflammation to severe local and general symptoms, sometimes even with fatal outcomes. He cultured and characterized bacteria from necrotic pulps and studied their pathogenic potential in animal experiments (54, 55).

In some cases of infected necrotic pulp, an open pathway for the entry of bacteria is found in the form of pulp exposure due to caries or fractures. On the other hand, infections and apical periodontitis also occur in cases of closed necrosis, even in apparently intact teeth. Although there are potential forms of entry (e.g. dental caries, trauma, periodontal disease and anachoresis), no data is available concerning the relative frequency of these entry routes in clinical situations.

Nevertheless, the intact dentine-pulp complexes are considered a highly efficient defense system, often capable of preventing and suppress the entry of microorganisms.
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

Therefore, with the event of bacteria entering a vital pulp, their survival depends on their number and virulence as opposed to the defense mechanisms of the pulp (56).

The necrotic pulp, in turn, provides excellent growth conditions for microorganisms and is the major location for bacteria causing periapical lesions. Mixed microbial masses consisting of cocci, rods, filamentous bacteria, spirochetes and yeasts have been demonstrated in necrotic pulps using light microscopy of histological tissue sections, as well as by transmission electron microscopy of root fragments (57-59).

Generally, microorganisms adhere to some areas of the root canal walls, either as dense aggregates or as thin, single, or multilayered condensations. When the pulp becomes non-vital, the organisms can also extend for some distance into the dentinal tubules. In fact, the presence of bacteria in the dentinal tubules of infected teeth has been seen as reaching approximately half the distance between the root canal walls and the cement-dentinal junction (60-62). If bacteria are confined usually to the inner third, Sen et al. reported bacteria and fungi penetration between 10 to 150µm in most of their samples (63).

The invasion or penetration of the dentinal tubules by bacteria is essentially due to microbial multiplication and not necessarily by their movements. External pressure can also encourage migration, in which the microbes that were in the external part of the dentinal tubes penetrate into the interior - passive migration. Bae and colleagues have inclusively shown that most pulp infections are related to immobile bacteria progression (64).

Thus, in cases of periapical lesions, microorganisms are always found in these intra-radicular locations, often walled off by neutrophil granulocytes or an epithelial plug at the apical foramen (57).

The resident oral microflora compromise more than 300 species of cultivable bacteria and an unknown number of species that is impossible to grow with usual methods. The special environment of the root canal, however, selects for certain frequent species. A mixture of several typical ones has been cultured from necrotic pulp samples prior the start of treatment (65, 66).

The microorganisms in the root canal samples from deciduous and permanent teeth are predominantly the same bacteria as those found in dental plaque, periodontal pockets and carious lesions. Most isolates in initial cultures are obligate anaerobic bacteria. These have constituted 91% of the isolates from closed necrosis (67), 90% of isolates from necrotic pulps of deciduous teeth (68), and 68% from the apical part of necrotic pulps in carious teeth (69). A large proportion of these anaerobes are asaccharolytic, peptide and amino acid-degrading bacteria (70).
Thus, many genera and species currently identified in root canal samples comprise obligate anaerobic and facultative anaerobic oral bacteria. Among the streptococci, species of the anginosus group (S. anginosus, S. intermedius, S. constellatus) and mitis group (S. mitis, S. oralis, S. gordonii, S. sanguis, S. parasanguis) are common, and in carious teeth S. mutans is also prevalent. It has been suggested that S. sanguis and S. salivarius often occur in root canal cultures due to contamination with saliva or invasion through leaking temporary fillings (71). Actinomyces israelii and other Actinomyces species may be present and actinomycotic periapical lesions may sometimes develop for periodontal reasons (72).

The Enterococcus faecalis is known to be a facultative Gram-positive anaerobic coccus and endodontic-related pathogen, as it is frequently recovered from the root canals of teeth with post-treatment infections (73, 74). It is frequently isolated from saliva, mucosal surfaces, and supra-gingival dental plaque; consequently it has been associated with several conditions, such as oral mucositis, aggressive periodontitis and other infections including endodontic–related ones (75, 76).

It is thought that the biological properties of E. faecalis allow the bacterium to survive in adverse conditions inside the canal, where concentrations of nutrients are low and alkaline conditions are prevalent (it can resist high pH values, up to 11.5)(77). E. faecalis also has the particular characteristic that it can survive in canals as a single species without the support of other microorganisms (78). In addition, they are able to form intra and extra-radicular biofilms making it even harder to control or eradicate them (79, 80).

Bacteria may also invade the dentinal tubules, depending on the morphological factors of the bacterial species. Some bacteria including E. faecalis might migrate deeper into the lateral root canal or dentine tubules than others, which may protect them from effective disinfection (81).

Although experimental demonstrations have shown that this specific bacteria can invade as far as 800µm into the dentinal tubules of root canal walls (28), it has also been reported that this microorganism has the ability - under specific conditions - to infect the full length of the tubules within two days (22). These characteristics often contribute to therapy resistant cases that end up as long-term failures after endodontic treatment.

Sodium hypochlorite and chlorhexidine, i.e., have proven to be effective against E. faecalis in vitro, but they require direct contact (82, 83). Furthermore, whereas pathogenic microorganisms are able to penetrate more than 1mm (Streptococcus mutans were found in dentinal tubules of open infected root canals up to a depth of 1100µm), rinsing solutions, such
as NaOCl, only reach a depth of 100µm and under special conditions (e.g. temperature) (26, 62).

E. faecalis has also been found to resist to inter-appointment medications, including the most common intracanal treatment, calcium hydroxide (22, 84-86).

An additional and crucial factor that may prevent the complete elimination of E. faecalis relates to the intricate anatomy of the root canal system, consisting of lateral canals, fins, apical ramifications (deltas), accessory canals and isthmuses (87, 88).

Altogether, such findings may clearly justify the necessity to develop effective means of removing the smear layer and all known pathogenic bacteria from root canal dentin following biomechanical treatment. Consequently, the development of an alternative to traditional disinfection protocols (such as a laser-assisted approach) may seem useful and appropriate to be under investigation in order to effectively eradicate microorganisms harbored deep within the dentinal tubules and complex root canal ramifications.
Apical Periodontitis: Microbiological aspects

Dental pulp reacts to the different external irritant factors (microbes, thermal, mechanical, and chemical injuries) through characteristic inflammatory responses including vasodilatation. It can frequently lead to an increase of internal pressure which affects the pulp circulatory system; as it is a terminal circulatory type, it is often associated as the direct cause for the pulp necrosis (89).

Necrotic root canal ambient can afford bacteria space and a moist of warm, nutritious, and anaerobic environment that is relatively well protected from host defenses. Even so, only a restricted assortment of oral bacteria can be found in an infected canal, suggesting that selective pressures can favor the establishment of some species and inhibit others to proliferate in the root canal system (90).

Following the formation of a periapical inflammatory lesion secondary to pulpal necrosis, chronic apical periodontitis (granuloma) is considered the next step in the progression of these inflammatory events showing replacement of adjacent tissue with an inflammatory cell infiltrate that usually occurs at the expense of the surrounding bone. In addition to the inflammatory cells, it typically contains fibrous tissue and cholesterol crystals (91).

Over time, due to inflammatory stimulation and proliferation of the epithelial rests of Malassez (residual epithelial cells in the periodontal ligament), an inflammatory cyst can develop at the root apex and through the bone (92). If the lumen of the cyst is continuous with the infection source at the pulpal entry, it may not be self-sustained (‘pocket’ cyst); this will heal following infection source elimination. On the other hand, if the cyst is completely encased by epithelium and removed from the source of infection, it may be self-sustained (‘true’ cyst) and become refractory to treatment except by surgical excision (93).

Cysts most appear as round or pear-shaped, unilocular, radiolucent lesions in the periapical region. They are usually classified when become bigger than 1cm in diameter, being bordered by a thin rim of cortical bone. Cysts may displace adjacent teeth or cause mild root resorption (94).

The differentiation between radicular cysts and granulomas is difficult or impossible by traditional radiographic techniques, even if several radiographic features have been proposed to make this distinction; these may include the lesion size and the presence of a radiopaque rim lining the cystic lesion (95, 96). While the probability of a lesion being a cyst may increase with its size, a reliable diagnosis still remains based on histology (95, 97).
Chronic Apical Periodontitis (CAP) is arguably one the most common forms of biofilm-induced human diseases (98). This condition is usually asymptomatic and can be often found by radiographic means in patients between 30 and 50 years old (99, 100).

This condition usually develops after dental pulp necrosis and infection as a result of caries, trauma, or iatrogenic clinical procedures. The environmental conditions in the necrotic root canal are conducive to the establishment of a microbiota typically dominated by anaerobic bacteria. However, bacterial profiles may vary from individual to individual i.e., each one harbors a unique biofilm in terms of species richness and abundance. This also indicates that apical periodontitis has a heterogeneous etiology, where no single species can be considered to be the main endodontic pathogen, and multiple bacterial combinations can play a demanding role in the disease development (101).

The literature contains significant divergence regarding histological results in terms of apical lesions, with the prevalence of granulomas ranging from 9% (102) to 87% (103) and cysts prevalence ranging from 7% to 59% (92, 104).

The discrepancy between prevalence in the literature is probably attributed to the different criteria used in the various histological studies. Histological diagnosis based on samples with few sections can also lead to the incorrect definition of epithelialized lesions as cysts. As example, Ricucci et al. established the diagnosis of cysts with the presence of a cavity completely or partially delimited by epithelium. This epithelium was found in 21 of 50 lesions, whereas only 16 were indeed classified as cysts (105).

In most studies, neither radiographic size nor the presence of radiopaque lamina alone can be considered sufficient evidence on which to base a diagnosis of periapical pathology. A histological study is mandatory to definitively identify the type of periapical lesion. Authors such as Hirsch et al. cited that most periapical lesions are either cysts or granulomas and only few are keratocysts. Therefore this controversy can be found quite irrelevant from a surgical point of view and - as that the prognosis does not depend on the type of lesion -histological diagnosis in considered unnecessary. While periapical cysts usually demonstrate the largest radiographic areas, chronic apical periodontitis is the most common type of apical lesions, followed by scar tissue (106).

Early studies of microbiota associated with apical periodontitis were conducted using a broad-range of culture methods. Culture studies have first demonstrated that primary endodontic infections are characterized by a mixed consortium dominated by anaerobic bacteria and composed of a mean number of 2.6 to 5.4 taxa per root canal (8, 107, 108).
Culture studies were followed by a number of studies employing molecular detection methods such as species-specific PCR and the original checkerboard DNA-DNA hybridization. These methods allowed the inclusion of some culture-difficult species in the set of candidate endodontic pathogens. Afterwards, the adoption of 16S rRNA gene clone library analysis allowed even more comprehensive investigations of bacterial biofilms in endodontic infections. Through this technique, not only cultivable species but also as-yet-uncultivated and uncharacterized bacteria could be identified, revealing that 40 to 55% of the bacterial taxa found in primary endodontic infections have not been cultivated and validly named/identified (109, 110).

After about 1 decade of application of molecular biology methods to endodontic microbiology research, specific knowledge regarding bacterial diversity involved with apical periodontitis has been substantially refined and redefined. In addition, to strengthen the associations of several cultivable species with chronic apical periodontitis, new findings using large scale analysis allowed to constantly improve the inclusion of some newly named species and as-yet-uncultivated phylotypes in the set of candidate pathogens associated with this disease.

The broad-range molecular analyses of bacteria present within the root canals of teeth with chronic apical periodontitis have revealed higher figures than those demonstrated by culture methods: 7 taxa in denaturing gradient gel electrophoresis analyses (101), 11 taxa in terminal restriction fragment length polymorphism analyses (109), 10 to 12 taxa in clone library analyses (111), and 20 taxa in combined culturing and clone library analyses (110). Therefore, data gathered from culture studies tend to underestimate the number of bacteria taxa in infected canals, as a result of difficulties or even impossibilities in cultivating a significant proportion of the endodontic microbiota.

Another important finding was related to the mean number of taxa per canal that was clearly in direct proportion to the lesion size: small lesions (<5mm) harbored 11,7 taxa, lesions from 5 to 10mm harbored 16 taxa, and lesions >10mm harbored about 20 species (112). These differences in species richness help explain the long-held concept that the endodontic treatment of teeth with large lesions have lower success rates than treatment of teeth with smaller or no lesions (113).

Another example of 16S rRNA gene-based nested or heminested PCR assays effectiveness is that O. uli only recently was recognized as a frequent member of the endodontic microbial consortium of teeth with apical periodontitis (114). In corroboration, and analyzing the presence and relative levels of 83 oral bacterial species, Siqueira et al. have adopted an innovating reverse-capture checkerboard hybridization assay; associations
between the most frequently detected taxa were also recorded. The most prevalent taxa detected were *O. uli*, *E. corrodens*, *P. endodontalis*, and *P. anaerobius*. *O. uli* was, in fact, present in about three-fourths of the samples, confirming that this species is a very regular member of the microbiota associated with chronic apical periodontitis (112).

*E. corrodens* has been detected in endodontic infections mainly by molecular methods and the 63% frequency - at which this species was found in Fouad et al. study - is probably the highest one, while assessing endodontic infections. *P. endodontalis* was primarily found in endodontic infections by culture means, but its association with apical periodontitis has been strengthened by findings obtained from molecular studies, where higher prevalence values have been reported (115).

It has also been demonstrated by scanning electron microscopy that periapical bacterial plaque is a coating of various microbial forms embedded in a structureless material on the outer root surface, near the main apical foramen. In resorption lacunae bacteria and yeast cells sometimes could be detected (116, 117).

In ultrastructural studies, micro-organisms are generally not found in the soft tissue lesion in cases of chronic apical periodontitis or in periapical cysts (when the cavity completely encases in an epithelial lining so that no communication to the root canal exists). An exception to this rule is the occasional finding of typical actinomyces-containing colonies in granulomas, radicular cysts (118) and in cases of periapical abscess with or without sinus (fistula), which sometimes various bacterial forms and yeasts may be present inside the lesion and/or inside phagocytes (119).

The stages in development and healing of chronic apical periodontitis, *granulomas* and cysts are, depending on several circumstances, reflected by changes in the radiographic appearance of periapical areas. Generally, the prognosis for complete healing of endodontically treated teeth with diagnosis of apical periodontitis is approximately 10%-15% lower than teeth without apical periodontitis (34, 120). Thus, if with ideal conditions for root canal therapy the success rate can reach over 90%, for teeth with periapical radiolucency, the success rate can decrease to about 80% (121). So, the real challenge for endodontists regarding disinfection of the root canal system would be gangrenous teeth in addition with chronic apical periodontitis which the pathological reaction caused by certain (and yet to be determined) bacteria harbored in the root canal thrive to produce the clinical picture of periapical infection (122).
Apical Periodontitis: Radiological features & Anatomical considerations

**Radiological features:** In several dental situations, the use of radiographs is considered mandatory for precise diagnostic judgment. Specifically in endodontics, radiographs arguably represent the most important single diagnostic aid due to its reliability as method to obtain relevant information concerning the pulp canal space and periapical tissues.

The periodontal ligament provides the space for the initial cell infiltration. It serves as starting point for resorption processes as well as the end of healing ones. Then, a widened periodontal ligament space can be associated with either initial or residual chronic inflammation (123).

If teeth with increased mobility due to bruxism or marginal periodontitis may also present records of widened periodontal ligament, in the case of apical periodontitis it is restricted to the infected area near the apex (124).

The lamina dura appearance in radiographs varies, as it is a continuation of the jawbone cortex, which encases the root in a cortical bone socket. There is a considerable intra- and inter-individual range in its thickness and density. Even related to the fact that the bone is frequently thin in the region of maxillary canines, the lamina dura is almost impossible to discern around these teeth.

*Lamina dura* may be described as irregular indistinct or serrated, but none of these changes is pathognomonic in the early or healing stages of apical periodontitis (125).

Thus, its appearance is mainly determined by the shape and position of the root in relation to the X-ray beam rather than the density and integrity of the lamina dura itself. As it can also vary with the amount of occlusal load or stress, some lesion in lamina dura may produce radiographic detection earlier than in cancellous bone because more minerals are removed at that site (126).

Unfortunately, normal variations in thickness and continuity of lamina dura make the diagnosis by these single criteria inappropriate. In more advanced stages however, other radiographic changes become prominent and those can offer better distinction between stages and degrees of inflammation in terms of pathognomonic signs of apical periodontitis.

As inflammatory reactions of periapical tissues often proceed without any clinical symptoms, apical periodontitis cases are frequently diagnosed just by radiographic means. Numerous investigators have studied the sensitivity of periapical radiography; a common approach was to create artificial bone lesions in cadavers to access the minimum amount of bone loss necessary to result in a visible radiolucency. One reference study set up by Bender &
Seltzer reported that bone lesions were not visible until the cortex or the interface between cortical and cancellous bone was involved. In addition bone destructions were always found larger than suspected from studying the correspondent radiographs (127).

In fact, changes in mineralization and structure of the bone adjacent to the site of inflammation constitute the basis of radiographic diagnostic procedures for the detection and monitoring chronic apical periodontitis. Nevertheless, radiographic appearance depends as well on its evolution stage. While studying human autopsies, Brynolf compared the radiology and histology of periapical areas in upper incisors and reported a high frequency of radiographically undetectable inflammatory lesions (128).

Figgord et al. generally address to apical periodontitis as a very prevalent problem (98). Typically, these lesions are located at the root apex, but communications may exist at various levels along the root surface, and though can develop from lateral and furcal locations to sinus or further adjacent structures.

By its turn, Moreira et al. in 1998 performed a portuguese epidemiological study - in Porto area – and their results indicated a prevalence of apical periodontitis in 27% of the 322 residents which were assessed (129).

**Anatomical considerations:** Although being very prevalent, the location of periapical lesions in the oral cavity was found quite similar in different populations. A study by Nobuhara and Del Rio (130) found the majority in the anterior maxilla (47,3%), followed by the posterior maxilla (28,7%), posterior mandible (15,3%), and anterior mandible (8,7%). These findings are in agreement with Spatafore et al., who found 46,5% of lesions in the anterior maxilla, 20,7% in the posterior maxilla, 18,3% in the posterior mandible, and 14,3% in the anterior mandible (131). These prevalence results are also similar to the distribution found and reported by Carrillo et al. (132).

Radiographic imaging of CAP is presented on a background of superimposed, normal osseous and other structures. By its turn, radiographic diagnosis of apical periodontitis is based on deviations from the normal anatomy. So, periapical changes can only be interpreted properly considering some important anatomical aspects that are closely related to the location of the lesions and different types of bone.

Because cortical bone is more mineralized than cancellous bone, the demineralization or resorption process will manifest radiolucent changes, i.e. minerals are lost sooner and more readily in more calcified tissues than in less calcified ones. Though, lesions in cortical bone are easier to detect and assume prominence for their clarity and size in radiographs.
Several *in vitro* studies addressed this thematic and it seems that the lesion may visualized in earlier stages when its near, or in the bone cortex (127, 133). On the contrary, it may not or is less likely to become apparent in the cancellous bone (134).

However, in presence of root canal infection, changes in the structure of cancellous bone may be a primordial sign of apical periodontitis. The radiolucency may be seen clearly adjacent to the root apex but may blend or become rarefied with the surrounding bone at the periphery of the lesion, indicating moderate or severe inflammation (123).

The cancellous bone is thick over the deeper portions of anterior teeth and premolars palatal alveoli. The apex of the lateral incisor, however, is frequently located in apposition to the palatal cortical bone.

In the mandible the alveolar process in very thin in its anterior portion, around the incisor teeth roots. The canine alveolar process, however, is strong and heavier than over the incisors. The buccal cortex of the alveoli is relatively thin, whereas the lingual cortex is rather thick.

In the anterior maxilla, the trabeculae are thin and numerous, forming a fine, granular dense pattern; consequently, marrow spaces are typically small and numerous. In the anterior mandible, the trabeculae are thicker than in the maxilla, resulting in a rough pattern with horizontally oriented trabecular *striae*. There are also less trabeculae than in the maxilla, with the marrow spaces correspondingly larger (135).

Specificity of periapical radiographic diagnosis is considered higher than sensitivity; though, several investigations pointed out problems with the sensitivity of radiographic diagnosis, supporting guidelines for selective use of periapical views based on both patient symptoms and clinical signs. Although dentists have presented a good – or satisfactory - degree of reliability on repeated evaluations through time, only fair agreement when compared with other dentists reading the same radiograph was obtained. In complement, and despite the high risk of false-negative recordings, clinicians can also arrive at false-positive diagnoses by erroneous interpretation of normal anatomical structures as major blood vessels and spaces in the bone marrow that, as example, might simulate images of inflammatory periapical lesions (136, 137).

Related to false-positive appearances, the incisive foramen – opening of the incisive canal onto the roof of the hard palate – may cause diagnostic problems while appearing on radiographs as a radiolucent area related to the apex of the maxillary central incisors. Therefore, we may follow the *lamina dura* or by taking angled radiographs we may shift the radiolucency in relation to the apex, revealing its true nature.
Concerning the mental foramen - that usually opens on the facial aspect of the mandible in the region of the premolars – it also may be projected over the premolars apices simulating periapical disease. If possible, one may follow the mandibular canal extension to the suspected radiolucency or trace the lamina dura around the root apex, in order to perform a correct assessment (135).

Although not being sufficient to overcome radiographic sensitivity issues, negative reactions to vitality tests are considered necessary and preponderant as information to act on diagnosis. However, in combination with a discolored crown or periapical radiolucency, an access preparation to the pulp chamber might be justified and the diagnosis confirmed with the finding of a non-bleeding pulp (138).

There are several radiologic modalities and techniques, digital or not, which permits and/or help in the diagnosis and assessment of periapical pathology. After the introduction of periapical and panoramic digital radiographs researchers have tried to compare all the techniques. However, because of their improved quality, low radiation and ease of use, panoramic radiographs have become inevitably popular in dental diagnosis. As an extra-oral method it may be more comfortable for the patient and may allow a more vertical alignment of the structures than do series of intraoral radiographs.

Recent improvements in electronic radiographic imaging systems, such as digital panoramic radiography, have introduced many potential benefits into clinical dentistry. Digital panoramic radiography systems include a 50-80% reduction in radiation exposure, wider exposure latitude, immediate image generation and manipulation with elimination of chemical processing of radiographs. Disadvantages include size, shape and stiffness of the sensor and arguable image resolution depending on the system itself.

Conventional intraoral films have a special resolution exceeding 20 line pairs per millimeter (139), whilst the corresponding resolution for photostimulable phosphors is less than 7 line pairs per millimeter (140). Until recently, just some exclusive charged-coupled devices could go up to 20 line pairs per millimeter (141).

Some results also suggest that conventional periapical radiographs allowed the assessment of higher percentage of apices, with the exception of maxillary second and third molars, that were better viewed in panoramic radiographic assessments’ (142). Rohlin et al. also described that panoramic radiography frequently underestimate periapical lesions compared with periapical radiography (143).
By this, it is possible to conclude that differences in resolution of details may have an effect on subtle structures such as thin trabeculae, lamina dura and the periodontal ligament. However, the identification and assessment of lesion size appear to be influenced not only by the technology available in office; the percentage of mineral loss within the path (perpendicular) of the X-ray beam may be more critical than the size or shape of the lesion which produces the image itself. This may explain why a change in angulation of the X-ray can cause the disappearance or augmentation of the lesion (135).

It has also been proposed that panoramic radiography, followed by appropriate periapical radiographs (taken with the paralleling technique), is an alternative to complete mouth intra-oral surveys in terms of both diagnostic yield and radiation thrift; despite its limitations the areas to which radiography can make a significant contribution for diagnosis are: the assessment of bone loss, mobility, occlusal trauma, calculus, marginal overhangs and crown-root ratio. Hirschmann et al. also evaluated in detail the validity of three criteria that have been proposed for the radiographic assessment of early periodontitis: loss of crestal bone height, marginal widening of the periodontal ligament and crestal irregularity. He concluded that only the first is of any diagnostic worth, if provided by two sequential radiographs at least (144).

Although the bisected-angle method may be used, long-cone techniques are preferred, particularly for endodontic and periodontal evaluations. The paralleling technique provides images with a minimum of geometric distortion, but with some enlargement of structures. The bisecting angle technique introduces some image distortion, particularly in the bucco-lingual direction. Differences in were not found between the two techniques in the assessment of periapical lesion size, but paralleling technique had better reproducibility of repeated exposures (145).

Film holders are commercially available and became useful for long-cone techniques, as they are considered an excellent method to obtain reliable radiographs, particularly for posterior teeth. Because of the angle of the hard palate, radiographs that are held by thumb or finger usually demonstrate short buccal roots and long palatal roots. For mandibular teeth assessment, film in a holder is exposed with the teeth closed rather than open as in finger-held views. As the mylohyoid muscle is relaxed when the jaws are closed, the film within the film holder has less chance to gouge the tissues on the mouth floor.

The film holder ensures that the film is placed perpendicular to the occlusal surfaces of teeth and aids in the positioning of the x-ray tube. Though, it is easier to reproduce film angles for a series of the same area; it also provides a good method by which a particular tooth may be centered in the film. Because many articles have been written in nonprofessional magazines
concerning the dangers of radiation, patients are even more comfortable when some device rather than their own finger is used to hold the film (Figure 2) (146).

Independently of the technique applied, in follow-up studies of individual cases, identical or at least similar conditions for radiation exposure are recommended; moreover, the use of periapical film holders, bisecting angle and paralleling techniques and rectangular collimation are often associated to dentists which have postgraduate qualifications and. The use of film holders for intraoral radiography is inclusively advocated in current guidelines to British general dental practitioners’ (147-149).

Figure 2: Paralleling technique using a film holder and exposure settings recorded for each subject (authors’ property).
Sodium hypochlorite (NaOCl) as irrigation solution

Hypochlorite was first produced in 1789 in Javel, France, by Claude Louis Berthollet, as an aqueous chlorine solution, by passing chlorine gas through a solution of sodium carbonate. The resulting liquid, known as “Eau de Javel”, was a week solution of sodium hypochlorite. However, this process was not very efficient and alternate production methods were sought. One such method involved the extraction of chlorinated lime (known as bleaching powder) with sodium carbonate to yield low levels of available chlorine. This method was commonly used to produce hypochlorite solutions for use as hospital antiseptic that was sold under the trade names “Eusol” and “Dakin’s solution”.

Near the end of the nineteenth century, E.S. Smith patented a method of hypochlorite production involving hydrolysis of brine to produce caustic soda and chlorine gas, which then mix to form hypochlorite. Both electric power and brine solution were in cheap supply at this time and various enterprising marketers took advantage of this situation to satisfy the market’s demand for hypochlorite.

Today, an improved version of this method, known as the Hooker process, is the only large-scale industrial method of sodium hypochlorite production. In this process sodium hypochlorite (NaOCl) and sodium chloride (NaCl) is formed when chorine is passed into a cold and dilute sodium hydroxide solution. It is prepared industrially by electrolysis minimal separation between the anode and the cathode. The solution must be kept below 40°C to prevent the undesired formation sodium chlorate: Cl₂ + 2NaOH \rightarrow NaCl + NaOCl + H₂O.

Sodium Hypochlorite mechanism of action is indeed, related to a dynamic balance as shown by the following reaction (150):

$$\text{NaOCl} + \text{H₂O} \rightleftharpoons \text{NaOH} + \text{HOCl} \rightleftharpoons \text{Na}^+ + \text{OH}^- + \text{H}^+ + \text{OCl}^-$$

In the human body, chlorine compounds are part of the nonspecific immune system. They are generated by neutrophils via the myeloperoxidase-mediated chlorination of a nitrogenous compound or set of compounds.(151) But, in fact, interpreting these chemical reaction lead to a better understanding into how sodium hypochlorite can act as a solvent for organic and fat degrading fatty acids, transforming them into fatty acid salts (soap) and glycerol (alcohol) that reduces the surface tension of the remaining solution.

Hypochlorous acid (HOCl), a substance present in sodium hypochlorite solution, when in contact with organic tissue acts as a solvent and releases chlorine that, combined with the protein amino group, forms chloramines (chloramination reaction) that interfere in cell metabolism, leading to amino acid degradation and hydrolysis. Moreover, chlorine (which is a
strong oxidant) presents antimicrobial action inhibiting bacterial enzymes leading to an irreversible oxidation of SH (sulphhydryl) groups of essential bacterial enzymes - cystein (150).

The antimicrobial effectiveness of sodium hypochlorite, based in its high pH (hydroxyl ions action), is similar to the mechanism of action of calcium hydroxide. This particularity, interfere in the cytoplasmic membrane integrity with an irreversible enzymatic inhibition, biosynthetic alterations in cellular metabolism and phospholipid degradation observed in lipidic peroxidation (152).

Sodium Hypochlorite is since long time used in endodontics during root canal treatments. It is considered the medicament of choice due to its efficacy against pathogenic organisms and pulp digestion. Beside their wide-spectrum and non-specific killing effects on all microbes, hypochlorite preparations are sporicidal, viricidal (153) and show far greater tissue solving effects on necrotic than on vital tissues (154).

These features, associated to the facts that it’s of cheap production, easily available, and demonstrate good shelf life, prompted the use of aqueous sodium hypochlorite in endodontics as the main irrigant as early as 1919 as recommended by Coolidge (155).

Historically, Henry Drysdale Dakin’s solution (0,5%) has been the mostly used. However, its concentration for endodontic propose, can vary from 0,5% to 5,25%. Thus, several in vitro studies have been performed on the antibacterial activity of NaOCl. Walker (156), in 1936, introduced the use of double-strength chlorinated soda (5% NaOCl) solution as root canal irrigant in endodontic practice, which as continued worldwide ever since with no study definitively showing any other irrigant to be more effective.

Siqueira et al. compared the antibacterial activity of several irrigants against four black-pigmented anaerobic bacteria and four facultative bacteria through an agar diffusion test. Their findings showed that the antibacterial effectiveness of 4% NaOCl and 2.5% NaOCl was significantly greater than other tested agents (32). However those same authors, in another study, showed that there was no difference in the antibacterial activity of 1%, 2.5% and 5.25%NaOCl (157).

This subject is still a matter of big controversy as researchers try to find the ideal concentration for the NaOCl solution. Another demonstrative example, such as Berber et al. (158) which assessed the efficacy of 0.5%, 2.5% and 5.25% NaOCl as intracanal irrigants associated with hand and rotary instrumentation techniques against E. faecalis within root canals and dentinal tubules. They found that 5.25% concentration was the most effective solution followed by 2.5% concentration.
In vivo the controversy still remains. A classical study by Bystrom and Sundqvist (159) evaluated the antibacterial effect of 0.5% NaOCl of fifteen single-rooted teeth. Each tooth was treated at five appointments, and the presence of bacteria in the root canal was studied on each occasion. No intracanal dressings were used between appointments. When 0.5% hypochlorite was used no bacteria could be recovered from the twelve of fifteen root canals at the fifth appointment. This should be compared with eight of fifteen root canals when saline solution was used as irrigant.

In another study, Siqueira et al. (160) investigated the bacterial reduction after instrumentation using 2.5% NaOCl as an irrigant and further interappointment dressing with calcium hydroxide/camphorated paramonochlorophenol paste. Results showed that chemomechanical preparation with 2.5% NaOCl significantly reduced the number of bacteria in the canal but failed to render the canal free of cultivable bacteria in more than one-half of the cases. Nevertheless, with 7-day intracanal dressing with Ca(OH)₂/CPMC paste further significantly increased the number of culture negative cases.

Fungi have occasionally been found in primary root infections, but they seem to be more common in root canals of obturated teeth in which the treatment has failed (161). Overall, the occurrence of reported yeasts, such as *C. albicans* (found the most prevalent) in infected root canals varies between 1% and 17% (162).

The antifungal activity of sodium hypochlorite is then well known, and it is generally accepted that even when significantly diluted, it’s still effective (163). Radcliffe et al. (164) demonstrated that four concentrations of NaOCl lowered CFU below the limit of detection after 10s in the case of *C. albicans*. This finding is also supported by Ayhan et al. (165).

However, it has to be stated that, in intact dentine, the hydroxyapatite has a protective role by embedding collagen and other proteins against the oxidative activity of NaOCl. As mentioned earlier the antibacterial effect of sodium hypochlorite is well established. But, in contradiction, the sum of significant number of *in vivo* studies has clearly shown that instrumentation and irrigation with sodium hypochlorite failed to predictably produce sterile root canals. In order to reveal the reasons for such difficulty, studies with dentine powder were performed and have shown that dentine has an inhibitory effect on the antibacterial effectiveness of 1%NaOCl. Dentine powder (18% v/w) greatly delayed the killing process of *E. faecalis*, which as used as a test organism (86).

On the whole, despite the fact that dentine seems to reduce or inhibit the NaOCl antibacterial activity, it can be concluded that in both *in vitro* and *in vivo* conditions, it exhibits excellent antibacterial and antifungal activity. Moreover, at low concentrations it will dissolve
mainly necrotic tissue; whereas at higher concentrations tissue dissolution is better but it also dissolves vital tissue, a generally undesirable effect (166).
Hazardous effects of sodium hypochlorite

Toxicity and injection beyond the apical foramen:

Most complications from the use of sodium hypochlorite appear to result from its accidental injection beyond the apical foramen which can cause violent tissue reactions characterized by pain, hemorrhage, swelling, and in some cases the development of secondary infections, necrosis and paresthesia. This can be attributed to the fact that sodium hypochlorite has a pH of approximately 11-12 in addition to its excellent tissue-dissolving capability. When it comes into contact with tissue proteins, nitrogen, formaldehyde and acetaldehyde are formed within a short time and peptide links are broken resulting in protein dissolution. As a consequence, NaOCl is highly toxic at high concentrations and tends to induce tissue irritation merely on contact (167).

Heggers et al. studied biological tissue toxicity related to NaOCl irrigation in correlation with its bactericidal properties, both in vitro and in vivo. It was found that tissue toxicity was observed at 0.25% but not at 0.025%. Below this concentration, NaOCl solution was no longer bactericidal. Thus, the 0.025% NaOCl concentration would be the safest concentration to use in biological tissues since it preserves bactericidal properties while eliminating potential negative side-effects on wound healing (168).

Furthermore, sodium hypochlorite solution with 0.5% concentration (Dakin’s solution) already had proved to be detrimental to neutrophil chemotaxis and toxic to fibroblasts and endothelial cells (169).

Unfortunately, reports of sodium hypochlorite injection after iatrogenic perforations or solution forced beyond the apex can be found with rather higher concentrations, affecting different areas of the mouth. The symptoms are often immediate and exacerbated with long-term consequences such as extended periods of necrosis and paresthesia. Obviously, the most severe cases are those related to the endodontic use of 5.25% NaOCl (170).

In a rare and yet unpublished case report, one undergraduate student from our Faculty recently performed an iatrogenic root canal perforation in an upper left pre-molar. He inadvertently injected between 0.5ml and 1ml of 3% sodium hypochlorite into the peri-radicular tissues through the lateral perforation. The consequences were severe, with immediate pain, swelling, edema and loss of sight; the patient required hospital treatment and the long-term consequences have yet to be determined (Figure 3: 3% sodium hypochlorite accident following iatrogenic perforation - photograph gently provided by Prof. Doutor J.A. Capelas.)
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Regarding such dangers, dental practitioners should carefully check both clinically and via x-rays for immature apices, root resorption, root perforations or any other conditions that might permit larger than normal volumes of irrigant to be extruded from the root canal into the surrounding tissues or periradicular structures.

Due to these risks, endodontic irrigation should be performed slowly with gentle movements of the needle in order to ensure that it is not completely obstructing the root canal.

**Eye damage:** It was reported that irrigant in contact with the patient’s or operator’s eyes result in immediate pain, profuse watering, intense burning and erythema. Loss of epithelial cells in the outer layer of the cornea may also occur. If so, eyes should immediately be flushed with large amounts of tap water or sterile saline solution and the patient must promptly be examined by an ophthalmologist and treatment provided is deemed necessary (29).

**Allergic Reactions:** Cases of hypersensitivity and allergic reactions to sodium hypochlorite have also been reported in which patients immediately reported severe pain and burning sensations, swelling of lips and cheeks, accompanied by ecchymosis and profuse hemorrhage from the root canal (171). Pain may diminish after few minutes but breathing problems may result and emergency care may potentially be required (172).

![Figure 3: 3% sodium hypochlorite accident following iatrogenic perforation](image)

- photograph gently provided by Prof. Doutor J.A. Capelas.
Calcium hydroxide (CaOH) paste as inter-appointment dressing

Radiolucencies are often amenable to nonsurgical treatment when sufficient time for multiple appointments is available or the patient is recalled at a later date for healing evaluation. On the other hand, in single-appointment endodontic treatments of teeth with chronic apical periodontitis, post-operative exacerbation may occur leading to surgical alternatives usually performed under acute conditions.

The second appointment is therefore often necessary to stop the hemorrhage or suppuration at the apical third, and to allow an effective removal of the elements of the predentin layer packed against the walls during preparation. Moreover, teeth associated periapical radiolucencies must be prepared up to the apical constriction to better rid the canal of necrotic debris, metabolites, bacteria, and other irritants that are responsible for adjacent bone inflammation. It was reported that sharp apical enlargement of the apical foramen should be obtained; otherwise, those cases showing radiolucency may yield a strong possibility of failure (146, 173).

Whether adequate microbial control can be obtained in one appointment is an ongoing source of controversy. Although there are multiple scientific arguments to prefer multiple appointments in root canal therapy of infected teeth with apical periodontitis, clinical research to date has been equivocal (174, 175).

Calcium hydroxide paste is one of the most commonly used intracanal medications for multiple appointment root canal therapy. Nevertheless, there is a growing body of evidence that questions the effectiveness of calcium hydroxide against several microorganisms commonly associated with persistent apical periodontitis (176, 177).

Although some studies have demonstrated improved healing when calcium hydroxide is used in multiple appointment treatment as an intracanal medication (19, 178), others have found little or literally no benefit (179, 180). Therefore, the quest for an effective scientifically supported one-visit procedure has been carried out from principally two angles: (1) the exclusion of an antibacterial intra canal dressing or (2) the inclusion of a short time dressing.

Interestingly, a recent systematic review and meta-analysis by Sathorn et al. (181) excluded all but three clinical studies that met the standards for highest level of evidence. These investigators concluded that there was no statistically significant difference in the healing rate between one-visit and two-visit root canal therapy. However, conclusions should be taken with caution as there are few studies and sample size is still relatively small (only 146 cases all together).
Root canal preparation techniques

The cleaning and shaping of a root canal is the foundation of successful endodontic therapy. However, until now, no chemo-mechanical technique has shown the ability to clean and/or disinfect the root canal system completely.

In addition, the generalized concept of root canal enlargement up to the apical part for disinfection purpose is considered controversial. Some studies suggest that if a sufficient coronal taper is achieved it may not be necessary to remove dentine in the apical part of the root canal to allow satisfactory irrigation of the root canal system with antimicrobial agents such as NaOCl and EDTA (182).

The action of the file is to scrape the flutes against the canal walls to gouge a portion of the dentin and pull it form the canal. This action requires periodic cleaning of the instrument by the operator so the dentin shavings do not clog the flutes. Files are efficient removers of tooth structure because the multiplicity of cutting edges and may be adequately used in canal preparation.

Hand instruments combined with the most effective sodium hypochlorite irrigation can hardly attain a thoroughly clean surface without any smear layer (183).

So, in the past 15 years a plethora of new file system designs have been introduced for preparation manly of curved canals. As usual, with most products, several of these have been very useful and efficacious, but others have been worthless, and some have been potentially detrimental. There have been three major areas of development for these systems: (1) increase in flexibility by changes in file designs, (2) increase in flexibility by changes in file metals, and (3) files that do not zip because of flute removal or modified tips.

Siqueira and colleagues studied five methods of instrumentation, including activation of irrigants with ultrasonic devices, and found that no method achieved complete debridement. Other studies have also shown the inability to debride root canals predictably and completely despite use of several methodologies, including various combinations of sonics, ultrasonics, irrigation and instrumentation techniques (184-187).
The role of different lasers in Endodontics

Until the present practitioners have lacked a simple and effective way of cleaning and disinfecting the entire root canal system, including lateral dentinal tubules, accessory canals and anastomoses. The aim to achieve a sterile ambient that could be tridimensional filled by a biocompatible material without major complications or risks, has lead to several laser-related investigations in order to assess possible advantages when compared with either traditional disinfection methods or other promising therapies that may be currently under investigation (e.g. ozone, photodynamic therapies).

Conventional procedures using mechanical tools and toxic disinfection agents usually present several disadvantages. Firstly, the complexity of the root canal system makes debris removal and effective disinfection impossible to achieve. More than 30% of the canal’s surface area generally remains covered by a smear layer, protecting bacteria in the dentinal tubules from intra-canal disinfection agents. Moreover, the local microenvironment of the root canal system favors the selection of a few bacterial species that can survive and proliferate where they are out of reach of the host’s immune system response (188-190).

Conventional rinsing solutions and intra-canal medications have shown a limited antibacterial spectrum and a limited ability to diffuse into the dentinal tubules, affecting bacteria only partially, a fact which is attributed to treatment failures or relapses (191).

Mainly because of such limitations (including the imprecise or slim chances of successfully treating necrotic/infected teeth), the use of lasers for endodontic applications has been extensively reported and increasing in impact for the last 15 years. As laser-assisted treatments have been demonstrating several advantages over conventional methods, its noninvasive approach - which is always more agreeable for the patient - can be considered crucial or preferably to surgical interventions (192-194).

In conformity, the rapid development of laser technology as well as a better understanding of laser interaction with biological tissues has also broadened the spectrum of possible laser applications in endodontics. The development of new delivery systems, including thin and flexible fibers as well as newly designed endodontic tips, have enabled the use of this technology in almost all range of endodontic procedures.

Relevant literature suggests that some laser wavelengths are, indeed, an effective tool for the removal of debris, the smear layer and obturation materials, as well as providing effective disinfection. Gutknecht et al. (1996) found that few of problematic endodontic cases (21%) were successfully treated with conventional methods, without laser support. On the other hand, 82% unsuccessful conventionally treated cases (either using corticoids, different
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...rinsing solutions and preparation techniques including re-treatments), were successfully treated with laser-assisted therapy; a 1064nm Nd:YAG laser was used and this was one of the pioneer clinical studies addressing the potential use of near-infrared lasers to assist root canal treatments (195).

Based on the specific bactericidal effect proven by clinical experience and follow-up results, laser procedures have been integrated, step-by-step, into conventional endodontic procedures in order to improve conventional therapy outcomes.

In fact, as a result of the accumulation of pre-clinical studies addressing laser supported endodontic treatments, numerous laser wavelengths have been tested and distinct laser protocols have been successfully reported.

It is well known that during canal preparation instrument fractures can occur increasing the possibility of an endodontic failure. This risk is dependent of numerous factors including those related to the tooth anatomy (e.g. canal curvatures, permeability) operator experience, files material (e.g. stainless steel, Ni-Ti...), type of irrigants or lubricant materials, technique selection (e.g. Roanne, crown-down, step-back) and other several circumstances (196-198). However, the breakage of an optical fiber seems to be extremely rare, and most interestingly it does not necessarily lead to treatment failure as it can be easily removed or by-passed.

Another positive contribution of laser irradiation is the capacity, accordingly to the wavelength and tip configuration, to disinfect strongly curved root canals and those susceptible to small enlargement. Conventionally used irrigation solutions have limited effects on such small lumina; this represents a particularly important limitation specially when addressing the disinfection of the apical third. Due to either absorption or transmission properties in dentin, laser energy was found to be still effective in deep dentine layers adjacent to the canal lumen as well as in periapical regions (199-202).

Another important area to address is the patient acceptance of laser therapy, considering both personal and psychological factors. In practice, little or no additional time is needed for the laser assisted treatment. Furthermore, the positive prognosis the medium/long term post-treatment periods combined with the possibility of saving the tooth have been contributing to increase patient’s acceptance.

As in most cases, there are indications and limitations for the use of a laser assisted treatment. Lasers may be specially recommended in the following situations: teeth with purulent pulpitis or pulp necrosis, with periapical lesions (from 1mm up to 5mm granulomas),...
abscesses, with lateral canals, with reabsorption of the apex caused by inflammation or trauma and for teeth retreatments with low prognosis of success.

However, there are other factors that could exclude laser therapy as a treatment option, such as: the consideration of whether it is worth conserving the tooth for functional/aesthetic reasons, whether its crown has been destroyed or has root caries, and whether the patient’s acute symptoms and health condition might delay the endodontic treatment to be performed. Further serious contra-indications include the case of advanced periodontitis, deep crown or root fracture, and when preliminary procedures have confirmed the existence of obliterated root canals.

So, although the interest for the clinical applications of laser systems for root canal treatments is increasing, some concerns about their use remain, specifically, the lack of well-designed clinical studies clearly demonstrating the advantage of lasers over current conventional methods and techniques.

In fact, until the present date, few clinical studies were reported within this field. However, clinical reports and in-vitro investigations have supported the safety of laser assisted protocols and have provided verification over time, leading to preliminary conclusions regarding the potential benefits and long-term outcomes after such treatments.
Different Lasers for Endodontic Treatments

Lasers assisting root canal disinfection

In various laser systems used in dentistry, the emitted energy can be delivered into the root canal system by a thin optical fiber (Nd:YAG, KTP-Nd:YAG, Diodes, etc.) or by a hollow tube (CO2 and Er:YAG). Thus, the potential bactericidal effect of laser irradiation can be effectively used for additional cleaning and disinfection of the root canal system following biomechanical instrumentation. These effects have been extensively studied over the years using lasers such as CO2 (203, 204), KTP-Nd:YAG (205), Excimer (206), Diode (207), Er:YAG (208, 209) and Nd:YAG (195, 210, 211).

Considering that the primary use of lasers in endodontics is focused on eradicating of micro-organisms within the root canal, namely in the lateral dentinal tubules, it may prove necessary to adopt a wavelength easily transmittable through the dentine. Therefore, the transmission/absorption coefficients in hydroxyapatite and/or water may be considered as the basic wavelength-selection principle.

Since the early 1980s, several studies have been published regarding the impact of different laser systems on the root canal and the surrounding dentin. The carbon dioxide (CO2) laser, with a wavelength of 10.600nm, has long been used in oral surgery and, in 1986, Zakariasen and colleagues showed for the first time that this wavelength could be used in endodontics due to its good bactericidal effects (203).

In 1997, Moritz et al. aimed and achieved a partial closure of dentinal tubules also using the CO2 laser on root canal surfaces. Nevertheless, owing to the fact that the emitted far-infrared wavelength can be transmitted into the root canal exclusively by using a hollow, rigid wave guide, the canal lumen must be greatly enlarged and the laser could only be used in straight root canals (212).

An in-vitro study done by Pini and colleagues focused on the use of the xenon chloride (XeCl) excimer laser, which emits ultraviolet radiation at 308nm. This ultraviolet wavelength satisfactorily removed hard tissues but also shown interesting bactericidal effects and few thermal side effects. However, the technical requirements were tremendous and the use of the XeCl excimer laser remains restricted primarily to basic research (213).

Moshonov et al. demonstrated in turn the efficacy of the Argon laser in removing intracanal debris by means of computerized scanning electron microscopy (214), whereas
Blankenau and colleagues illustrated this procedure’s safety regarding the temperature rise at the root surface when using power settings of 1 and 2 watts (215).

The neodymium:yttrium-aluminum-garnet (Nd:YAG) laser, which emits a wavelength of 1064nm can be considered the most widely used laser in endodontics. Owing to its wavelength at the near infrared spectrum, flexible conductors (i.e. thin optical fibers) can be used in narrow and curved root canals. The pulsed Nd:YAG laser systems were, until the present, considered to be the first choice for deep root canal disinfection. This wavelength has shown the best measures in dentin transmission and consequently the best bacterial reductions within the deep dentin. In fact, even at penetration depths exceeding 1000µm, a mean of 85% germ reduction was accomplished (195, 216).

Rooney et al. in 1994, described reductions of 99% using a Nd:YAG laser in different experimental designs and bacterial combinations (217). Also interesting are the results reported by Gutknecht et al. (1996) with Nd:YAG laser on infected root canals. The average of 99.92% bacterial reduction (Enterococcus faecalis) with standard settings of 15Hz/1.5W (100mj per pulse), repeating the irradiation cycles of 5-8seconds, four times (195) may be considered a remarkable finding.

The 810nm diode laser can be comparable to the Nd:YAG one in terms of effectiveness and is considered to represent the second best choice due to its comparable bactericidal capabilities. Micro-biological studies have shown that this wavelength can achieve the second highest germ reduction at 1000µm in depth, with a mean of 63% (207).

The 980nm diode laser is often reported as a suitable wavelength for endodontic use. Although this wavelength presents high transmission in hydroxyapatite, the energy transmission within the root canal system is compromised due to a higher absorption peak in water if compared either with the 810nm, 940nm or 1964nm lasers (218). Therefore, it might seem logical that at 500µm the mean CFU (Colony Forming Units) reduction in several studies varied between 57% to 86% depending on the energy applied during irradiation (202). Its higher absorption coefficient in water also helped to explain why this laser wavelength could only achieve a mean of 30 to 40% germ reduction at a depth of 1000µm (202, 219).

The apparent consensus is that laser irradiation emitted from distinct laser systems used in dentistry has bactericidal potential and their efficiency is demonstrated in areas that were formerly inaccessible (dentin tubuli). In most cases, their efficiency is directly related to the energy outputs, mode of operation and adopted protocols (211).
Lasers ability to remove debris & smear-layer

Numerous studies have documented the different abilities of lasers such as CO₂ (220, 221), Nd:YAG (220, 222, 223), Argon (210, 220), Er:YAG (224, 225) and Er,Cr:YSGG (226) wavelengths to remove debris and smear-layer from the root canal walls following biomechanical instrumentation.

Gutknecht (1991) found that the smear-layer could be completely removed and the dentinal tubules were, for the most part, closed through the inorganic melting when the Nd:YAG laser was applied with 15Hz/1.5W settings. Similar results were found with the 810nm diode laser under similar irradiation protocols (50, 227).

The doubled frequency Nd:YAG laser (often known as KTP laser) was also reported as effective to remove the smear-layer and debris from previously instrumented root canals. However, few studies could attest such capacity (228).

The wavelengths of both Er:YAG and Er,Cr:YSGG lasers show absorption coefficients in hydroxyapatite and water so high that germ reduction would theoretically take place predominantly in the main canal(s). Therefore, these lasers were initially found to be useful only to remove organic tissue and smear-layer. However, several researches such as the one conducted by Franzen et al. reported that, through light penetration into dentinal tubules that act as light optical conductors, Erbium lasers could still be considered efficient up to depths of 300-500µm (201).

In fact, following the adjunctive application of such Erbium laser systems, the smear-layer is effectively removed, widely exposing dentinal tubules. However, some features potentially associated with the intra-canal use of such infrared lasers should not be overlooked...

Blanken et al. (229, 230) showed that, with 75mJ pulse energy, the Er,Cr:YSGG laser fiber remaining stationary 5mm far from the apex, with six irradiation cycles of 5 seconds each or, alternatively, being removed out from the canal in five cycles of 5 seconds each the treatment managed to remove all the colored water from an artificial root canal model. In addition, while activating 2.5% NaOCl, the same authors showed that - with the 200µm endodontic bare fiber (Z2) - four cycles of 5 seconds each, the cavitation effects were sufficient to remove large dentin plugs without damaging or widening the unprepared last millimeter (apical constriction) (231). It was also one demonstrated that laser activation of irrigants with the Er,Cr:YSGG laser is more effective at removing artificially placed dentin debris from a root
canal groove than conventional hand irrigation with 2.5% NaOCl or Passive Ultrasonic Irrigation (with *Irrisafe®* tip) when the irrigant is activated for 20 seconds.

These findings stimulated new perspectives for cleaning and detaching smear plugs or smear layer in those areas that usually cannot be reached by root canal instruments and/or insufficiently accomplished by irrigation solutions’ application. Interesting and relevant endodontic features associated to the adjunctive application of infrared lasers such as Er, Cr:YSGG laser can be found in the “Er, Cr:YSGG laser in Endodontics” chapter.

**Lasers’ Effects on Adhesion and Leakage of Endodontic Sealers**

The necessity of complete obturation of the root canal system though a proper interface between obturation material and root canal surface, is still topic of controversy and scientific debate. Sabeti *et al.* (232), for instance, questioned its importance and reported no difference in apical periodontitis healing when root canals were left without obturation, after cleaning and shaping procedures. Other authors, however, have argued for the importance of filling root canals with thermoplastized gutta-percha and sealer, as such procedures may statistically influence endodontic outcomes (233, 234).

Although disinfection of the root canal system could be unanimously considered to be the most important factor in root canal therapy, the importance of the obturation should not be overlooked in the attempt to maintain the root canal free of contamination, avoiding re-colonization and increasing the chances of long-term clinical success (235).

Endodontic sealers are intended to adhere to the root canal walls, promoting the interface and union of gutta-percha to these walls, and to seal the space between the gutta-percha cones (236).

Several authors have pointed out the need for properties that an ideal root canal sealer must have to provide adequate adhesion (237-239) as possible leakage between the interface of dentin wall and the root canal filling material can lead to recurrent contamination and consequent periapical disease (240, 241). As researchers and lecturers, we find that, although not critical for the initial success of the endodontic therapy, the sealer detachment and posterior leakage may easily compromise long-term outcomes in cases of coronal restoration or rehabilitation failure that are, unfortunately, frequent.

According to White *et al.* the smear layer may play a negative role in root canal sealing due to the layer of organic/inorganic debris that forms an interface between the sealer, the root canal walls and dentinal tubules reducing the material’s bond strength to dentin (242).
In adjunct to commonly used irrigation substances to promote disinfection, distinct surface treatments have been investigated for smear layer removal, such as laser irradiation. If with Nd:YAG and Diode Lasers no promising results were achieved, under restricted parameters and irradiation protocols, several studies have demonstrated that the applicability of Erbium lasers for cleaning debris and smear layer could be effectively achieved, leaving open dentinal tubules without any reported hazardous effects (243-248).

A review of the literature shows that Erbium lasers, namely the Er,Cr:YSGG laser, are capable not only to remove smear layer but also of literally exposing lateral dentinal tubules. They can also promote positive morphologic changes such as the formation of irregularities, the increase of dentin area, the liquefaction of hydroxyapatite crystals and the alteration of the carbonate amount in dentin, which could improve some sealers’ adhesion (in “Er,Cr:YSGG laser in Endodontics” chapter).
Limitations Associated to Laser Tips

The morphology of root canal systems can present great challenges to the practitioner as mechanically prepared canals usually have regions inaccessible to endodontic instruments (such as accessory canals, anastomoses, and fins). Furthermore, bacteria are capable to invade dentinal tubules, resulting in persistent infections due to limited penetration of irrigation solutions such as sodium hypochlorite (35, 62, 249). In fact, it is commonly accepted that most conventional antibacterial agents may not affect microorganisms within the deeper dentin layers, due to poor penetration ability (26).

However, owing to the adjustable penetration depth according to each wavelength, laser irradiation can reach further into the complex and inaccessible regions of the root canal system.

Nowadays, a wide variety of highly scientific and technically refined dental laser systems are available with a broad spectrum of wavelengths, pulse widths, and optical fibers for distinct applications. These innovations have enabled their applicability in endodontics and all endo-related procedures including direct pulp capping, drying of the canal, canal disinfection and canal wall modifications as shaping and preparation (250). Yet, there are still restrictions that may be found regarding the intra-canal use of lasers that cannot be disregarded and seen as laser-limitations (251).

In fact, topics such as laser disinfection, preparation and shaping of the root canal have always been subjects of controversy and discussion, ever since their introduction in the early 1990s (252, 253).

The emission of laser energy from the tip of the optical fiber or the laser-guide is usually directed along the root canal and not necessarily laterally to the canal walls. Thus, with plain fibers, it was found almost impossible to obtain uniform coverage of the canal surface using a laser source (254).

Optical fibers in endodontics need to be small and flexible in order to negotiate the complex, curved and tortuous anatomy of the root canal but existing optical fibers are less flexible than the Ni-Ti instruments. Moreover, optical fibers usually have bare tips so the energy is transmitted forward with a relatively small divergence (collimation is, in fact, a laser property). This limitation often requires the clinician to move the fiber in a plunging, withdrawing and rotating action in order to attempt a uniform coverage of the root canal walls.

The direct emission of the laser from the tip of the optical fiber near the root end may result in the transmission of the irradiation beyond the apical foramen and lead to undesirable
effects on the supporting tissues of the tooth, a fact which could harm either teeth in close proximity to the mental foramen or the mandibular nerve (255).

Bahcall et al. were the first to study the effects of (Nd:YAG) laser irradiation upon the periapical tissues in dog experiments. Despite the questionable protocol, their results indicated that laser treatment might cause cell necrosis in the periodontal ligament 1 day after treatment. Irradiated teeth could also suffer later ankylosis, since lyses of the root cement and signs of bone resorption were observed within 30 days (256). Nevertheless, it should be pointed out that the power settings used were inordinately high and the absorption of this particular wavelength in dentin is very low when compared to Erbium lasers, for instance.

Matsumoto’s review “Lasers in endodontics”, also addressed some possible limitations to the use of lasers in the root canal system, including the fact that the “removal of smear layer and debris by laser is possible (...) however it is difficult to clean all root canal walls, because the laser is emitted straight ahead, making it almost impossible to irradiate lateral canal walls”. More recent calls have been made for the need to improve endodontic tips in order to allow the tridimensional irradiation of the root canal walls (252).

Although they are believed to be athermal in nature, bactericidal effects were attributed to instant evaporation of intracellular water, bacterial turgescence, thermal necrosis, and bacterial dehydration (208). However, limitations regarding the intracanal use of straight forward emitting tips and also some wavelengths (e.g. 10600nm CO2 laser) were identified and highlighted in the literature (257).

Erbium lasers, such as the Er,Cr:YSGG laser, now have thin and flexible endodontic fiber tips of various diameters and lengths that allow to be properly inserted inside root canals, independently of their curvatures (258).

As stated previously, the emission of laser energy from the tip of the optical fiber or the laser guide is in most cases directed along the root canal. Ideally, lateral emission would cover tri-dimensionally the root canal walls and penetrate directly into the dentinal tubules (259). Therefore, it is proposed that the laser fiber should be moved repeatedly in a spiraling motion along the root canal walls in order to maximize the area exposed to the laser beam. Nevertheless, and despite good bactericidal results were obtained by several investigators, with straight forward emitting tips it was found almost impossible to obtain uniform coverage of the canal surface, leading to the inconsistency of results.

In addition, there is also the potential risk of thermal damage to surrounding structures in the event of misuse of lasers or if adopting an erroneous protocol (252).
Direct emission of laser irradiation from the tip of the optical fiber may also result in energy transmission beyond the apical foramen, being potentially hazardous to periapical tissues.

Extensive research has been devoted to overcome such limitations and alternative approaches (using radial/side-firing tips) are now under extensive investigation, being approved for clinical use.

The use of high pressure air and water spray instruments together with lasers could cause clinical complications such as emphysemas, which are related to the presence of air within the soft tissue; such adverse effects may be detected by the occurrence of crepitation upon palpation (260). In other words, greater danger exists for apical pressure with water escape for large canal diameters. However, results obtained by Ishizaki et al. reported that lasers may be safely used with fibers up to 400µm in diameter on canals instrumented up to ISO#45 (261).

Furthermore, care must be taken when using the bare fibers in the root canal, as apical extrusion of irrigants after laser activation has been described even while using sub-ablative settings. George et al. (262) showed that there was twice dye extrusion beyond the apical constriction if the fiber tip is placed at 4mm than at 5mm from the apical stop.

In in-vitro studies temperature elevation assessment’s might not represent true clinical conditions as, in healthy periodontal tissues, the circulating blood and periodontal structures help to dissipate much of the heat (263). For this reason, in-vitro models to measure temperature changes probably only approximate - in excess - those seen in clinical trials (264).

Regarding the assessment of temperature changes on radicular thirds, the apical third is generally found more susceptible due to the fact that dentine acts as a thermal isolator; thus, less dentine thickness would allow more heat transmission (225, 264, 265).

Morphologically speaking, although the achievable ablated surface qualities can be distinct, it has already been shown that the use of the use of lasers, namely the Er,Cr:YSGG laser, enables the removal of intra-canal dentine while maintaining that of surrounding tissues (266, 267).

The risk of creating ledges from the use of erbium lasers at ablative settings has been reported during the attempts of performing root canal preparation (268). Hence, the use of lasers for shaping procedures using currently available fibers is, until the present, hypothecated.

However, with the Er,Cr:YSGG laser, no significant occurrence of ledges up to a canal curvature of <10° has been found (269) and undesirable side-effects such as dentin
carbonization are moderate; this laser treatment has, therefore, been shown to be safe (270, 271).

Minas et al. stated that due to the small laser beam angulation obtained during the movement of plain endodontic tips inside the canal lumen, and the axial beam distribution pattern incorporated in the tested tip design (Z2, Z3 and Z4), the specific tip energy density seems to have a minor effect on canal dentine surface alteration. More pronounced morphological changes were found by increasing the tip diameter, mainly owing to the higher average power, offered by larger tips. These results showed that all bare tips must be placed by at least 2mm short of the estimated working length, if the apical root region is to be preserved (272).

Regarding root canal preparation to date, there is still insufficient information about the choice of the optimum canal preparation parameters that will lead to the desired clinical treatment goals without causing iatrogenic damage to the root canal system and the surrounding tissue (269, 271, 273).

In conclusion, although lasers are currently acceptable either as an alternative or adjunct to conventional root canal therapies (laser assisted endodontic treatment concepts) and for endodontic surgery (i.e. incision, bone cutting, granulation tissue removal, disinfection, apicectomy and retrograde preparation), it does not seem reasonable to rely totally on lasers for complete root canal treatments due to the incapacity of performing root canal preparation without risks of ledging, zipping or perforating (271, 273, 274).

As more conservative approaches for root canal preparation have been proposed to prevent extensive substance loss and preserve the original shape of the canal, the range of available endodontic equipment has broadened. Thus, the alteration in tip design to ensure an increased annular profile of side firing beam inside the canal is thought to be mandatory in order to produce a more uniform and homogeneous ablation pattern that could improve the final outcome.

However, none of these studies have yet used Radial Firing Tips that would distribute homogeneous irradiation along the root canal walls. Further studies should address this specific limitation, increasing the acceptance for such investment.
Erbium Lasers

Erbium doped YAG (Er:YAG) laser and Erbium, Chromium doped YSGG (Er,Cr:YSGG) laser

The wavelength emission of this kind of laser systems ranges from 2.6µm to 3µm and in this spectrum region the absorption coefficient is perfectly matched to the water molecules and hydroxyl (OH⁻) groups, often found as components of dental tissues (275). This particular characteristic supports the fact that, since 1988, Erbium lasers have been the most used lasers in dentistry for hard tissue ablation such as cavity preparation (276-278).

Like neodymium, erbium also belongs to the group of rare earth elements (lanthanides) that is embedded in a host crystal. In general, erbium lasers are excited by flash lamps, and the actual laser process takes place in the erbium ion (Er³⁺). The two host crystals (doped by erbium or erbium and chromium) consist of Yttrium-Aluminium-Garnet (YAG, Y₃Al₅O₁₂) and Yttrium-Scandium-Gallium-Garnet (YSGG, Y₃Sc₂Ga₃O₁₂) and they are used to generate similar but distinct wavelengths (Figure 4).

Therefore, these are the two Erbium laser systems which are currently prominent in conservative dentistry: the Erbium:YAG laser emitting light at exactly 2940nm (279) and the Erbium, Chromium:YSGG laser, which emits light at 2780nm (280, 281).

![Figure 4: Simplified Erbium laser set-up configuration [adapted from (282)].](image)

Neither erbium laser (flash pumped) can run in continuous-wave mode due to the long lifetime of the lower laser level. However, in pulsed mode, Erbium lasers can be operated at average powers of 30W up to a pulse repetition rate of 50Hz, reaching pulse energies of 1000mJ.

For the removal of hard dental tissue, these lasers act through photoablation since their wavelengths correlate closely with the absorption maximum of hydroxyapatite and water. The absorption coefficients (µa) for these dental constituents range from 5000cm⁻¹ to 13000cm⁻¹, depending on the wavelength and tissue composition (275). Photoablation, however, occurs when irradiated water contained in the hard dental tissue evaporates,
promoting the instantaneous ablation of the surrounding tissues with minimal thermal/side effects (277).

However, a close observation of the absorption peak associated with Erbium lasers shows a 300% difference between the absorption coefficients between the Er,Cr:YSGG (4000 cm⁻¹) and Er:YAG (12000 cm⁻¹) wavelengths in water (Figure 5). As a direct consequence the Er:YAG laser wavelength is able to penetrate around 7µm in enamel, and 5µm in dentine. The Er,Cr:YSGG laser wavelength however, can penetrate more deeply, 21µm in enamel, and 15µm in dentine.

Although being used primarily for the preparation of dental hard substances, Erbium lasers can also be used for endodontic purposes. In 1997, Hibst and colleagues first proposed that Er:YAG lasers can be used in endodontics (283); later studies performed by Schoop and colleagues, confirmed Er:YAG laser’s findings (209).

If Er:YAG laser is thought to be more efficient for hard tissue ablation due to it’s higher absorption in water, the Er,Cr:YSGG laser is able to penetrate further into the dentin. This penetration ability plays a crucial role for endodontic disinfection purposes as it allows deeper penetration into the dentinal tubules. Then, the development of superior, thin and light-conductive materials was the key factor in allowing the irradiation of narrow and curved root canals.

Primordially, researchers were only focused on caries removal and cavity preparation using the Er,Cr:YSGG laser. However, Yamazaki et al. and Kimura et al. described positive morphological changes encountered while irradiating root canal walls and endodontic applications for such wavelengths were then increasingly developed (226, 284). The literature review regarding the applications of the Er,Cr:YSGG laser in endodontics can be found on the following chapter.

![Absorption curve of water in the middle infrared region](adapted from (282)).
The Er,Cr:YSGG Laser
(WATERLASE® MD, Biolase Technology, San Clemente, CA, USA)

While lasers have been involved in dentistry for more than twenty years, until the recognition of the capacity of the 2.78µm wavelength, no other single-wavelength laser had been cleared for use with all oral tissues, including hard tissue, soft tissue, endodontics, periodontology and bone management. In fact, the introduction of the Er,Cr:YSGG laser pierced that limitation, being the first laser to obtain commercial clearance for use in all oral tissues.

After receiving clearance for hard tissue applications, researchers began to work on soft tissue with the YSGG laser (Millenium®, Biolase CA, USA). With the water spray minimized or turned off, this laser could effectively cut and coagulate soft tissue with more control and, in many cases, more quickly with improved wound healing results. By 2000, the FDA had expanded clearances for soft tissue indications and clinicians were able to work on all oral tissues and on patients of all ages.

In the same year, BIOLASE released a second version of the YSGG laser (the Waterlase®) and the Waterlase MD® in 2004 (Figure 6). Soon the FDA would go on to issue ground-breaking clearances for laser in endodontics (2002), endodontic surgery (2002), cutting and ablation of oral osseous tissues (2003), as well as procedures related to periodontal therapy, including laser curettage and osseous crown lengthening (2004).

The Waterlase MD® uses an Erbium, Chromium: Yttrium, scandium, gallium, garnet laser-powered hydrokinetic system (HKS). The HKS debridement is the process of removing organic and inorganic materials through the use of high-speed water spray, and is known to produce minimal plume and almost no increase of pulp temperature during cavity preparation (285, 286).

Figure 6: Waterlase MD®, Biolase CA, USA.
According to Dederich et al. in 2004 February’s edition of the Journal of the American Dental Association, the advent of the Er,Cr:YSGG laser represents the pinnacle of the dental laser revolution and could be considered the “most important recent development in laser dentistry” (287).

Although lasers have not yet replaced all conventional protocols such as endodontic instrumentation, the clinical applications of the 2.78µm wavelength have clearly demonstrated that no other instrument has the versatility and clinical utility of the Er,Cr:YSGG laser for dentistry.
Light Transmission System(s)

It is essential to point out, first of all, that light transmission systems are selected on the basis of the wavelength, the pulse energy and the power to be transmitted. However, the tip design and diameter, usually vary according to the desired clinical application (288).

Quartz glass optical fibers with diameters of 150-1000µm can be used for the near UV to near IR range (300-2500nm). This kind of fiber is unsuitable for bigger wavelengths, however, because they contain OH- groups that absorb 3000nm wavelength. That is why other materials, such as fluoride or oxide compounds, are necessary fiber materials for the 3000nm emission range.

Articulated mirror-arms are used to transmit high pulse energies and powers in the mid-infrared region (2000-12000nm). These are simple hollow tubes with mirrors positioned in the joints for deflection. They are used in Erbium and CO2 lasers, as well as in picosecond and femtosecond lasers.

In order to guarantee optimum light transmission, the inside wall of these hollow waveguides are specially coated for the particular wavelength to be transmitted. In fact, these hollow waveguides can only be used for specific laser devices. For CO2 lasers, such waveguides are now capable of transmitting up to 25W (289).

In order to better understand the laser-tissue interaction mechanism, we must also be aware how light behaves after leaving the optical fiber. It normally emerges from the end of the fiber in a conical fashion, with a Gaussian beam-like pattern (290, 291). Based on the irradiated area, it is then possible to determine the energy or power densities per pulse that are responsible for the interaction of the light with the tissue.
Tapered Fibers and the Radial Firing Tip (RFT)

In addition to using a lens for beam focusing, high intensities of laser light can be obtained by guiding the beam through the tapered end of a fiber or a tapered rod (292). As the cross-sectional area of the rod decreases, the light intensity increases until the beam starts to refract out of the tapered tip at a highly divergent angle. In contact with tissue, the high power density of the light at the point of the tapered end initiates tissue ablation. Because of the large divergence angle of the exit beam, the irradiance decreases rapidly distal from the tip, minimizing possible injuries to adjacent tissue (293).

The geometry of the tapered fibers and laser scalpels, composed of either silica or sapphire, can be defined by the taper angle (α) and the entrance diameter. The taper angle may vary from 1 to 45 degrees. The taper may end in a point, hemisphere, or may be flat, resulting in different beam profiles (294).

Optical characteristics of tapered fibers: The optical behavior of tapered fibers can be simulated with ray-tracing software. A ray reflects several times within the tip before its terminal angle. With respect to the tip surface, it exceeds the angle of total reflection (Figure 7). Then the ray refracts out of the tip (or scalpel). It is then reflected up and down inside a taper point until the angle of incidence exceeds the critical angle and the ray is refracted out of the tip – so called “leaking” point. Thus, the “leaking length” can be defined as the distance between the positions where rays start to refract out at the end point of the taper.

![Figure 7: Ray-traced beam profile for a 10° taper. (Left: ray trace 10° taper. Right: power density distribution) [adapted from (1)].](image-url)
The angle and the position at which a ray is refracted out, or leaks, changes in discrete steps, depending on the taper angle and angle of the ray in the fiber (295).

Figure 8: The “leaking” length of tapered fiber normalized to taper length in relation to the taper angle in air and water [adapted from (1)].

The steps are related to the number of reflections inside the tip. A small decrease in the taper angle can result in an additional internal reflection of the ray (Figure 8). The ray will then refract out of the tip - or scalpel - on the opposite side and further toward the tip. The same will happen between two rays with slightly differing starting angles. Consequently, rays are emitted out of the tip in conically shaped beams at discrete angles. The angle of a cone depends on the position and angle with the optical axis of the rays at the beginning of the taper. The discrete cones dilute when the taper ends in a hemispherical tip (295).

Figure 9: Small decrease in the angle of the taper point may result in an additional reflection of the ray; decreasing the angle of a ray with the optical axis may result in a similar effect [adapted from (1)].

For taper angles as small as 5°, a power density increase of over 300 times can be achieved at the entrance of a tapered tip. However, the practical use of thin such tip (scalpel) - with a very small taper angle - will be limited by its lack of mechanical strength.
Analogous to ball-shaped fibers, tapered fibers can be prepared from a bare tip using several methods, such as heat drawing, etching or polishing, depending on the optical properties needed and the desired tissue effect. The Radial Firing Tip for endodontic use is one of them and the manufacturing process for such fiber profiles can be found described below.
Endodontic Radial Firing Tip: concept and state of art

Generally, the key factor in endodontic therapy is the disinfection of the main root canal and the three-dimensional network of dentinal tubules. Moreover, as the infected pulp tissue and bacteria penetrate into deeper layers of root dentine, it becomes clear that some cases can present therapy resistances from the beginning. In other perspective, in the presence of bacterial persistence, results can end-up as long-term failures (296, 297).

Distant areas of the tubular system are the biggest challenge in current endodontic treatment and they are of fundamental importance for achieving prolonged preservation of endodontically treated teeth.

Dental lasers aim to provide greater access to formerly unreachable parts of the tubular network, due to better penetration of specific wavelengths into dentinal tissues (216, 298, 299). However, most of these devices have commonly reported disadvantages: (1) most of the laser energy is directed only in the axial direction, and little energy can be obtained perpendicular to the fiber; and (2) most of these lasers cannot eliminate the smear layer and the bacteria in the root canal wall, making the use of lasers less applicable (Figure 10).

Although primarily used for the preparation of hard dental tissues, Erbium wavelengths can also be effectively applied in the field of non-surgical endodontic therapy. Thus, the development of both thin and flexible fiber tips has enabled the irradiation of even narrow or bent root canals, thought to be unreachable until recently.

Figure 10: Z-2 standard tip emission profile in air (courtesy of Biolase™, CA, USA).
Despite the favorable results achieved with several wavelengths, findings regarding laser fiber tips still reveal the need for further improvements. Owing to total reflectance at the fiber walls, the laser beam can be expanded to a certain degree when leaving the end of the fiber tip. However, most laser light will be still propagated straight forward and, theoretically, towards the apex of the root.

By conducting the irradiation of the root canal in spiral movements and through a certain tilting of the fibers, one can minimize this effect to a certain extent, and direct sufficient energy density to the root canal walls. Nevertheless, after assessing the results obtained by such protocols, studies examining the removal of smear layer with conventional (forward emission) optical fibers often report a lack of consistency.

To overcome the above-mentioned drawbacks associated with the root canal enlargement, investigators such as Cohen et al. designed a tip for the Holmium:YAG laser, capable of transmitting energy with an angular ring shape. The idea arose from the assumption that conical-ended fibers would provide better debridement in the root canal when delivering energy from a water-absorbing middle infra-red laser (Er:YAG or Er,Cr:YSGG) into an aqueous irrigant, as the absorption of laser energy would induce shock waves into the irrigant.

Blanken et al., reported that an Er,Cr:YSGG laser used within the canal with a plain endodontic tip (Biolase Z4; Biolase Technology, Irvine, CA) could generate fluid movement at speeds up to 100km/h. With pressure waves causing such fluid movement, the possibility of accidental extrusion of fluid beyond the apical constriction must be considered.

Since then, innumerous investigators have been attempting to improve the shape and delivery properties of endodontic tips namely in erbium lasers; a number of modifications for optical fibers for medical use or industrial applications have been reported.

Interestingly, Shoji et al., first developed a cone-shaped irradiation tip for the Er:YAG laser, which transmits the laser beam annularly. It was meant to both enlarge the root canal effectively and provide a cleaner surface without smear layer, demonstrating as well that 30mj at 10Hz was the most efficient set of parameters for root canal preparation.

Stabholz and colleagues reported the development of a conceptual endodontic tip coupled to an Er:YAG laser system (Figure 11). The beam of this laser was delivered through a hollow metal tube, making it possible to develop an endodontic tip allowing lateral emission of the radiation (side-firing) rather than direct emission.
This new endodontic side-firing spiral tip (RCLase; Lumenis, Opus Dent, Israel) was designed to fit the shape and the volume of root canals prepared by Ni-Ti rotary instrumentation. It emits the Er:YAG laser irradiation laterally to the walls of the root canal through a spiral slit located along the tip.

![Figure 11: Side Firing Tip #60 prototype for Er:YAG laser (RCLase®; Lumenis, Opus Dent, Israel); Courtesy of Prof. Dr. Adam Stabholz (Dean of the Hebrew University’s, Faculty of Dental Medicine, Jerusalem).](image)

The tip is sealed at its far end, preventing the transmission of irradiation to and through the apical foramen of the tooth. Distal and palatal root canals of freshly extracted human molars following instrumentation with nickel-titanium files (ProTaper®; Dentsply, Tulsa Dental, Tulsa Oklahoma) up to size F3 were subsequently treated with the endodontic side-firing spiral tip to remove debris and smear layer; the efficacy was then examined. The lased roots were removed, split longitudinally, and submitted for SEM evaluation. The main canal walls revealed clean surfaces, free of smear layer and debris, with clearly distinguishable open dentinal tubules (259).

However such metal wave-guides and conical tips with slits for lateral emission have shown limited clinical interest in situations rather than in large and straight root canals because of their size and inherent rigidity.

It was later found that the use of flap fiber tips resulted in significantly higher temperature increase than the cone-shaped fiber tip and it was speculated that the latter might dissipate the energy homogeneously to the periphery, avoiding the concentration of the energy at one single focus point.

In industrial settings, either heating and/or pulling processes may modify fiber tips. The end of a fiber can be stripped from the buffer coating over a length of 10-15mm. The fiber is positioned vertically pointing down while a weight of several hundreds of grams is fixed to the tip. While heating the fiber (at a half away point along the stripped end) with a focal torch flame perpendicular to the fiber, gravity pulls the melting silica into a taper shape as the weight falls. The taper angle form heavily depends on the diameter of the fiber, the melted volume, and the pulling weight. The very tip of the tapered fiber will be irregular, but it can easily be shaped into a small hemisphere by melting or polishing to a flat surface (304, 305).
The end of optical fibers can also be modified by fixing certain materials, such as titanium dioxide, to the fiber end to disperse the energy. Such isotropic tips may be used in photodynamic therapy (i.e. photodynamic disinfection) in endodontics (306).

Conical ends can also be prepared from a bare tip by other methods like grinding and/or polishing (special equipment may produce a taper shape by polishing a fiber end positioned under an angle with the grinding surface while rotating the fiber) (266, 303, 307) or by etching with chemicals such as strong acids (308).

To etch the fiber tip, the stripped end of the fiber is positioned vertically in a solution such as hydroxyl fluoride (HF). This acid will dissolve a volume of silica linearly in time. By retracting the fiber end slowly and at a constant speed from the solvent, the diameter of the fiber will become smaller (tapered) toward the end since more silica has been etched away. Although this method is very controlled, caution should be taken since HF is highly toxic and must be carried out under a fume vent.

Indeed, this is a simple and inexpensive method of altering the tip to obtain greater lateral distribution of energy. Reported etching methods include static etching, dynamic etching and tube etching (309).

By way of example, an 84° degree top angled cone-shaped fiber tip delivered approximately 80% of the energy laterally and only 20% in a forward direction. Thereby, it proved to remove the smear layer and debris more efficiently and with fewer morphological changes than the bare one, because the former could transmit light directly to the canal wall (310).

George and colleagues recently reported the use of a simple tube etching method to create long conical-ended fibers with lateral emissions, specifically for endodontics (266). This method provided better fiber shape control than earlier methods such as polishing or heating and pulling (304, 311, 312).

To prepare modified conical fiber ends, batches of fibers were etched with a 50% hydrofluoric acid solution in a tube etching technique during either 90 or 160 minutes for Er:YAG and Er,Cr:YSGG fibers respectively. It was apparent that the laser improved the action of EDTAC and that the conical fiber design performed better than plain fibers when matched for the same laser system and the same irrigant for the smear layer removal. There was no statistical difference in performance between the 2 laser systems (262).

Another study by the same group of investigators, used not only Erbium lasers but also the Nd:YAG laser, to find that modifications to existing optical fibers using relatively simple chemical and physical methods improved the lateral emission of both visible and infrared laser
energy. While conventional fibers produced little divergence, etching of the fiber with hydrofluoric acid to create a conical tip increased this divergence more than 100° in all fiber types, producing a fan-shaped beam (Figure 12). These conical tips were though to be useful for disinfection applications in both root canals and periodontal pockets. However, to obtain a uniform effect the fiber would need to be moved (e.g. withdrawn in a coronal direction whilst lasing) at a constant speed.

Fiber ends modified through abrasion gave lateral emissions of visible red laser energy but not of longer wavelengths such as in the near and middle infrared regions.

Conical fiber modification using a simple etching protocol led to a variety of etching times according to the chemical composition of the fiber. Quartz glass fibers used with infrared lasers are typically doped with one or more metal oxides to increase the transmission of longer wavelengths (Danh, C.T., Bethesda, M. Infrared fiber systems Inc, Silver Spring, MD assignee. Heavy metal-oxide glass optical fibers for use in laser medical surgery and process of making. US patent 5796903. August 18, 1998.).

Biolitec™ and Biolase™ fibers, for example, are doped with fluoride, whereas Er:YAG (Kavo Key3®) fibers were doped with germanium. Previous studies reported that germanium-doped fibers etch more easily than plain silica fibers (313).

No tip damage was expected with low intensity applications such as photodynamic therapy, disinfection, or detection. However, conical tips did sustain minor damage at the distal 0.5mm of their terminus when higher laser pulse energies (e.g., for ablation) were used for more than 10 cycles; their optical performance was not apparently affected and re-etching for 5 minutes was even able to restore the geometry of the fiber tip (262).

The combination of etching and abrasion gave rise to novel “honeycomb” topography with multiple facets and with excellent lateral emissions for visible, near infrared, and middle infrared wavelengths. Such fibers would be useful for ablative applications where placement in a root canal would achieve a relatively even effect along the length of the modified zone, since the gradation in size of the fiber tip is not dissimilar of that of a root canal.

The emission profile of the honeycomb fiber ends may be of particular benefit for ablative applications in hard and soft tissues, compared to a conical tip, because it emits and collects light from both lateral and forward directions. Its higher lateral emissions should give a stronger signal from the bacteria on root canal walls, given the intensity of the irradiating light.

Due to the strong lateral emissions, such tips would be useful for both endodontic procedures such as the ablation of debris, smear layer and dentine from the root canal walls, and periodontal applications, such as the disinfection of periodontal pockets around teeth or dental implants (314).
Moreover, the tips of Er:YAG and Er, Cr:YSGG lasers generated photo-acoustic and ablative effects which removed thick smear-layers that had been created intentionally to provide a challenge for the laser system. This has provided the initial “proof of concept” needed for the clinical utility of lateral emitting fibers in endodontics (262).

Striving even more for the improvement of the established delivery systems, this new generation of fiber tips has been commercially developed, aiming for the unique property of homogeneous irradiation through the three dimensions of the root canal. The end of these radial-emitting tips shows a conical outline with a cone angle around 60°. Therefore, laser light is expanded to a broad cone, facilitating an even coverage of the whole root canal wall (Figure 14) (Figure 15)(315).

![Figure 12: RFT2 - 270µm Radial Firing Tip (quartz tip). (Illustration and photography gently provided by Biolase™, CA, USA)](image)
**Figure 13:** Radial firing tip cone angle 55° in air.
(Illustration provided by Biolase™, CA, USA)

**Figure 14:** 320µm Radial Firing Tip (RFT3) profile(s);
Authors’ property.
**Figure 15:** Radial Firing Tip in air - footprint on target at 1mm.

(Illustration gently provided by Biolase™, CA, USA)
Scaini et al. in turn reported that a temperature increment of over 10ºC can induce changes in the fibers of the periodontal ligament and even lead to their necrosis (316).

Cohen et al. were more precise in their assessment, since they pointed out that the probability of vitality loss affecting the cementoblasts, periodontal ligament and alveolar bone is practically insignificant in the case of root surface temperature increments of no more than 5ºC (317).

Quantification of thermal tissue effects of laser irradiation can be made by determining the corresponding temperature increment, for which some measurement options are available: Thermocouples, infrared pyrometry and infrared cameras. Most experiments published in literature have employed thermocouples, since the later are easy to use, inexpensive and highly reliable (318). On the other hand, thermographic studies have also been conducted using infrared cameras with similar results to those afforded by thermocouples (319).

In order to determine that no thermal damage occurs in pulsed Nd:YAG or diode lasers treatments, Behrens and Gutknecht conducted experiments on dentine slices with laser settings that took into account extreme situations. By measuring the temperature on the root surface, the maximum result of 38ºC was achieved after 45 second of irradiation at 15Hz/1.5W. This result turns it even more secure if we consider that this value lies within the physiological area, in which in an in-vivo situation the dental tissue is more efficiently cooled by the blood flow surrounding the root surface (227). Through the Finite Element Model technique the high temperatures in the apical area of the root can induce the kill of microorganisms harbored at the root branches. However the temperature rapidly decreases when the fiber-optical waveguide is moved into coronal direction which guarantees that surround tissues are only marginally affected and bone damage should not be expected.

In what concerns to erbium lasers, Hibst et al. and Keller et al. early reported and therefore recommended pulse energies of 50mj, with frequencies between 6-15Hz, in direct relation to the root thickness of the irradiated tooth (276, 277).
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Reporting findings while using an Er:YAG laser, which is the most similar system to the Er,Cr:YSGG laser, Theodoro et al. showed that an increment of only 2.2°C is obtained while applying 100µJ at 10Hz for a maximum of 30 seconds (320).

As expected, a previous report from Romero et al., stated significant differences that were recorded between different teeth with different root thickness, since lower incisors logically showed greater temperature increments than those results obtained while irradiating thicker teeth roots as in canines (321).

Regarding the Er,Cr:YSGG, the temperature rise during laser irradiation found to be minimal, not causing any damage to periradicular bone or adjacent tissues (261).

Another relevant in vitro study with incisors and canines was performed in order to simulate the most extreme working conditions using this wavelength and the glass fiber tip specifically developed for endodontic practice (200µm diameter and 28mm length). No water spray or air was used and the root canal interior was always dry. The laser was activated at a power setting of either 1 or 2W, 140µs pulse duration, 20Hz, 1mm far from the apex (working length), and subsequently moving coronal at a speed of approximately 2mm per second. This movement was made in circular manner and repeatedly for 30 seconds. All conditions were taken in order to simulate the intraoral environment, and has been reported as both effective and safe methodology for endodontic disinfection purposes (318).

In one study performed by Gay-Escoda et al. with the Er,Cr:YSGG laser, 200µm tips were used, implying a power loss of approximately 70% (calibration factor). Accordingly, on irradiating at 1W, the effective power setting was actually 0.3W. Since the 20Hz frequency was involved in all cases, the effective working energy was 25µJ p.p. – i.e. similar to the energy delivered by the Er:YAG laser in previous reports. In result, the irradiation of the root canal with the Er,Cr:YSGG laser using 200µm tips at a power setting of either 1W or 2W for 30 seconds, with continuous circular movements of about 2mm.s⁻¹., induced a statistically significant but clinically irrelevant temperature increment at the external surface of the root canal. Their results suggested that the average energy needed for endodontic purposes would never be sufficient to damage neighboring tissues (e.g. periodontal ligament and supporting alveolar bone) (322).

Another experimental work from the same research team, Arnabat and colleagues found that the range of temperature increases showed that the use of the Er,Cr:YSGG laser to disinfect root canals will not cause tissue damage in the regions surrounding the teeth. Temperatures values increased around 3°C in an environment recreating the oral cavity -
which is often 3°C cooler than the normal physiological temperature - leading to surface root temperatures near 37°C (323).

In a broader perspective, the Er,Cr:YSGG laser system uses the hydrokinetic energy (HKS-hydrokinetic system) in which the laser energy heats the air and water directly in front of the laser beam to deliver energy onto the rear surface of atomized water molecules with the aim of accelerating them to a higher speed. As result the HKS have in fact great utility, and eliminate the overheating problems during irradiation. So, it is used to prepare cavities, namely in minimally invasive dentistry in which pulpal temperature increases by only 2°C, without pulpal inflammatory responses associated, either immediately or after 30 days following its use (324). The low power settings recommended for endodontic irradiation allied to the HKS property were, as matter of fact, the real arguments to support the safety adjunctive application of the Er,Cr:YSGG laser in root canal therapy.

Historical perspective of literature related to Er,Cr:YSGG safety in Endodontics

2001. Yamazaki et al. showed that with energies exceeding 2W specimens irradiated with Er,Cr:YSGG laser (750µm tip, 140µs, 20Hz, 3x1second) without water spray resulted in carbonized root canal walls and cracking. However, if used up to 6W with water spray (50%water,48%air) specimens temperatures did never exceed 8°C (226).

2004. Ishizaki et al. evaluated the performances of 200, 320 and 400µm fibres when the irradiation was performed during 2 seconds (1mm shorter than the apex) followed by 5seconds moving coronally with the Er,Cr:YSGG laser (20Hz, 140µs, 50%water&air) at 2W, 3W and 5W. It was reported that the temperature rise during Er,Cr:YSGG laser application on root canals in the presence of water spray is minimal (<8°C with 400µm fiber at 5W) and does not cause any damage to periradicular structures. The morphology of enamel prisms and dentinal tubules were preserved without evidences of carbonization or melting (261).

2004. Evaluating four wavelengths and their bactericidal effects on deeper layers of dentin inoculated with E. coli and E. faecalis, Schoop et al. demonstrated that all four laser systems, including the first report for Er,Cr:YSGG laser, were capable of significant reduction in both strains. Using 400µm fibers, these authors compared 810nm Diode laser (66.7mJ and 100mJ output, 15Hz), 1064nm Nd:YAG laser (66.7 and 100mJ output, 15Hz), Er:YAG laser (66.7 and 100mJ output, 15Hz) and Er,Cr:YSGG laser (50mJ and 75mJ output, 20Hz), without any water
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spray or air cooling. Lasing cycles comprised five irradiations of 5s each (25s total) with 10° incidence angle. Er:YAG yielded the best results eradicating E. coli. The increase of power from 1W to 1.5W slightly increased the average temperature from 8.3±0.7°C to 8.7±0.7°C (325).

2007. The same investigation team detected the mean average temperature rise was 2.7°C and 3.2°C for 1W and 1.5W outputs respectively (300µm fiber, 20Hz, 0%water&air, 5x5seconds). In addition, the excessive heating could not be appointed as reason for the antibacterial effect (326).

2008. In an in vitro dye visualization study, George et al. reported interesting findings concerning the possible apical extrusion of root canal irrigants while using Er:YAG or Er,Cr:YSGG lasers either with bare or modified (conical) optical fibers. Apical foramen sizes were standardized either at ISO#15 or ISO#20 and canals were all prepared to the working length up to Protaper® F5 (0.50mm). Er:YAG laser irradiation (4W, 200mJ,20Hz, 200µs, 0%W+A, 400µm bare [1W output at 10mm] and 330µm [0.75W output at 10mm] conical tips) and Er,Cr:YSGG laser irradiation (1.25W, 62.5mJ, 20Hz, 140µs, 0%W+A, Z4-400µm bare [1W output at 10mm] and 330µm conical [0.75W output at 10mm] tips) were performed during 5s with the tips placed into the canal at a distance of either 5 or 10mm from the apex. Results were compared under the same conditions with a 25-G conventional needle and a 25-G side-vented needle. In all laser groups, considerable apical extrusion occurred. Neither both lasers, tip designs or distance to the apex gave significant differences regarding extrusion. All variables shown to be similar when compared to results obtained with a conventional needle. Apex size was set determinant concerning apical fluid extrusion (ISO#20>ISO#15). Therefore, caution should be used when using such lasers in combination with irrigants such as NaOCl and H₂O₂ (266).

2009. As bactericidal efficiency improved in dry conditions, Abad-Gallegos et al. evaluated the temperature increase at the external root surface after Er,Cr:YSGG laser (200µm fiber, 140µs, 20Hz, 0%water&air, WL-1mm, 2mm.s-1≈30seconds) irradiation and its possible correlation with the teeth with different dentinal layer thickness. Measurements recorded during irradiation showed that the average temperature increased significantly 3.84°C and 5.01°C while using 1W(0.3W) and 2W(0.6W) respectively. In addition, average temperature increments measured after 30seconds were 1.21°C and 1.78°C for the same settings. Despite
the fact that in lower incisors the temperature increments were higher than in canines, these results suggest that the temperature increment is clinically irrelevant for both cases (322).

2009. Regarding the application of radial-firing tips in endodontics, Schoop et al. conducted temperature measurements when using the RFT2 (200µm) tip, 20Hz, for 5x5seconds with 20seconds between each irradiation, in dry conditions. At 2W (0.6W output) the average temperature rise was 1.3°C while at 3W (0.9W output) the increase was 1.6°C at the root surface. In consequence, the excessive heating of the dentin obviously could not be attributed as the decisive factor for the high bactericidal effect observed in this study (315).

2010. While accessing and comparing the thermal effects of plain and modified (laterally emitting conical) laser tips in the apical thirds of root canals, George et al. reported that, for both Er:YAG (4W, 200mJ, 20Hz, 470µm fiber) and Er,Cr:YSGG (1.25W, 62.5mJ, 20Hz, 400µm fiber) lasers, the use of modified tips increase smear layer removal with lower average temperatures increment (<2°C). Irradiations were performed with 0%water&air, WL-1mm, moving coronally at 1mm.s⁻¹, 6x10 seconds, and canals were filled with distilled water before each irradiation which was considered to be essential to attenuate completely hazardous thermal effects in apical thirds (327).
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Er,Cr:YSGG laser Bactericidal Properties

As a result of advances in the field of laser assisted endodontics, the Er,Cr:YSGG laser (λ=2780nm) have been constantly modifying and manufacturing specialized thinner and more flexible endodontic tips of various lengths providing easier access to radicular canals, and a uniform coverage of the root canal system. The bactericidal effect of 2780nm laser irradiation is based on the high absorption coefficient in hydroxyl (OH) groups (µa=5000cm⁻¹) and H₂O molecules (µa=7000cm⁻¹) (275). In similarity with the Er:YAG laser, the bactericidal effect of the Er,Cr:YSGG laser occur under the same basic principles of energy absorption and consequent biophysical interactions. When the energy is directly or indirectly absorbed into the plasmatic volume of the bacteria, it induces membrane disruption and consequent bacterial eradication. The same principle is applied when water present within aggregated bacteria would serve as energy target to disrupt biofilms of endodontic pathogens (328).

Another reasonable explanation for the germ eradication can be associated either to thermal necrosis or dehydration of the germ, which was found more affected by the repetition rate rather than the pulse energy (329).

Historical perspective of literature related to Er,Cr:YSGG bactericidal efficacy

2004. Evaluating four wavelengths and their bactericidal effects on deeper layers of dentin inoculated with E. coli and E. faecalis, Schoop et al. demonstrated that all four laser systems, including the first report for Er,Cr:YSGG laser, were capable of significant reduction in both strains. Using 400µm fibers, these authors compared 810nm Diode laser (66.7mJ and 100mJ output, 15Hz), 1064nm Nd:YAG laser (66.7 and 100mJ output, 15Hz), Er:YAG laser (66.7 and 100mJ output, 15Hz) and Er,Cr:YSGG laser (50mJ and 75mJ output, 20Hz), without any water spray or air cooling. Lasing cycles comprised five irradiations of 5s each (25s total) with 10° incidence angle. Er:YAG yielded the best results eradicating E. coli. The increase of power from 1W to 1.5W slightly improved bacterial reduction for all lasers and both stains. However, all four lasers encountered greater difficulties to eliminate E. faecalis when compared with other species (325).

2006. Although the experimental model was based on cavity preparation, Turkun et al. demonstrated that with lower power outputs, Er,Cr:YSGG laser (0.75W and 1W outputs,0%
water and air, 5x5 seconds) reduced to a similar extent as 2% chlorhexidine gluconate in contact for 60 seconds, cavities inoculated with S. mutans. The antibacterial effect was thought to be due to the evaporation of cellular water leading to abrupt disintegration of the bacterial cell wall. Another explanation stemmed from the thermal necrosis or dehydration of the germ, which was more affected by the repetition rate rather than the pulse energy (329).

2006. In Jha et al. investigation, researchers have concluded that the “Er,Cr:YSGG laser instrumentation was not able to eliminate an E. faecalis infection in root canals and that “the laser was completely ineffective in disinfecting root canals when sterile saline was used as an irrigating solution”. However, criticism should be taking into account regarding the turbidity model used by these researchers as they recovered viable bacteria after laser treatment of infected root dentin and transferred/incubated them in broth tubes. In fact this model is not quantitative sensitive in the sense that a single surviving bacterial cell would give the same turbidity result as a million surviving organisms. Therefore, according to this model it would be impossible to determine whether any disinfection had occurred (330).

2007. In an ex vivo pilot study, Eldeniz et al. first reported the bactericidal efficacy of Er,Cr:YSGG laser against E. faecalis in teeth with straight roots with different sizes of apical foramen. They found that, after removing smear layer with 17%EDTA for 3 minutes, the Er,Cr:YSGG laser was capable to reach up to 96% disinfection working with Z2 plain fiber (200µm), 0.5W, 20Hz, 20%water+20%air, 20 seconds approximately (2 cycles moving at a speed of 1.5 mm.s^{-1}, 1mm from the apex to coronal). The size of apical foramen did not show to be significant in terms of bacterial reduction. 3%NaOCl in contact for 15 minutes was used as comparison group and achieved complete sterilization values (331).

2007. Schoop et al. evaluated the bactericidal efficacy of Er,Cr:YSGG laser on E. coli and E. faecalis, while using two different settings, 300µm fibers, and irradiating for 5x5 seconds from apical to coronal each sample. In the same time, temperature and morphological changes were recorded and visualized. If regarding E. coli elimination all samples (10/10) were below detection level with 1.5W output, 20Hz, 0%water&air, regarding E. faecalis elimination similar results were obtained with 1W output when compared to 1.5W output (8/10 below detection level). The mean average temperature rise was 2.7°C and 3.2°C for 1W and 1.5W respectively. As consequence the excessive heating of the sample could not be appointed as reason for the antibacterial effect. Concerning morphological changes, with 1W output exposed dentinal
tubules were clearly discernible whereas with 1.5W output some areas had partially closed dentinal tubules, partial melting and recrystallization (326).

2007. Comparing the bactericidal effects in the main canal of Er,Cr:YSGG laser with Nd:YAG laser and 2.5% NaOCl in infected root canals with *E. faecalis*, Wang *et al.* found statistical differences between lasers. Although both lasers achieved significant reductions, Nd:YAG laser revealed to be more effective. Irradiating each sample for 40 seconds (4x10 seconds), the Er,Cr:YSGG laser (20 Hz, 0% water & air) gave 77% and 96% reductions with 1W and 1.5W respectively, while Nd:YAG laser (15 Hz) gave reductions of 97% and 98% with same power settings. The 2.5% NaOCl irrigation for 2 minutes gave complete sterilization of the main canal walls. These results were attributed to the endodontic tip which didn’t enable to irradiate all areas of the root canal (332).

2007. Trying to solve the problem of tridimensional root canal decontamination Gordon *et al.* conducted a study to evaluate the efficacy of Er,Cr:YSGG laser using an improved radial emitting tip (200 µm, cone angle of 60±5 degrees) when compared to 2.5% NaOCl to eradicate *E. faecalis* in dentin cylinders. Using 350mW of real output power (68% tip calibration factor), 20 Hz, 150 µs pulse, moving at 1 mm.s⁻¹, 240 seconds in incremental steps, it was obtained the greatest CFU reduction of 99.7%. Even with the lowest power/time exposure (175 mW, 20 Hz, 150 µs, 15 s) 94.7% reduction occurred. To be enlightened that the mean effects of all three predictors, time, wattage and wet/dry technique were significant. Between all groups evaluated, the dry method provided most effective results. These tips were reported to be “capable of penetrating narrow, long and curved canals more efficiently, in areas that sodium hypochlorite irrigations may not be able to reach”. The measured tip power loss after 10 treatments was between 10 and 15% (333).

2009. To evaluate the ability of deep dentin decontamination of the Er,Cr:YSGG laser, Franzen *et al.* reached very interesting results using a model with dentin slices of 100 µm to 1000 µm, *E. faecalis* suspension and low pulse energies (0.25 W, 3.13 mJ, 20 Hz, 0% water & air, 200 µm fibre, 4x10 seconds). Irradiating at an incidence of 5° to the dentin slices surface, laser irradiation resulted in significant bacterial reduction in slice thickness up to 500 µm. In addition, despite the low pulse energies used, irradiated surfaces showed absence of smear layer and opened dentinal tubules (201).
2009. Trying to achieve more accurate protocols for the application of radial-firing tips in endodontics, Schoop et al. reported interesting bactericidal results after using the 200µm (RFT2) tip, in dry conditions (0% water & air), 20Hz, 5 x 5 seconds from apical to coronal, with 20s break in between. These authors used 2W and 3W, corresponding to a real power output of 0.6W (30mJ) and 0.9W (45mJ). On E. coli a major reduction was found at the lower output of 0.6W and at the higher power of 0.9W the impact was even more considerable yielding results below detection level. Regarding E. faecalis however, the increase from 0.6W to 0.9W conferred only a slight improvement in terms of disinfection and none of the samples achieved results below detection level. At 2W (0.6W output) the average temperature rise was 1.3°C while at 3W (0.9W output) the increase was 1.6°C at the root surface. In consequence, the dentin heat deposition could not obviously be attributed and correlated as the decisive factor for the high bactericidal effect observed in this study (315).

2010. Using plain 200µm (Z2) fibers, Arnabat et al. used the Er,Cr:YSGG laser to access the bactericidal effect on E. faecalis when compared to 0.5% and 5% NaOCl in contact for 30 seconds. Results demonstrated superior performances than 0.5% NaOCl when irradiating with 20Hz, 140µs pulse, 1W (0.3W, 15mJ output) and 2W (0.6W, 30mJ output) either for 30 and 60 seconds. However, laser treatment was as effective as 5%NaOCl when applied with 2W (0.6W) for 60 seconds or 1W (0.3W) for 120 seconds (334).

2010. Dewsnup et al. reported a study regarding the bacterial reduction (E. faecalis) in straight and curved canals enlarged up to size #40/0.06 taper, using radial firing tips (RFT2) when compared to 6.15% NaOCl. After 3 minutes in contact with 17%EDTA, and during 60 seconds in contact, 6.15% NaOCl samples achieved complete sterilization in all samples. By its turn, the Er,Cr:YSGG laser (0.75W, 20Hz, 10%air+0%water) used for 12x10 seconds (120 seconds) was also capable to reach sterilization in straight canals. Although there was no statistical significance between treatment groups, in curved canals laser irradiation did not achieve sterilization levels in all samples. Authors postulated that this difference is due to the “deflection of the laser tip away from the canal wall during activation” (335).

2010. Among 300 different microbial species which can be found in root-canal infections, yeast can be isolated either as pure cultures or together with other bacteria in primary, secondary and persistent root canal infections (336-338). Having contact sensing (trigmotropism) as characteristic, Candida albicans (C. albicans) demonstrates an invasive affinity to dentin being the most common yeast isolated from root canals (161, 337, 339, 340). It was also
demonstrated that smear layer increase the adhesion of *C. albicans* to dentin (341) delaying or stopping the antifungal effects of NaOCl and CHX (342). Thus, Onay *et al.* (343) aimed to evaluate the antifungal effects of Er,Cr:YSGG (300µm-Z3 tip, 0.75-1W, 20Hz, 0%water&35%air, 5x5seconds) and the possible combination with 5.25%NaOCl. At the SEM the 0.75W output power “with or without NaOCl combination exhibited completely disintegrated yeasts cells and scattered cell debris”. In microbiological analysis the 1W output power combined with the NaOCl for 1minute exhibited the greatest reduction of 92% of CFUs followed by 1W laser alone with 90.6% reduction. Furthermore, the 0.75W laser group demonstrated superior bactericidal efficacy to NaOCl alone. All treatment modalities showed to be statistically effective.

2010. Yavari *et al.* showed, once more, the limits of plain tips while using Er,Cr:YSGG laser to kill *E. faecalis*. After removing the smear-layer from instrumented root canal walls with 17%EDTA for 3 minutes, irradiation was performed with Z2 (200µm) tips, 140µs, 20Hz, 20%water&air, with 2W(100mJ) and 3W(150mJ), 1mm shorter than the working length, for 2x8seconds. Despite 1%NaOCl for 15min has demonstrated no bacterial growth, the laser groups at 2W and 3W for 16seconds, reduced bacterial growth 97.6% and 98.5% respectively. This bactericidal inconsistence could be though attributed to the excessively high energies and to the uneven irradiation pattern tip-related (344).
Er,Cr:YSGG laser mechanisms for debridement and smear layer removal

At the basis, root canal instruments are commonly used for shaping and cleaning. In addition, irrigants are needed for cleaning and disinfecting especially in these areas that cannot be reached by instruments or are insufficiently cleaned. Irrigation is then considered a very important part of the root canal treatment procedures (345).

Unfortunately, it is well known and scientifically accepted that hand irrigation is not so effective, namely in the apical part of the root canal, in oval extensions, isthmuses and anastomoses (346, 347).

Nowadays lasers have been proposed either as an alternative for conventional approaches in cleaning, disinfecting and shaping the root canal or as an adjuvant to conventional chemo-mechanical preparation in order to enhance debridement and promote higher disinfection levels (268, 348).

Almost all studies done with several wavelengths in order to remove or modify the smear layer, and/or promote disinfection have in common that the desired effects are the result of photo-thermal effects since the laser devices were mostly used without water and/or air cooling and depending on the laser-tissue/target interaction also more or less on absorption (252).

On the other hand, to enhance the spreading of the irrigant and to activate irrigation solutions, sonic and ultrasonic activation have been under investigation with interesting results (349, 350).

It was then demonstrated that ultrasound might result in the acoustic cavitation, that is, the creation of new bubbles or the expansion, contraction, and/or distortion of pre-existing bubbles in a liquid. By definition, cavitation is the formation of vapor bubbles of a flowing liquid in a region where the pressure of the liquid falls below its vapor pressure. Cavitation is usually divided into two classes of behavior: inertial (or transient) cavitation, and non-inertial cavitation. Inertial cavitation is the process where a void or bubble in a liquid rapidly collapses, producing a shock wave. Such cavitation often occurs in pumps, propellers, and in the vascular tissues of plants. Non-inertial cavitation is the process in which a bubble in a fluid is forced to oscillate in size or shape due to some form of energy input, such as an acoustic field. Such cavitation is often employed in ultrasonic cleaning baths.

Since the shock waves formed by cavitation are strong enough to significantly damage moving parts, cavitation is usually an undesirable phenomenon, specifically avoided in the design of turbines, and eliminating cavitation is a major field in the study of fluid dynamics.
The process of bubble generation, subsequent growth and collapse of the cavitation bubbles results in very high energy densities, resulting in very high temperatures and pressures at the surface of the bubbles for a very short time. The overall liquid medium environment, therefore, remains at ambient conditions. When uncontrolled, cavitation is damaging; however, by controlling the flow of the cavitation the power is harnessed and non-destructive. In (mainly industrial) cleaning applications, for example, cavitation has sufficient power to overcome the particle-to-substrate adhesion forces, loosening contaminants. The threshold pressure required to initiate cavitation is a strong function of the pulse width and the power input. This method works by generating controlled acoustic cavitation in the cleaning fluid, picking up and carrying contaminant particles away so that they do not reattach to the material being cleaned.

Moreover, controlled cavitation can be used to enhance chemical reactions or propagate certain unexpected reactions because free radicals are generated in the process due to disassociation of vapors trapped in the cavitating bubbles.

So if explosions and implosions generating pressure waves that create shear stress along the root canal walls, they may be sufficient to remove smear layer and biofilms are of interest.

A study conducted by Blanken and Verdaasdonk estimated that when an Er,Cr:YSGG laser is used within the canal with plain endodontic tip (Biolase Z4 endotip), fluid movement within the root canal occurs immediately following each pulse, with fluid speeds up to 20m/second - 72km/hour (302). Then it can be stated that, in part, the working mechanism of the Er,Cr:YSGG laser in the root canal could be therefore attributed to vapor bubble expansion and implosion with secondary cavitation effects inducing high-speed fluid motions into and out the canal. In addition, as expected, it was also demonstrated that thermal components were moderate.

These same authors recently published a visualization study, where the laser induced vapor and cavitation result in effective irrigation properties (230).

Using exposure times of 130microseconds (pulse length of the H-mode), 125mJ and 20Hz, the water is instantly turned into vapor. At the beginning of the laser pulse, the energy is absorbed in a 2µm-thick layer that is instantly super-heated to boiling temperature (100°C) at high pressure and turned into vapor. As the laser continues to emit energy, the light passes through the bubble and evaporates the water surface at the front of the bubble. In this context it “drills” a channel through the liquid until the pulse ends after about 140 microseconds. This
mechanism is well known and has been referred to as “the Moses effect in the microsecond region” by van Leeuwen et al. (351).

The small canal prevents the vapor from expanding freely laterally, pushing the water both forward and backward in the canal. Since the water obstructs the expansion of vapor in forward direction, the bubble also grows backwards along the fiber. Lateral and forward expansion in the root canal is limited by the root canal wall, while the backward expansion is blocked by the fiber making the lumen of the canal even smaller.

The pressure inside the bubble remains high for a long time, since it has to fight against the resistance of the irrigant that has to be displaced in the small canal. This process delays the dynamic of expansion and implosion compared to a free water situation and it can take three times longer. During implosion of the vapor bubble, the formation of new bubbles can be observed near the apex and are attributed to cavitation effects due to low pressure as a secondary effect of the imploding vapor bubble.

The high speed imaging method applied enabled the capture of images with microsecond resolution and the dynamics of the bubble formation has proven. It was found that fluid turbulence remains for a longer time after the actual laser pulses up to several milliseconds. The findings were identical for the cavitation effects in both water and NaOCl in the root canals, which as an interesting finding from endodontic point of view. At 75mJ fluid velocities of 21m/second were derived.

The implosion of the primary and secondary bubbles creates micro-jets in the fluid aimed at the wall with very high forces locally. All these effects result in the creation of shear stress along the wall of the canal, which contributes and should be sufficient to the disruption of cells and remove smear-layer.

Data obtained with the dye solution model showed that the difference between keeping the fiber stationary in the canal and moving it out the canal was absent for higher pulse energies. This means that the fiber can be kept a number of millimeters away from the apex of a tooth to have the secondary bubble effect. After 260 microseconds, the process of implosion is finished and the bubble has vanished. This bubble mechanism has shown to be reproducible at each pulse in a free water environment. When the wavelength is not absorbed in water, no bubble formation occurs, no pressure is build-up and, therefore, no cavitation or fluid motion is produced (230, 231).

The working hypothesis that conical tips should outperform conventional tip designs was also confirmed, with the contrived but standardized situation of a thick smear layer created by conventional rotary instruments used with water. This can be attributed to the
more even irradiation of the irrigant fluid and of the canal walls with these tips. Such tips are also likely to be easier to navigate into curved canals (262).

Although the contribution of smear layer to long-term outcomes in endodontic treatment remains under debate, results demonstrate that the debriding action of middle infrared laser energy is much better when delivered through conical modified fibers than unmodified plain fibers, while the same irrigant is used. The divergent laser energy can better interact with the canal walls, causing direct and indirect ablation by photomechanical effects (303, 352). In fact, some scientific reports have shown that pulsed mid-infrared lasers can induce shock waves in aqueous solutions inside root canals but the conical shape positively influence the configuration of this shock wave which would further enhance its action on debris and microorganisms (353).

Historical perspective of literature related to Er,Cr:YSGG for smear Layer removal

1999. Hossain et al. first recommended output powers below 3W for the removal of smear layer and debris from root canal walls, avoiding the production of craters on root walls, because the ablated depth of dentin irradiated at 3W with water spray reach up to 143µm (354).

Some other authors in the early stages of research dedicated to the Er,Cr:YSGG - such as Yu et al. in 2000 (355), Hossain et al. and Kimura et al. in 2001 (284, 356) - provided the first evidences for the Er,Cr:YSGG laser ability in terms of smear layer removal.

2001. Although the irradiation angle used cannot be compared directly, Yamazaki et al. showed that with energies exceeding 2W specimens irradiated with Er,Cr:YSGG laser (750µm tip, 140µs, 20Hz, 3x1second) without water spray resulted in carbonized root canal walls and cracking. However, if used up to 5W with water spray (50%water,48%air) specimens showed clean surfaces with open dentinal tubules with little or no carbonization observed. With water spray temperatures did not exceed 8°C. Authors described that Er,Cr:YSGG laser irradiation with water spray cooling could be a useful method for smear layer and debris removal from root canals (226).

2004. Ishizaki et al. evaluated the performances of 200, 320 and 400µm fibers concerning their efficiency on smear layer and debris removal. The irradiation was performed during 2 seconds (1mm shorter than the apex) followed by 5 seconds moving coronally with the Er,Cr:YSGG laser
(20Hz, 140µs, 50%water&air) at 2W, 3W and 5W. Despite obtaining heterogeneous results regarding the smear layer removal, interesting results were achieved with the 400µm fiber even at 5W. It showed most areas of effectively removed smear layer, with no evidence of carbonization. Furthermore, it was described the formation of apical stops due to the initial 2 seconds irradiation 1mm from the apex (261).

2006. Altundasar et al. compared the ultramorphological and histochemical changes after Er,Cr:YSGG laser (3W output power, 20Hz, 140µs, 50%water&air, 10seconds) irradiation with results obtained by preparing the canals in conjunction with Rc-Prep (Primier Dental, Norristown PA). It was demonstrated that the Ca/P ratios remained statistically similar for all groups, suggesting absence of changes at molecular level. Although Er,Cr:YSGG laser irradiation resulted in dentin characterized by partial and total removal of smear layer, few areas of carbonization and melting were also detected (300).

2006. Verdaasdonk et al. estimated that when using Er,Cr:YSGG laser with a 400µm plain endodontic tip (Biolase Z4 Endotip), fluid movements within the root canal occurs immediately following each pulse. In fact, fluid speed could reach up to 72Km/hour. Despite thermal components were considered moderate, the expansion and implosion of vapour bubbles with secondary cavitation effects induced fluid motion into and out the canal (357).

2007. Schoop et al. evaluated the bactericidal efficacy of Er,Cr:YSGG laser on *E. coli* and *E. faecalis*, while using two different settings, 300µm fibers, and irradiating 5x5 seconds from apical to coronal each sample. At the same time, temperature and morphological changes were recorded and visualized. Concerning morphological changes, with 1W output exposed dentinal tubules were clearly discernible whereas with 1.5W output some areas had partially closed dentinal tubules, partial melting and recrystallization (326).

2008. George et al. experiments provided a “proof-of-concept” for endodontic procedures using lateral emitting fibres and the activation of endodontic irrigants for smear layer removal. While irradiating with the Er,Cr:YSGG laser (1.25W, 62.5mJ, 20Hz, 0%water&air, 10 cycles moving at 1mm.s⁻¹, in the last apical 5mm’s) and 400µm plain (1W output) or modified-conical fibers(0.75W output) it was demonstrated that conical fibers performed better than plain fibers when matched for the same laser system and the same irrigant. The activation of water and 3% hydrogen peroxide provided similar results as the use of 15%EDTAC alone for 2minutes. The laser activation of EDTAC also improved its action in dissolving smear layer, providing the best results. The conical shape may influence the configuration of the shock
waves inside the root canal, which enhanced its action on debris through their ability to better transfer energy to the root canal walls (262).  

2009. With the application of radial-firing tips in endodontics, Schoop et al. showed detailed views after irradiating with the 200µm tip (RFT2), 20Hz, 5x5seconds in dry conditions either at 2W (0.6W, 30mJ output) and 3W (0.9W, 45mJ output). With 0.6W dentinal tubules were found partially exposed while with 0.9W most of dentinal tubules have been exposed without any signs of cracking or melting. As conclusion, the expansion of the beam by the tip geometry could favour a homogeneous energy distribution along the root canal walls (315).  

2009. Concerning the fact that little was known about laser inducing cavitation bubbles in rinsing solutions and whether a fiber as to be moved or kept stationary in the root canal, Blanken et al. performed a visualization study with Er,Cr:YSGG laser (Z2-200µm fiber, 140µs, 20Hz, 0.25-2.5W) at pulse energies of 75, 125 and 250mJ in glass models with artificial root canals filled with water, 2.5%NaOCl and/or coloured dye. The creation of bubbles was similar in both water and NaOCl solution. The difference between keeping the fiber stationary in the middle or moving it out of the canal was irrelevant for 125mJ and higher pulse energies. With 75mJ the repetition of 5 times irradiating for 5 seconds moving along the canal was proved to remove all dye from the canal, while for 125mJ and 250mJ, 3 and 1 repetitions were enough (230).  

2009. Following Blanken's study, De Moor et al. accessed how Er,Cr:YSGG (Z2-200µm, 75mJ, 20Hz) laser assisted irrigation (LAI) resulted in smear layer removal when compared with Passive Ultrasonic Irrigation (358) (Irisafe®, 30KHz, 30µm amplitude, 20seconds, 1mm from the apical stop) in straight roots prepared up to ISO#40 and filled with 2.5%NaOCl. With the laser fiber kept stationary 5mm from the apical stop and applied 4 x 5seconds, LAI resulted in significantly less debris than PUI and cavitation effects were sufficient to remove a large, artificially placed dentin plug. No signs of carbonization or collateral ablative removal of dentin were detected (231).  

2010. de Moor et al. proceeded with his previous investigations, being the first to compare both Er,Cr:YSGG and Er:YAG laser activated irrigation (LAI) with the intermittent flush technique for PUI. Using 200µm fibers, kept at 5mm from the apical stop, with sub-ablative settings (75mJ, 20Hz), 4 times 5 seconds, similar results were obtained for both wavelengths being as efficient as PUI for 60s (Irisafe®, 30Khz, WL-1mm, 3x20s) with the intermittent flush
technique. In addition, no statistically differences were found between Er:YAG and Er,Cr:YSGG laser systems (359).

2010. Yavari et al. showed the limits of plain tips while using Er,Cr:YSGG laser attempting to kill *E. faecalis*. After removing the smear-layer from instrumented root canal walls with 17% EDTA for 3 minutes, irradiation was performed with Z2 (200µm) tips, 140µs, 20Hz, 20%water&air, with 2W(100mJ) and 3W(150mJ), 1mm shorter than the working length, for 2x8seconds. Despite the laser groups at 2W and 3W reduced bacterial growth 97.6% and 98.5% respectively, SEM pictures showed dentinal tubules partially or completely closed. While a large amount of intertubular dentin was removed, peritubular dentin has remained as protruding tubules. The dentin pattern and bactericidal irregularities could be though attributed to the excessively high energies and the uneven irradiation tip-related (344).

2010. Analysing the morphology of root canal dentin after Er,Cr:YSGG irradiation (Z4, 400µm, 140µs, 20Hz, 24%water&34%air, 4 times at 2mm.s⁻²) and different power settings (0.75W, 1.5W and 2.5W), Silva et al. reported that increased dentinal permeability was obtained if irradiation was performed after 15ml 17%EDTA rinsing. While the combination with 0.75W did not show any morphological and permeability differences to 15 ml 17%EDTA group alone, the 1.5W and 2.5W combinations presented evidently more open dentinal tubules with no signs of meting or carbonization. If generally the 2.5W irradiation group gave the best permeability results for all root canal areas, the plain tip (Z4) and irradiation technique used appeared not to distribute the radiation homogeneously (360).

2011. Peeters et al. studied the efficacy in activating 17%EDTA solution with Er,Cr:YSGGG laser and 600µm plain fiber (MZ6 tip, 1W, 35Hz, 0%water&air) for 30s and 60s placing the tip above the orifice of the pulp chamber which served as reservoir, and continuous irrigation to maintain the hydration level. Results were compared with those obtained by PUI (*Irrisafe*®, 30KHz, 60s, WL-2mm). Completely clean root canals were found just in teeth irradiated for 60s in canals prepared up to a size #30/0.02. For this technique, it was demonstrated that the duration of laser irradiation is more demanding factor than the size of apical enlargement (361).
We could also find few but relevant references regarding the possible interference between the modified root canal surfaces and sealers adhesion/leakage after Er,Cr:YSGG laser irradiation.

2007. Varella et al. (270) prepared teeth up to size #40/0.06 and accessed the number of canals and isthmus filled after Er,Cr:YSGG laser (Z3-320µm fibre, 1.5W, 20Hz, 30%water & 50%air, 40s) treatment comparing with conventional treatment strategy (6%NaOCl and 3 minutes rinsing with 17% EDTA). Root canal filling was performed with gutta-percha points, AH Plus® root canal sealer (Dentsply), downpacking using SystemB® unit (SybronEndo, Orange, CA) and backfilling with ObturaIII® (Obtura Corporation, Fenton, MO). The group treated with Er,Cr:YSGG laser resulted in the obturation of greater number of canals and isthmus (p<0.01).

2010. Improvements in adhesive technology have incorporated adhesive dentistry in endodontics, as alternative root filling materials (e.g. Resilon/Hybrid Root Seal). After preparing teeth up to #30/0.04 to the WL, and through a computerized fluid filtration meter, Onay et al. (362) aimed to compare the apical sealing abilities of AH Plus/gutta-percha and Resilon/HRS combinations, at 1 and 4 weeks, after treating root canal surfaces either with 17% EDTA solution (3-minute rinse with 2mL) or Er,Cr:YSGG laser (1.5W, 75mJ, 20Hz, 30%W+50%A, 40s, 320µm Z3tip). Findings suggest that under these conditions the Er,Cr:YSGG laser did not influence the sealing ability when compared with EDTA application. For both root treatment groups, obturation with AH Plus/Gutta-percha showed the lowest apical leakage values.

From the majority of these studies we can conclude that here are no significant differences among the different parameters for laser usage with respect to the efficacy of removing smear layer. When they compared teeth which have been irradiated with those that had not, generally they all reach to conclusion that - namely under wet conditions - laser irradiation made a significant positive effect in terms of smear layer and debris removal.

Hence, it is comprehensible why laser irradiation of the root canal walls would demonstrate a positive interference with the root canal filling and adhesion mechanisms.
MATERIALS AND METHODS

No amount of experimentation can ever prove me right;  
A single experiment can prove me wrong.

Albert Einstein (1879-1955)  
Theoretical Physicist
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

**TRIAL DESIGN**

This investigation was designed as a prospective, blind randomized controlled superiority trial. There was the intention to compare conventional endodontic treatment with a similar protocol supplemented by the Er,Cr:YSGG laser irradiation for smear layer removal and decontamination propose. Patients were randomly assigned in two groups (control group and test group). Treatment modalities were compared through radiographic evaluation and followed up over time (6 and 12 months) in order to trace the bone healing in the apical region.

**OUTCOMES & HYPOTHESIS**

We hypothesized that necrotic teeth with Chronic Apical Periodontitis (CAP) treated with Er,Cr:YSGG laser and Radial Firing Tips (RFT’s) would demonstrate similar outcomes in comparison with teeth treated with 3% NaOCl and calcium hydroxide dressing interappointment with a similar root canal preparation protocol. The periapical index scoring system (PAI) was chosen as –scientifically validated- evaluation method (363).

**Primary Outcome(s):** The clinical effectiveness of the Er,Cr:YSGG Laser Assisted Endodontic Treatment. In accordance, the aim of this blind randomized clinical trial (RCT) was to compare radiographic evidences of periapical healing after root canal therapy and suggest the possibility to achieve predictable outcomes using the Er,Cr:YSGG laser (Waterlase MD® – EndoLase Root Canal Therapy System®- Radial Firing Tips) for root canal disinfection without the aid of any chemical substances.

The PAI was used to evaluate radiographic healing such as proposed by Orstavik et al.(363). This radiographic scoring system has shown to be one of the most reliable scoring systems, becoming widely accepted and validated by scientific reviewers (364).

**Secondary Outcome(s):** Proportion of teeth in each group that could be considered unchanged (same PAI score), improved (decrease in PAI score) or healed (PAI≤2) was analyzed. In addition, the presence of clinical symptoms or abnormal findings after treatments (e.g. spontaneous pain, presence of sinus tract, swelling, mobility, sensitivity to percussion or palpation) was checked through visual inspection, palpation and brief patient report. Up to date these data were registered but not submitted to analysis. However, there is the intention to develop the present investigation, through clinical findings’ scientific report(s), concerning the immediate post-operative features of laser therapy.
CONFIDENTIALITY AND ETHICAL CONCERNS

University Ethical Committee Approval

First we proceeded with the demonstration that for this study there was strong basic evidences available, with enough in vitro studies and few promising clinical case reports about the addressed topic. As incentive, the lack of well-designed controlled clinical trials referring the efficacy of Er,Cr:YSGG laser assisted endodontic treatments, served as logical interest to proceed with the clinical investigation.

All claims and settings for the Waterlase MD® & EndoLase Root Canal Therapy System® (Biolase™ Technology, CA, USA) laser assisted endodontic treatment were supported by previously published peer reviewed reports.

FDA clearance (#K071363 – 01/02/2008) for the laser application in endodontics after root canal preparation and CE approval for medical and dental treatments enabled dental practitioners to treat patients without being in conflict with the Helsinki Declaration (Annex I).

An agreement of minimal interference was set up regarding the F.M.D.U.P. interests, methods and the daily undergraduate’s clinical protocols for endodontic clinical practice.

The Ethics Committee pre-requirement of proper liability insurance was on time fulfilled (Annex III). Following insurance approval, the study protocol was re-submitted and approved by the Faculty Scientific and Ethics Committee [Annex II (missive nº0682, 22nd July 2009 and missive 0068, 17th January 2011)].

During this clinical investigation all the rules expressed by the Helsinki Declaration and national legislation were regarded concerning the confidentiality of all gathered personal information. All safety requirements for lasers Class IV irradiation were regarded (e.g. safety goggles during irradiation procedures).

Only the main investigator or his direct research collaborators dealt with the data which identified the participants, being treated separately on a data base model.

The results of the study or endpoint measurements had to be presented in a global way or by a numeric reference, ensuring both confidentiality and individual anonymity.

Each patient (and legitimate representatives of underage participants) agreed to participate in the study and was - at the same time - clarified about the need of radiographic follow-ups, by signing a properly detailed informed consent form (Annex IV).
PARTICIPANTS

Method and Period of Recruitment

With the approval of the study protocol obtained from the University of Porto Ethics Committee, eligible participants were recruited from October 2009 until April 2011 from among patients who attended spontaneously the Faculty Dental Clinic (Clínica Professor Fernando Peres, Faculdade de Medicina Dentária da Universidade do Porto) and referred for initial nonsurgical root canal treatments.

Inclusion criteria

Patients with asymptomatic teeth, necrotic pulps and CAP, as verified radiographically (minimum size, ≥ 1.0mm x 1.0mm), were consecutively enrolled in the study being set as primary inclusion criteria.

Diagnosis was performed by undergraduate students and confirmed by negative responses to sensitivity pulp tests. Clinical and radiographic interpretations were verified by supervising faculty members. Anterior and premolar teeth with mature, fully formed apex were selected. Within-person design was allowed (2 patients contributed with more than one tooth) minimizing subject variability bias. All patients included (gender).

Exclusion criteria

Patients were excluded if they were younger than 12 years old, pregnant, had a positive history of antibiotic use within the past month, indication for antibiotic prophylaxis (e.g. bacterial endocarditis or immune-compromising disorders), suffering from uncontrolled hypertension or diabetes mellitus, chronic renal failure, hematologic diseases, HIV, osteoporosis treated with bisphosphonates, steroid therapy exceeding 5mg/day of prednisolone, prior head and neck irradiation therapy.

Molars, no restorable teeth, teeth with abnormal root canal anatomy, more than 26mm in length and teeth with advanced periodontal disease were also excluded from the study.

Rubber dam isolation technique was considered mandatory.

No compulsion was allowed (e.g. terminal stages, prisoners).
IMPLEMENTATION, ENROLLED INTERVENIENCTS & OPERATORS

Once eligibility was confirmed, the study was explained to the patient by one endodontic resident, and the patient was invited to participate.

In addition, all patients were advised that root canal treatment would be performed regardless of participation in the study.

No financial incentive was offered (i.e. patients were responsible for the usual root canal treatment fee) and no financial incentive was offered for patients in return for follow-up clinical and radiographic examination.

After verbal and written informed consent (Helsinki Declaration 1973, revised in Edinburg 2000) was acquired, participants were randomly assigned to either test or control group by using block sequences generated from a randomization computer program with a 1:1 ratio between groups.

Participants’ Enrollment

All patients were enrolled by undergraduate students (resident clinicians) following initial diagnostic appraisal. After diagnosis confirmation through clinical and radiographic examination, the main researcher (M.R.M.) secondary explained in detail the meaning and purpose of the investigation to the patient.

At the same period, both enrolled undergraduate clinicians and assistants received a brief oral explanation and written protocol to adopt.

Allocation Sequence Generation

Two groups with no restriction or previous selection regarding baseline demographic characteristics (stratified randomization) were set up; the allocation sequence was performed by one researcher (M.A.M.) by means of randomization allocation software.

Random Sequence & Patient Assignment:

The random sequence was implemented and followed by the main researcher, which assigned each patient to each group immediately after written and verbal informed consent was acquired; neither the undergraduate clinician nor the patient was aware of the group assignment before agreeing to participate in the study and completing the consent process.
Intervenients and Operators

All endodontic procedures were performed at Clinica Professor Fernando Peres (Faculdade de Medicina Dentária – Universidade do Porto) by enrolled 4th and 5th academic year undergraduate students, constantly supervised by graduated professors who were not aware of the patient group assignment.

Laser irradiation and radiographic assessments were performed by the main investigator (M.R.M.).

Two previously calibrated and blinded endodontic specialists (M.F.C., I.P.V.) independently evaluated and scored the radiographs. In case of disagreement, another blinded endodontic specialist (J.A.C.) was brought in to set the final scoring.

Statistical analysis was performed by someone independent and not aware of the study purpose (D.A.N.).
RANDOMIZATION PROCESS & ALLOCATION

“Preventing selection and confounding biases”

After acquiring verbal and written inform consent, each patient (tooth) was randomly assigned to either control (NaOCl + CaOH) or test (Er,Cr:YSGG laser) group by using tables generated by a randomization software (Random Allocation Software v.1.0.0., Department of Anesthesia, Isafhan University of Medical Sciences, Iran), which.

Neither the clinician nor the patient was aware of the group assignment before agreeing to participate in the study.

Randomization tables provided by the allocation program – sequence generation method - resulted in blocks with a 1:1 ratio between groups, with a 0.05 level of significance.

The stratified randomization method restricts and controls the randomization in order to achieve balance between groups in size or baseline characteristics; stratification has been shown to increase the power of small randomized trials (365, 366). So, if groups become demographically unbalanced with the sequential patient enrolment defined by randomization tables, we took into consideration the possibility to be necessary to implement a stratified randomization/allocation methodology.

Figure 16: First block of 20 patients. Sequence provided by the random allocation software.
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Figure 17: Second block of 10 patients. Sequence provided by the random allocation software.

Figure 18: Third block of 10 patients. Sequence provided by the random allocation software.
Figure 19: Forth block of 10 patients. Sequence provided by the random allocation software.

Allocation concealment

Standardized Allocation

Standard patient (control/test group) allocation was done accordingly to the sequence given by the computer randomized generated tables.

Within-Person allocation

When more than one tooth was allocated in the same patient, randomization was performed by assigning the right or more mesial tooth to the control treatment group, whereas the left or more distal tooth was allocated to the laser assisted group.
INTERVENTIONS

Following the Faculty’s conventional protocol, teeth in both groups were subjected to a common two-visit root canal treatment.

All treatment sessions were performed by undergraduate residents following the standardized protocol for each intervention, during approximately 3 hours in length. This allowed adequate time to complete all treatment demands.

Local anesthesia (2% lidocaine with 1:100000 epinephrine) was administered as needed for patient comfort.

The radiologic assessment was carried out by the main investigator (M.R.M.), using the long-cone paralleling technique, using the same film holder and radiation exposure settings for each patient; settings were recorded.

**Common Endodontic procedures (for both groups)**

During the first visit, all caries lesions or previous restorations were removed. If necessary, the crown was restored using either glass-ionomer cement or reinforced zinc-oxide eugenol intermediate restorative material (IRM®, Dentsply).

The tooth was isolated and an access cavity was then prepared; root canal was located, cleaned and shaped using 25mm length ISO standard stainless-steel manual files (Zipperer CC+, VDW GmbH, Munich, Germany).

Working length (WL) was radiographically established at 1mm short of the biological apex of the root, with an ISO #15 K stainless steel file. WL was confirmed and adjusted using straight and angled radiographs. The master apical file for each canal was set at least 3 consecutive sizes larger than the first file to bind at the WL. The minimum master apical file of ISO #35 was required for every canal.

After achieving the apical enlargement, “step-back” manual root canal preparation was standardized and performed regardless to the group assignment.

Finishing the first visit a sterile cotton pellet imbibed on Cresophène® (Septodont) solution was placed in the pulp chamber, and the access cavity was sealed with a reinforced zinc-oxide eugenol temporary cement (IRM®, Dentsply).

The second visit was scheduled from 7 to 24 days after the first visit and every patient was inquired for symptoms such as pain, sensitivity to percussion, or swelling. The tooth was
isolated and the temporary filling removed; then, root canal preparation was completed regardless to the test/control group assignment.

After the conclusion of the root canal preparation and before root canal filling, canals were dried with paper points, checking for any suppuration or exudate.

Cold lateral compaction technique (367) was performed with gutta-percha (Gutta-Percha Points, ISO Color Coded – Dentsply, Maillefer) and hand-made zinc-oxide eugenol as sealer (Zinc Oxide powder – regular setting, PD™, Switzerland).

In similarity with the first appointment, the access cavity was sealed with reinforced zinc-oxide eugenol temporary cement (IRM®, Dentsply) but followed by an immediate post-operative radiograph.

Figure 20: Undergraduates performing the standardized root canal treatment (Authors’ property).

All teeth were permanently restored by the referring dentists within 30 days period.
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

Parallel Interventions

**Group 1 » Control** [3% NaOCl + Ca(OH)$_2$]

During the first visit, root canal preparation was performed using the manual step-back technique, and irrigated with 5.0mL of 3% NaOCl after each cycle until reach the minimum enlargement of ISO #30 K file (Zipperer CC’, VDW GmbH, Munich, Germany).

As inter-appointment medication, root canals were dried with sterile paper points and dressed with calcium hydroxide paste.

On the second visit, calcium hydroxide paste was removed using circumferential movements, Hedstrom-type files (Zipperer, VDW GmbH, Munich, Germany) and copious irrigation with 3% NaOCl; main removal of the calcium hydroxide paste was confirmed by visual inspection and through the completion of manual instrumentation.

After final irrigation with 5.0mL of 3% NaOCl the root canal was checked for the absence of suppuration or exudate, dried with sterile paper points and filled according to the previously described technique.

**Group 2 » Test** [Er,Cr:YSGG Laser Assisted Group]

During the first visit, root canal instrumentation was performed in similarity with the protocol described for group 1. In contrast, irrigation was performed with 2.0mL of sterile saline solution between files.

After reaching the ISO #30 K file (Zipperer CC’, VDW GmbH, Munich, Germany), the main canal was filled with distilled water and laser irradiation was performed with the 2780nm Er,Cr:YSGG laser (Waterlase MD; Biolase Technology, Inc, San Clement, CA) and a 270µm in diameter radial firing tip [RFT2 Endolase, Biolase Technology, Inc; calibration factor of 0.55 (Figure 22: 270µm Radial Firing Tip (RFT2); Authors’ property.Figure 22)] with panel settings of 0.75W, 20Hz 37.5mJ, 140µs pulse, 0% water and air (Figure 21). The tip was placed at the working length and irradiation was performed approximately at the speed of 2mm.s$^{-1}$ until reach the most coronal part of the canal. This procedure was repeated four times (two with the main canal filled with distilled water and the following two in dry conditions), resting approximately 15 seconds between each irradiation (Figure 25).

Root canals were dried with sterile paper points; no dressing with calcium hydroxide paste was applied as inter-appointment medication.
On the second visit, canal instrumentation was completed until reach at least an ISO #45 K-file, with intermittent saline solution as irrigant.

The main canal was filled with distilled water and laser irradiation was performed with a 320µm radial firing tip [RFT3 Endolase, Biolase Technology, Inc; calibration factor of 0.85 (Figure 24)] with panel settings of 1.25W, 20Hz 62.5mJ, 140µs pulse, 0% water and air (Figure 23). The irradiation protocol was identical to the first visit.

Following final irrigation with 5.0mL of saline solution, the root canal was dried with sterile paper points, checking for the absence of any suppuration or exudate and filled according to the previous obturation protocol.
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

Figure 23: Panel settings for the 320µm Radial Firing Tip (RFT3); Authors’ property.

Figure 24: 320µm Radial Firing Tip (RFT3); Authors’ property.

Figure 25: Smear layer removal aspect while irradiating with the main canal filled with distilled water; Authors’ property.
OUTCOME CLASSIFICATION AND DATA ANALYSIS

The primary outcome measures for this study were change in apical bone density after 6 and 12 months.

Teeth with chronic apical periodontitis were radiographed by the same investigator (M.R.M.) in separate periods: (1) before treatment for diagnosis; (2) immediately after treatment for baseline comparison; (3) 6 month follow up; (4) 12 month follow up.

The long-cone paralleling technique coupled to a film holder was used for both immediate postoperative and follow-up radiographs. Radiographic exposure settings were recorded for each tooth and follow-ups were reproduced under similar conditions; radiographic images were coded and stored by 2 investigators (I.P.V. and J.A.C.).

Follow-up radiographs were compared with those taken immediately after treatment and the Periapical Index (PAI) was used to evaluate radiographic healing. Instructions for grading images with the PAI scoring system were (363):

1. Find the reference radiograph where the periapical area most closely resembles the periapical area you are studying. Assign the corresponding score to the observed root.
2. When in doubt, assign higher score.
3. For multi-rooted teeth, use the highest of the scores given to the individual roots.
   (Not applied in this study)
4. All teeth must be given a score.

Figure 26: PAI with examples for scoring (adapted from Ørstavik et al., 1986; Ørstavik et al., 2004; and Huumonen et al., 2003).
Figure 27: PAI with verbal descriptions for each score (adapted from Ørstavik et al., 1986; Ørstavik et al., 2004; and Huumonen et al., 2003).

Each category used in the PAI represents a step on an ordinal scale for the radiological aspect of apical periodontitis. Thus, the viewing method of periapical radiographs was standardized accordingly to the following parameters:

1. Radiographs were evaluated in a darkened room using an illuminated viewer box whilst mounted in a cardboard slit to block off ambient light;
2. Radiographs were set up in a random order;
3. Scoring was performed by two previously calibrated endodontic specialists (M.F.C. and I.P.V.), without knowing the treatment protocol adopted for each patient;
4. If different scoring occurred, another independent specialist (J.A.C.) set the final scoring;

Reviewers Calibration

The use of a graded scale provides statistical power in comparative studies. However, depending on the observers and between observers that can be easily lost during the transformation. Though, extensive calibration and training in scoring periapical images is considered to be appropriate in order to improve the accuracy between and within examiners (368-371).

Both reviewers were initially calibrated through the evaluation of a set with 30 radiographic images associated with the study sample and representing a wide range of chronic apical periodontitis/periapical bone densities (Figure 28).
To access intra-rater agreement, 1 week after the first session, each examiner scored the same images. This method generated 4 PAI scores for each image: 2 from each of the 2 examiners.

The examiners then met as a group to reach consensus on cases that did not receive unanimous agreement and reviewed all scores to enhance calibration and inter-rater agreement. Consensus score for each image was considered the definitive score to be used for calibration statistical analysis.

The identifying code for each image was not broken until after consensus score was determined.

Agreement between and within examiners was determined using the interclass correlation coefficient (ICC). Intra-rater reliability was measured with the single measure ICC (SPSS 17 for Windows; SPSS Inc, Chicago, IL), and inter-rater agreement was measured with the average measure ICC (also known as the inter-rater reliability coefficient).

The criteria proposed for strength of agreement by Landis et al. (372) was adopted: 0.00-0.20, slight agreement; 0.21-0.40, fair agreement; 0.41-0.60, moderate agreement; 0.61-0.80, substantial agreement and 0.81-1.00, almost perfect agreement.

One week following the final calibration session, both reviewers randomly scored the assembled study images, blinded to the treatment protocol used for each patient. Examples of participant´s data used for blind outcome evaluation can be found on Annex VI.
RESULTS

The RCT is a very beautiful technique of wide applicability, but as with everything else there are snags. When humans have to make observations, there is always the possibility of bias.

To evaluate the efficacy of the Er,Cr:YSGG radial firing tips within the laser assisted endodontic treatment compared with conventional root canal treatment, radiographic follow-up was used to assess periapical bone healing. For calibration, radiographic images of periapical lesions were randomly evaluated from a set of radiographs [Table(s) 1].

Follow-up radiographs obtained after 6 and 12 months, were viewed together and compared with those taken immediately after treatment for the same patient. Evolution or changes in the radiolucency of each periapical lesion was evaluated and immediately scored.

Two main reviewers were used; both experienced and previously calibrated endodontists (M.F.C. and I.P.V.). All reviewers were initially calibrated by evaluating one set of similar radiographs and blinded to the group to which a given tooth belonged.

Each reviewer evaluated and scored each set of radiographs independently. When both reviewers agreed, then the score was registered. When disagreement occurred, another reviewer (J.A.C.) was brought in and the final scoring for each radiograph was determined.

Scoring was later dichotomized so that the decrease in PAI score could be considered “improved”, whereas either advanced healing or complete healing could then be considered together as “healed” (PAI<2).
CALIBRATION RESULTS

Table(s) 1: PAI scores of 20 randomized radiographs representing a wide range of CAP.

<table>
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<tr>
<th>Reviewers</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<th>12</th>
<th>13</th>
<th>14</th>
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<td>2</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
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<td>3</td>
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<tr>
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<td>4</td>
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<td>4</td>
<td>4</td>
<td>3</td>
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<td>2</td>
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<td>4</td>
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<td>3</td>
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<td>3</td>
</tr>
<tr>
<td>M.F.C.2 (25-10-2010)</td>
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<td>4</td>
<td>4</td>
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<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Agreement</td>
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</table>

Before the consensus scoring meeting, intra-rater reliability (observer consistency in scoring the same image in two different periods) and inter-rater agreement (scoring differences between the two observers at the same period) were analyzed.

The intra-rater reliability result was 0.95.

The inter-rater agreement result was 0.85.

Accordingly to the criteria proposed for strength of agreement by Landis et al. (372), results obtained between reviewers and within each reviewer were both considered in the category of “almost perfect agreement” (0.81-1.00).
6 MONTHS FOLLOW-UP RESULTS

Thirty-six patients met the inclusion criteria and consented to participate in the trial. Due to deviation from protocol (no compliance) 4 patients were excluded from statistical analysis.

Twenty-nine patients were examined and subjected to statistical analysis at the 6 months follow-up; 12 in group 1 (NaOCl+CH) and 17 in group 2 (Er,Cr:YSGG laser). Patient progresses during the phases of the trial are expressed at the CONSORT flow diagram for better comprehension (Figure 29).

![Figure 29: 6 months Flow Diagram – Provided by Consolidated Standards for Reporting Trials (CONSORT 2010)](image-url)
No adverse effects were found. As abnormal clinical finding, one patient (group 2) complained about swelling and sensitivity immediately after obturation which has disappeared in approximately 1 week with antibiotic and anti-inflammatory prescription.

We defined failure as the need for any additional treatment. The single failure (occurred in group 1) was related to the presence of swelling, and sensitivity to percussion after endodontic treatment. In group 2, one tooth needed to be extracted due to prosthetic reasons before the 6 months examination and was considered lost to follow-up. One additional patient was lost to follow-up.

Treatment failures were not included in the primary data analysis.

Demographic characteristics and baseline data for each group, analyzed at the 6 months follow up can be found in Table 2.

<p>| Table 2: Demographic characteristics (age, gender, and tooth type) for each group at 6 months. |
|-------------------------------------------------|-------------------------------------------------|-------------------------|---------------------------------|-------------------------|</p>
<table>
<thead>
<tr>
<th>Control Group1 (n=12)</th>
<th>Male</th>
<th>Female</th>
<th>Age</th>
<th>Anterior</th>
<th>Premolar</th>
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<tr>
<td></td>
<td>4</td>
<td>8</td>
<td>mean=49 (range 12 to 76 y.o.)</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Test Group2 (n=17)</td>
<td>7</td>
<td>10</td>
<td>mean=42 (range 24 to 67 y.o.)</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Treatment failure</td>
<td>0</td>
<td>1</td>
<td>45 y.o.</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Lost to Follow-up</td>
<td>1</td>
<td>1</td>
<td>26 &amp; 56 y.o.</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Totals</td>
<td>12</td>
<td>20</td>
<td>mean=45 (range 12 to 76 y.o.)</td>
<td>21</td>
<td>11</td>
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</tbody>
</table>

At the immediate postoperative examination the mean PAI score for group 1 was 3.83 (SD=1.19) and 2.17 (SD=1.47) at the 6 months follow-up, a decrease of 1.66;

At the immediate postoperative examination the mean PAI score for group 2 was 4.49 (SD=1.05) and 2.47 (SD=1.23) at the 6 months follow-up, a decrease of 2.02;

Both groups exhibited a statistically significant decrease in PAI score ($p < 0.05$).

There was no statistically significant difference between groups at either the immediate postoperative examination ($p= 0.28$) or the 6 months evaluation ($p=0.38$).
In group 1, 66.67% of teeth could be considered healed (PAI ≤2) after 6 months, 83.33% have improved (lower PAI score) and 16.67% kept unchanged (same PAI score). In addition, there was one treatment failure that could not be accessed to follow-up.

In group 2, 58.82% of the teeth could be considered healed after 6 months, 82.35% have improved and 17.65% kept unchanged. No treatment failures or increased PAI score were recorded. There was no statistically significant difference between groups \(p=0.69\).

The “Efficacy of Er, Cr:YSGG laser with endodontical radial firing tips on the outcome of endodontic treatment: blind randomized controlled clinical trial with six-month evaluation” can be currently found published in *Lasers in Medical Science* peer-reviewed journal (373).
12 MONTHS FOLLOW-UP RESULTS

Sixty-two patients were assessed for eligibility. Forty-three patients met the inclusion criteria and consented to participate in the trial.

At the 12 months follow-up, 30 patients were examined and subjected to statistical analysis, 12 in group 1 (NaOCl+CH) and 18 in group 2 (Er,Cr:YSGG); Patients’ progress during the phases of the trial are expressed at the CONSORT flow diagram for better comprehension (Figure 30).

No adverse effects were found. As abnormal clinical finding, one patient (group 2) complained about swelling and sensitivity immediately after obturation which has disappeared in approximately 1 week with antibiotic and anti-inflammatory prescription; this was the same patient record found in the 6 month results appraisal.

Figure 30: 12 months Flow Diagram - Provided by Consolidated Standards for Reporting Trials (CONSORT 2010)
We defined failure as the need for any additional treatment; two failures were detected in group 1 and were related to the presence or maintenance of swelling, and sensitivity to percussion after endodontic treatment.

In group 2 one tooth needed to be extracted due to prosthetic reasons before the 12 months examination and was considered lost to follow-up; 5 additional patients were lost to follow-up.

Treatment failures were not included in the primary data analysis.

Demographic characteristics and baseline data for each group, analyzed at the 12 months follow up can be found in Table 3.

<table>
<thead>
<tr>
<th>Table 3: Demographic characteristics (age, gender, and tooth type) for each group at 12 months.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Control Group1 (n=12)</td>
</tr>
<tr>
<td>Test Group2 (n=18)</td>
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<tr>
<td>Treatment failures (n=2)</td>
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<tr>
<td>Lost to Follow-up (n=5)</td>
</tr>
<tr>
<td>Analysed (n=30)</td>
</tr>
</tbody>
</table>

At the immediate postoperative examination the mean PAI score for group 1 was 3.83 (SD=0.89) and 1.33 (SD=0.14) at the 12 months follow-up, a decrease of 2.50;

At the immediate postoperative examination the mean PAI score for group 2 was 4.49 (SD=0.24) and 1.72 (SD=0.16) at the 12 months follow-up, decreasing 2.61;

Both groups exhibited a statistically significant decrease in PAI score ($p <0.05$).

There was no statistically significant difference between groups at either the immediate postoperative examination ($p=0.14$) or the 12 months evaluation ($p=0.11$).

In control group two treatment failures were detected and could not be accessed at the 12 months follow-up. However, all remaining teeth were considered healed (PAI ≤2); in the test group no treatment failures were detected; 88.90% of the teeth were considered healed, and
11.1% have improved (lower PAI score). There was no record of teeth with unchanged or increased PAI scores at the 12 months follow up. There was no statistically significant difference between groups ($p=0.503$).

The first year report entitled “Outcome of Er,Cr:YSGG Laser Assisted Treatment of Teeth with Apical Periodontitis: A Blind Randomized Clinical Trial” was accepted for publication in *Photomedicine and Laser Surgery* peer-reviewed journal, and can be found in Annex VIII.

**Additional records**

Time was not recorded (or taken into consideration) either in control or test groups. However, we may consider that no additional time is spent to perform the laser irradiation protocol if compared to the control group. In fact, intermittent irrigation with sodium hypochlorite along with the placement and removal of calcium hydroxide dressing take by principle several minutes.
STATISTICAL ANALYSIS

If your experiment needs statistics, then you ought to have done a better experiment.

Ernest Rutherford (1871-1937)
Nobel Prize, English physicist
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

Despite the exclusion of participants from any analysis can lead to erroneous conclusions, we have restricted both outcome assessments to the patients who have fulfilled the protocol in terms of eligibility, interventions, schedule plan and follow-up periods. This is known as an “on-treatment” or “per protocol” analysis (374). Thus, for this investigation the “per protocol” methodology was implemented as method of collecting data.

In all appraised teeth the periapical status was assessed, and scoring results recorded.

Investigator-determined exclusion(s): Teeth that were considered as endodontic failures ($n=1$ at 6 months and $n=2$ at 12 months, all in control group) were not possible to follow-up and were excluded from statistical analysis.

Presence of clinical symptoms or abnormal findings (e.g. spontaneous pain, swelling, mobility and sensitivity to percussion or palpation) were recorded but not submitted to statistical analysis but kept for further developments.

- **Confidence interval** for the estimated effect indicates a range of uncertainty for the true treatment effect. In the present studies a 95% confidence interval was set;

- **Statistical significance**: the $p$ Value represents the probability that the observed data could have arisen by chance when the interventions did not differ (statistical significance if $p < 0.05$);

- **Interim analysis** usually compares groups while the trial is still in progress to determine whether the recruitment should be stopped or proceed. For this clinical study no interim analysis was performed. However, results obtained from the six-month analyses suggested that the test group procedures were efficient enough to proceed with the trial without being in conflict with ethical concerns;
Parametric/non-parametric tests selection

To analyze differences obtained between and within the two groups, the normality of sampling distribution and population homogeneity of variance were primordially verified for both initial and final outcome scorings.

➢ Immediate Post-operative (T₀): Normality and Homogeneity

Table 4: Case processing summary (mean, median and variance descriptive) for each group at T₀.

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<thead>
<tr>
<th>Treatment applied</th>
<th>Cases</th>
<th>Statistics</th>
<th>Std. Error</th>
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<tr>
<td></td>
<td>Valid</td>
<td>Missing</td>
<td>Total</td>
</tr>
<tr>
<td>T₀ E₃,O,YSO₄ - RFT</td>
<td>18</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>3%NaOCl + Ca(OH)₂</td>
<td>12</td>
<td>0</td>
<td>12</td>
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</table>

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>Statistics</th>
<th>Std. Error</th>
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<tr>
<td></td>
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<tr>
<td>95% Confidence Interval for Mean</td>
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<tr>
<td></td>
<td>Upper Bound</td>
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<td>5% Trimmed Mean</td>
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<td>Median</td>
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<td></td>
<td>Upper Bound</td>
<td>4,87</td>
</tr>
<tr>
<td>5% Trimmed Mean</td>
<td>3,87</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>4,00</td>
<td></td>
</tr>
<tr>
<td>Variance</td>
<td>1,061</td>
<td></td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>1,030</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>2,00</td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>5,00</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>3,00</td>
<td></td>
</tr>
<tr>
<td>Interquartile Range</td>
<td>2,00</td>
<td></td>
</tr>
<tr>
<td>Skewness</td>
<td>-1,142,637</td>
<td></td>
</tr>
<tr>
<td>Kurtosis</td>
<td>1,232,1232</td>
<td></td>
</tr>
</tbody>
</table>
Table 5: Normality sampling distribution tests for T<sub>0</sub>.

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>Kolmogorov-Smirnov&lt;sup&gt;a&lt;/sup&gt; Statistic</th>
<th>df</th>
<th>Sig.</th>
<th>Shapiro-Wilk Statistic</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0 Er,Cr:YSGG - RFT</td>
<td>.353</td>
<td>18</td>
<td>.300</td>
<td>.687</td>
<td>18</td>
<td>.003</td>
</tr>
<tr>
<td>3% NaOCl + Ca(OH)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>.207</td>
<td>12</td>
<td>.163</td>
<td>.870</td>
<td>12</td>
<td>.065</td>
</tr>
</tbody>
</table>

Table 6: Homogeneity of variance test for T<sub>0</sub>.

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>Levene Statistic</th>
<th>df&lt;sub&gt;1&lt;/sub&gt;</th>
<th>df&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>Based on Mean</td>
<td>.049</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Based on Median</td>
<td>.256</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Based on Median and with adjusted df</td>
<td>.258</td>
<td>1</td>
<td>23,147</td>
</tr>
<tr>
<td></td>
<td>Based on trimmed mean</td>
<td>.076</td>
<td>1</td>
<td>28</td>
</tr>
</tbody>
</table>

The fact that the sample was n≤30 directly implied the Kolmogorov-Smirnov test exclusion and the Shapiro-Wilk test selection:

- SW<sub>18</sub>(Er,Cr:YSGG) = 0.687; p=0.000 (normal distribution not confirmed p<0.05)
- SW<sub>12</sub>(NaOCl) = 0.870; p=0.066 (normal distribution confirmed p>0.05)
- F(1,28) = 0.49; p=0.826 (homogeneity of variance confirmed p>0.05)
Immediate Post-operative (T₁₂): Normality and Homogeneity

Table 7: Case processing summary (mean, median and variance descriptives) for each groups at T₁₂.

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>Cases</th>
<th>Valid</th>
<th>N</th>
<th>Percent</th>
<th>Missing</th>
<th>N</th>
<th>Percent</th>
<th>Total</th>
<th>N</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month_12th</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Er:Cr:YSGG + RRT</td>
<td>18</td>
<td>100,0%</td>
<td>0</td>
<td>0,0%</td>
<td>18</td>
<td>100,0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3% NaOCl + Ca(OH)</td>
<td>12</td>
<td>100,0%</td>
<td>0</td>
<td>0,0%</td>
<td>12</td>
<td>100,0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>Statistic</th>
<th>Std. Error</th>
<th>95% Confidence Interval</th>
<th>Mean</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month_12th</td>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Er:Cr:YSGG + RRT</td>
<td>1,72</td>
<td>0,158</td>
<td>1,39</td>
<td>2,05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3% NaOCl + Ca(OH)</td>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

Table 8: Normality sampling distribution tests for T_{12}.

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>Kolmogorov-Smirnov</th>
<th>Shapiro-Wilk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Statistic</td>
<td>df</td>
</tr>
<tr>
<td>Month_{1-2th}</td>
<td>Er,Cr:YSGG - RFT</td>
<td>0.272</td>
</tr>
<tr>
<td></td>
<td>3% NaOCl + Ca(OH)</td>
<td>0.417</td>
</tr>
</tbody>
</table>

Table 9: Homogeneity of variance test for T_{12}.

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>Levene Statistic</th>
<th>df1</th>
<th>df2</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month_{1-2th}</td>
<td>Based on Mean</td>
<td>1,245</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Based on Median</td>
<td>0.781</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Based on Median and with adjusted df</td>
<td>0.781</td>
<td>1</td>
<td>27.949</td>
</tr>
<tr>
<td></td>
<td>Based on trimmed mean</td>
<td>1,574</td>
<td>1</td>
<td>28</td>
</tr>
</tbody>
</table>

The fact that the sample was n≤30 directly implied the Kolmogorov-Smirnov test exclusion and the Shapiro-Wilk test selection:

- SW (18)Er,Cr:YSGG = 0.788 ; p=0.001 (normal distribution not confirmed p<0.05)
- SW (12)NaOCl = 0.608 ; p=0.000 (normal distribution not confirmed p<0.05)
- F (1,28) = 1.245 ; p=0.274 (homogeneity of variance confirmed p>0.05)

Taking into account the previous results of normality distribution and homogeneity of variance, the hypothesis for parametric tests adoption (e.g. T-student test) was excluded; hence, to evaluate changes in PAI scores between groups and for each group from immediate postoperative (T₀) to 6 (T₆) and 12 (T₁₂) month follow-up radiographs, the Mann-Whitney U and the Wilcoxon signed rank tests were respectively selected.
6 MONTHS (T₆) FOLLOW-UP STATISTICS

Descriptive Analyses

Table 10: Age (global sample) at T₆.

<table>
<thead>
<tr>
<th>Age</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>23</td>
<td>12</td>
<td>76</td>
<td>45.43</td>
<td>15.427</td>
</tr>
<tr>
<td>Valid N (listwise)</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 11: Age distribution between groups at T₆.

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Er:Cr:YSGG - RFT</td>
<td>17</td>
<td>24</td>
<td>67</td>
<td>42.94</td>
<td>15.073</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valid N (listwise)</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3%NaOCl + Ca(OH)</td>
<td>12</td>
<td>12</td>
<td>76</td>
<td>49.08</td>
<td>18.228</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valid N (listwise)</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 12: Gender (global sample) at T₆.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>18</td>
<td>62.1</td>
<td>62.1</td>
<td>62.1</td>
</tr>
<tr>
<td>Male</td>
<td>11</td>
<td>37.9</td>
<td>37.9</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 13: Gender distribution between groups at T₆.

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Er:Cr:YSGG - RFT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valid</td>
<td>10</td>
<td>58.8</td>
<td>58.8</td>
<td>58.8</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>41.2</td>
<td>41.2</td>
<td>100.0</td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3%NaOCl + Ca(OH)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valid</td>
<td>8</td>
<td>56.7</td>
<td>56.7</td>
<td>65.7</td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
<td>33.3</td>
<td>33.3</td>
<td>100.0</td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 14: Appraised tooth number (global sample) at T₆.

<table>
<thead>
<tr>
<th>Valid</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>1</td>
<td>3.4</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>12</td>
<td>4</td>
<td>13.8</td>
<td>13.8</td>
<td>17.2</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>3.4</td>
<td>3.4</td>
<td>20.7</td>
</tr>
<tr>
<td>15</td>
<td>2</td>
<td>6.9</td>
<td>6.9</td>
<td>27.6</td>
</tr>
<tr>
<td>21</td>
<td>3</td>
<td>10.3</td>
<td>10.3</td>
<td>37.9</td>
</tr>
<tr>
<td>22</td>
<td>2</td>
<td>6.9</td>
<td>6.9</td>
<td>44.8</td>
</tr>
<tr>
<td>25</td>
<td>2</td>
<td>6.9</td>
<td>6.9</td>
<td>51.7</td>
</tr>
<tr>
<td>32</td>
<td>1</td>
<td>3.4</td>
<td>3.4</td>
<td>55.2</td>
</tr>
<tr>
<td>34</td>
<td>2</td>
<td>6.9</td>
<td>6.9</td>
<td>62.1</td>
</tr>
<tr>
<td>35</td>
<td>1</td>
<td>3.4</td>
<td>3.4</td>
<td>65.5</td>
</tr>
<tr>
<td>42</td>
<td>4</td>
<td>13.8</td>
<td>13.8</td>
<td>79.3</td>
</tr>
<tr>
<td>43</td>
<td>3</td>
<td>10.3</td>
<td>10.3</td>
<td>89.7</td>
</tr>
<tr>
<td>44</td>
<td>1</td>
<td>3.4</td>
<td>3.4</td>
<td>93.1</td>
</tr>
<tr>
<td>45</td>
<td>2</td>
<td>6.9</td>
<td>6.9</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 15: Appraised tooth number - distribution between groups at T₆.

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Er,Cr:YSGG - RFT</td>
<td>Valid</td>
<td>12</td>
<td>11.8</td>
<td>11.8</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>1</td>
<td>5.9</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1</td>
<td>5.9</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>1</td>
<td>5.9</td>
<td>5.9</td>
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<tr>
<td></td>
<td>22</td>
<td>2</td>
<td>11.8</td>
<td>11.8</td>
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<td></td>
<td>25</td>
<td>1</td>
<td>5.9</td>
<td>5.9</td>
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<td></td>
<td>35</td>
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<td>5.9</td>
<td>5.9</td>
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<tr>
<td></td>
<td>42</td>
<td>2</td>
<td>11.8</td>
<td>11.8</td>
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<td></td>
<td>43</td>
<td>3</td>
<td>17.6</td>
<td>24.4</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>1</td>
<td>5.9</td>
<td>29.2</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>2</td>
<td>11.8</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>3% NaOCl + Ca(OH)</td>
<td>Valid</td>
<td>11</td>
<td>8.3</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>2</td>
<td>16.7</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1</td>
<td>8.3</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>2</td>
<td>16.7</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>1</td>
<td>8.3</td>
<td>58.3</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>1</td>
<td>8.3</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>2</td>
<td>16.7</td>
<td>83.3</td>
</tr>
<tr>
<td></td>
<td>42</td>
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<td>16.7</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>
Table 16: Applied treatment relative frequencies at $T_6$.

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid</td>
<td>17</td>
<td>58.8</td>
<td>58.8</td>
<td>58.8</td>
</tr>
<tr>
<td>Er:C r:YSGG - RFT</td>
<td>12</td>
<td>41.4</td>
<td>41.4</td>
<td>100.0</td>
</tr>
<tr>
<td>3%NaOCl + Ca(OH)</td>
<td>20</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Table(s) 17: Immediate post-operative scorings - global sample at $T_6$.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Valid</th>
<th>Missing</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Valid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>29</td>
<td></td>
<td>0</td>
<td>4.10</td>
<td>1.113</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid</td>
<td>2</td>
<td>13.8</td>
<td>13.8</td>
<td>13.8</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>13.8</td>
<td>13.8</td>
<td>27.6</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>20.7</td>
<td>20.7</td>
<td>48.3</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>51.7</td>
<td>51.7</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 18: Immediate post-operative scorings - distribution between groups at $T_6$.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Valid</th>
<th>Missing</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Er:C r:YSGG - RFT</td>
<td>17</td>
<td></td>
<td>0</td>
<td>4.29</td>
<td>1.047</td>
</tr>
<tr>
<td>3%NaOCl + Ca(OH)</td>
<td>12</td>
<td></td>
<td>0</td>
<td>3.83</td>
<td>1.193</td>
</tr>
</tbody>
</table>
Table 19: Immediate post-operative scores - distribution between groups (frequencies) at $T_6$.

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Er,Cr:YSGG - RFT</td>
<td>Valid 2</td>
<td>11.8</td>
<td>11.8</td>
<td>11.8</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>5.9</td>
<td>5.9</td>
<td>17.6</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>23.5</td>
<td>23.5</td>
<td>41.2</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>58.8</td>
<td>58.8</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>3%NaOCl + Ca(OH)</td>
<td>Valid 2</td>
<td>15.7</td>
<td>16.7</td>
<td>16.7</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>25.0</td>
<td>25.0</td>
<td>41.7</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>16.7</td>
<td>16.7</td>
<td>58.3</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>41.7</td>
<td>41.7</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Table(s) 20: $T_6$ scores - global sample (mean, standard deviation & frequencies).

<table>
<thead>
<tr>
<th></th>
<th>Valid</th>
<th>Missing</th>
<th>29</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>2.34</td>
<td></td>
</tr>
<tr>
<td>Std. Deviation</td>
<td></td>
<td></td>
<td>1.317</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid</td>
<td>1</td>
<td>34.5</td>
<td>34.5</td>
<td>34.5</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>27.5</td>
<td>27.6</td>
<td>62.1</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>13.8</td>
<td>13.8</td>
<td>75.9</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>17.2</td>
<td>17.2</td>
<td>93.1</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>6.9</td>
<td>6.9</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 21: $T_6$ scores - distribution between groups.

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>N</th>
<th>Valid</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Er,Cr:YSGG - RFT</td>
<td></td>
<td>Valid</td>
<td>17</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>2.47</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td></td>
<td></td>
<td>1.231</td>
</tr>
<tr>
<td>3%NaOCl + Ca(OH)</td>
<td></td>
<td>Valid</td>
<td>12</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>2.17</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td></td>
<td></td>
<td>1.467</td>
</tr>
</tbody>
</table>
Table 22: T₀ scores - distribution between groups (frequencies).

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Er:Cr:YSGG - RFT</td>
<td>1</td>
<td>4</td>
<td>23.5</td>
<td>23.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6</td>
<td>35.3</td>
<td>58.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>17.5</td>
<td>76.5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3</td>
<td>17.5</td>
<td>94.1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1</td>
<td>5.9</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

| 3%NaOCl + Ca(OH) | Valid 1  | 5       | 50.0         | 50.0              |
|                  | 2       | 2       | 16.7         | 66.7              |
|                  | 3       | 1       | 8.3          | 75.0              |
|                  | 4       | 2       | 16.7         | 91.7              |
|                  | 5       | 1       | 8.3          | 100.0             |
| Total            | 12      | 100.0   | 100.0        |                   |

Statistical Tests for T₀ » T₆

The Mann-Whitney U test was used to evaluate differences in mean PAI score between groups for both immediate post-operative and 6 months follow-up evaluation.

Change in mean PAI score for each group from immediate post-operative to 6 months follow-up evaluation was tested with the Wilcoxon signed rank test.

Table(s) 23: Mann-Whitney U test for T₀ mean PAI outcome.

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>N</th>
<th>Mean Rank</th>
<th>Sum of Ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final</td>
<td>17</td>
<td>16.32</td>
<td>277.50</td>
</tr>
<tr>
<td>Er:Cr:YSGG - RFT</td>
<td>17</td>
<td>16.32</td>
<td>277.50</td>
</tr>
<tr>
<td>3%NaOCl + Ca(OH)</td>
<td>12</td>
<td>13.13</td>
<td>157.50</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mann-Whitney U</td>
<td>79.500</td>
</tr>
<tr>
<td>Wilcoxon W</td>
<td>157.500</td>
</tr>
<tr>
<td>Z</td>
<td>-1.082</td>
</tr>
<tr>
<td>Asymp. Sig. (2-tailed)</td>
<td>.279</td>
</tr>
<tr>
<td>Exact Sig. [2*1-tailed Sig.]</td>
<td>.325 a</td>
</tr>
</tbody>
</table>

(* not corrected for ties)

➢ The mean PAI score at T₀ (immediate post-operative) was not statistically different between groups

(U=79.5 ; W=157.5 ; p=0.279>0.05);
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

Table(s) 24: Mann-Whitney U test for T6 mean PAI outcome.

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>N</th>
<th>Mean Rank</th>
<th>Sum of Ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month_6th Er,Cr:YSGG - RFT</td>
<td>17</td>
<td>16,12</td>
<td>274,00</td>
</tr>
<tr>
<td>3%NaOCl + Ca(OH)</td>
<td>12</td>
<td>13,42</td>
<td>161,00</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table(s) 24:

- Mann-Whitney U: 83,300
- Wilcoxon W: 161,000
- Z: -5.72
- Asymp. Sig. (2-tailed): 0.383
- Exact Sig. [2*(1-tailed Sig.)]: 0.419

(* not corrected for ties)

The mean PAI score at T6 (6 month follow-up) remained not statistically different between groups

\( U=83.0; W=161.0; p=0.383>0.05 \);

Table(s) 25: Wilcoxon signed rank test – global sample.

<table>
<thead>
<tr>
<th>Month_6th - Final</th>
<th>N</th>
<th>Mean Rank</th>
<th>Sum of Ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Ranks</td>
<td>24a</td>
<td>12.50</td>
<td>300.00</td>
</tr>
<tr>
<td>Positive Ranks</td>
<td>0b</td>
<td></td>
<td>.00</td>
</tr>
<tr>
<td>Ties</td>
<td>5c</td>
<td></td>
<td>.00</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(* 6 months<immediate post-operative; 6 months>immediate post-operative;
6 months=immediate post-operative)

- Z: -4.329
- Asymp. Sig. (2-tailed): .000

(* based on positive ranks)

> Statistically significant differences were found between mean PAI scores from T0 to T6 for the global sample

\( p=0.000<0.05 \);
Table 26: Wilcoxon signed rank test – sample divided by treatment group.

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>Month_6th - Final</th>
<th>N</th>
<th>Mean Rank</th>
<th>Sum of Ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Er,Cr:YSGG - RFT</td>
<td>Negative Ranks</td>
<td>14a</td>
<td>7.50</td>
<td>105.00</td>
</tr>
<tr>
<td></td>
<td>Positive Ranks</td>
<td>0b</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ties</td>
<td>3c</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3%NaOCl + Ca(OH)</td>
<td>Negative Ranks</td>
<td>10a</td>
<td>5.50</td>
<td>55.00</td>
</tr>
<tr>
<td></td>
<td>Positive Ranks</td>
<td>0c</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ties</td>
<td>2c</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(a 6 months<immediate post-operative; b 6 months>immediate post-operative; c 6 months=immediate post-operative)

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>Month_6th - Final</th>
<th>Z</th>
<th>Asymp. Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Er,Cr:YSGG - RFT</td>
<td></td>
<td>-3.325a</td>
<td>.001</td>
</tr>
<tr>
<td>3%NaOCl + Ca(OH)</td>
<td></td>
<td>-2.848a</td>
<td>.004</td>
</tr>
</tbody>
</table>

*a based on positive ranks

➢ Statistically significant differences were found between mean PAI scores from T0 to T6 for both treatment groups (p=0.001<0.05 for test group and p=0.004<0.05 for control group);

Dichotomization of the Periapical Index (PAI) was proposed as secondary outcome measure to evaluate the proportion of teeth in each group that could be considered improved (decrease in PAI score) or healed (PAI ≤2).

Statistical significance between groups for the healed/not healed variable was analyzed with the χ² Monte Carlo simulation-test for the 6 month appraisal.

No statistical significance was found (p=0.69) in terms of healing distinction rates between groups.
12 MONTHS (T\textsubscript{12}) FOLLOW-UP STATISTICS

Descriptive Analyses

Table 27: Age (global sample) at T\textsubscript{12}.

<table>
<thead>
<tr>
<th>Age</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
<td>12</td>
<td>76</td>
<td>46,37</td>
<td>16,428</td>
</tr>
<tr>
<td>Valid N (listwise)</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 28: Age distribution between groups at T\textsubscript{12}.

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Er,Cr:YSGG - RFT</td>
<td>18</td>
<td>24</td>
<td>67</td>
<td>42,87</td>
<td>14,902</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5%NaOCl + Ca(OH)</td>
<td>12</td>
<td>12</td>
<td>78</td>
<td>51,98</td>
<td>17,753</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 29: Gender (global sample) at T\textsubscript{12}.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>17</td>
<td>56,7</td>
<td>56,7</td>
<td>56,7</td>
</tr>
<tr>
<td>Male</td>
<td>13</td>
<td>43,3</td>
<td>43,3</td>
<td>100,0</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100,0</td>
<td>100,0</td>
<td></td>
</tr>
</tbody>
</table>

Figure 31: Gender percentage distribution for 30 assessed participants at T\textsubscript{12}.
Table 30: Descriptive Statistics – Gender distribution between groups at T_{12}.

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Er,Cr:YSGG - RPT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>61,1</td>
<td>61,1</td>
<td>61,1</td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>35,9</td>
<td>35,9</td>
<td>100,0</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>100,0</td>
<td>100,0</td>
<td></td>
</tr>
<tr>
<td>3% NaOCl + Ca(OH)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>50,0</td>
<td>50,0</td>
<td>50,0</td>
</tr>
<tr>
<td>Male</td>
<td>6</td>
<td>50,0</td>
<td>50,0</td>
<td>100,0</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>100,0</td>
<td>100,0</td>
<td></td>
</tr>
</tbody>
</table>

Table 31: Appraised tooth number (global sample) at T_{12}.

<table>
<thead>
<tr>
<th>Valid</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>1</td>
<td>3,3</td>
<td>3,3</td>
<td>3,3</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>10,0</td>
<td>10,0</td>
<td>13,3</td>
</tr>
<tr>
<td>13</td>
<td>2</td>
<td>6,7</td>
<td>6,7</td>
<td>20,0</td>
</tr>
<tr>
<td>15</td>
<td>2</td>
<td>6,7</td>
<td>6,7</td>
<td>26,7</td>
</tr>
<tr>
<td>21</td>
<td>3</td>
<td>10,0</td>
<td>10,0</td>
<td>36,7</td>
</tr>
<tr>
<td>22</td>
<td>2</td>
<td>6,7</td>
<td>6,7</td>
<td>43,3</td>
</tr>
<tr>
<td>25</td>
<td>2</td>
<td>6,7</td>
<td>6,7</td>
<td>50,0</td>
</tr>
<tr>
<td>32</td>
<td>1</td>
<td>3,3</td>
<td>3,3</td>
<td>53,3</td>
</tr>
<tr>
<td>34</td>
<td>3</td>
<td>10,0</td>
<td>10,0</td>
<td>63,3</td>
</tr>
<tr>
<td>39</td>
<td>1</td>
<td>3,3</td>
<td>3,3</td>
<td>66,7</td>
</tr>
<tr>
<td>42</td>
<td>4</td>
<td>13,2</td>
<td>13,2</td>
<td>90,0</td>
</tr>
<tr>
<td>43</td>
<td>3</td>
<td>10,0</td>
<td>10,0</td>
<td>90,0</td>
</tr>
<tr>
<td>44</td>
<td>1</td>
<td>3,3</td>
<td>3,3</td>
<td>93,3</td>
</tr>
<tr>
<td>45</td>
<td>2</td>
<td>6,7</td>
<td>6,7</td>
<td>100,0</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100,0</td>
<td>100,0</td>
<td></td>
</tr>
</tbody>
</table>

Figure 32: Appraised tooth number (global sample) at T_{12}.
Table 32: Appraised tooth number - distribution between groups at $T_{12}$.

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Er,Cr:YSGG - RFT</td>
<td>12</td>
<td>5.6</td>
<td>5.6</td>
<td>5.6</td>
</tr>
<tr>
<td>13</td>
<td>2</td>
<td>11.1</td>
<td>11.1</td>
<td>16.7</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td>5.6</td>
<td>5.6</td>
<td>22.2</td>
</tr>
<tr>
<td>21</td>
<td>2</td>
<td>11.1</td>
<td>11.1</td>
<td>33.3</td>
</tr>
<tr>
<td>22</td>
<td>2</td>
<td>11.1</td>
<td>11.1</td>
<td>44.4</td>
</tr>
<tr>
<td>25</td>
<td>1</td>
<td>5.6</td>
<td>5.6</td>
<td>50.0</td>
</tr>
<tr>
<td>35</td>
<td>1</td>
<td>5.6</td>
<td>5.6</td>
<td>55.6</td>
</tr>
<tr>
<td>42</td>
<td>2</td>
<td>11.1</td>
<td>11.1</td>
<td>66.7</td>
</tr>
<tr>
<td>43</td>
<td>3</td>
<td>18.7</td>
<td>18.7</td>
<td>83.3</td>
</tr>
<tr>
<td>44</td>
<td>1</td>
<td>5.6</td>
<td>5.6</td>
<td>88.9</td>
</tr>
<tr>
<td>45</td>
<td>2</td>
<td>11.1</td>
<td>11.1</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3%NaOCl + Ca(OH)2</th>
<th>Valid</th>
<th>11</th>
<th>11.1</th>
<th>11.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>2</td>
<td>16.7</td>
<td>16.7</td>
<td>25.6</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td>8.3</td>
<td>8.3</td>
<td>33.3</td>
</tr>
<tr>
<td>21</td>
<td>1</td>
<td>8.3</td>
<td>8.3</td>
<td>41.7</td>
</tr>
<tr>
<td>25</td>
<td>1</td>
<td>8.3</td>
<td>8.3</td>
<td>50.0</td>
</tr>
<tr>
<td>32</td>
<td>1</td>
<td>8.3</td>
<td>8.3</td>
<td>58.3</td>
</tr>
<tr>
<td>34</td>
<td>3</td>
<td>25.0</td>
<td>25.0</td>
<td>83.3</td>
</tr>
<tr>
<td>42</td>
<td>2</td>
<td>16.7</td>
<td>16.7</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Figure(s) 33: Distribution of assessed teeth number in each group at $T_{12}$. 

Miguel Rui A. R. Martins 135
Table 33: Number of assessed patients and relative frequencies for each group.

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid ErCrYSGO - RFT</td>
<td>18</td>
<td>60,0</td>
<td>60,0</td>
<td>80,0</td>
</tr>
<tr>
<td>3%NaCl + Ca(OH)2</td>
<td>12</td>
<td>40,0</td>
<td>40,0</td>
<td>100,0</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100,0</td>
<td>100,0</td>
<td></td>
</tr>
</tbody>
</table>

Figure 34: Distribution of assessed patients between groups.

Table(s) 34: \( T_0 \) scores - global distribution (mean, SD and frequencies).

<table>
<thead>
<tr>
<th></th>
<th>Valid</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4,13</td>
<td></td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>4,042</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid</td>
<td>2</td>
<td>10,0</td>
<td>10,0</td>
<td>10,0</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>16,7</td>
<td>16,7</td>
<td>26,7</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>23,3</td>
<td>23,3</td>
<td>50,0</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>50,0</td>
<td>50,0</td>
<td>100,0</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100,0</td>
<td>100,0</td>
<td></td>
</tr>
</tbody>
</table>
Table(s) 35: $T_{12}$ scores - global distribution (mean, SD and frequencies).

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid</td>
<td>1</td>
<td>15</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>13</td>
<td>43.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>6.7</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Figure 35: Distribution of PAI scores (as percentages) for $T_0$ in all assessed patients.

Figure 36: Distribution of PAI scores (as percentages) for $T_{12}$ in all assessed patients.
**Table(s) 36:** PAI scores – control/test group distribution at $T_0$ (mean, SD and frequencies).

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>Frequency</th>
<th>Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Er,Cr:YSGG - RFT}$</td>
<td>Valid 2</td>
<td>11.1</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.8</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>22.2</td>
<td>22.2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>61.1</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>18</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>$3% \text{NaOCl + Ca(OH)₂}$</td>
<td>Valid 2</td>
<td>8.3</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>22.3</td>
<td>41.7</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>25.0</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>22.2</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>12</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**Figure(s) 37:** Distribution of PAI scores (in percentages) at $T_0$ for test and control group.
Table(s) 37: PAI scores - group distribution at T_{12} (mean, SD and frequencies).

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>N</th>
<th>Valid</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Er,Cr:YSGG - RFT</td>
<td>10</td>
<td>10</td>
<td>1.72</td>
<td>.956</td>
</tr>
<tr>
<td>3%NaOCl + Ca(OH)</td>
<td>12</td>
<td>12</td>
<td>1.33</td>
<td>.492</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>N</th>
<th>Valid</th>
<th>Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Er,Cr:YSGG - RFT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valid</td>
<td>1</td>
<td>7</td>
<td>38.9</td>
<td>38.9</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>56.0</td>
<td>56.0</td>
<td>100.0</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>11.1</td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>3%NaOCl + Ca(OH)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valid</td>
<td>1</td>
<td>8</td>
<td>66.7</td>
<td>66.7</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>33.3</td>
<td>33.3</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Figure(s) 38: Distribution of PAI scores (in percentages) at T_{12} for test and control group.

Statistical Tests
In similarity with the 6 month outcomes appraisal, the Mann-Whitney \( U \) test was adopted to evaluate differences in mean PAI score between groups at both immediate post-operative and 12 months follow-up evaluation.

Change in mean PAI score for each group from immediate post-operative to 12 months follow-up evaluation was tested with the Wilcoxon signed rank test.

**Table(s) 38:** Mann-Whitney U test for immediate post-operative (\( T_0 \)) PAI outcomes.

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>( N )</th>
<th>Mean Rank</th>
<th>Sum of Ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_0 ) \ Er\textsubscript{2}Cr\textsubscript{3}O\textsubscript{7} \cdot \text{YSGG} \ - \ RFT \</td>
<td>18</td>
<td>17.28</td>
<td>311.00</td>
</tr>
<tr>
<td>( T_0 ) \ 3% \text{NaOCl} + \text{Ca(OH)}\textsubscript{2} \</td>
<td>12</td>
<td>12.03</td>
<td>154.00</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[
\begin{array}{c}
\text{Mann-Whitney U} \\
\text{Wilcoxon W} \\
Z \\
\text{Asymp. Sig. (2-tailed)} \\
\text{Exact Sig. [2*(1-tailed Sig.)]} \\
\end{array} \begin{array}{c}
78,000 \\
154,000 \\
-1.403 \\
.144 \\
.185^* \\
\end{array}
\]

(* not corrected for ties)

- The mean PAI score at \( T_0 \) (immediate post-operative) was not statistically different between groups
  \((U=76.0 ; W=154.0 ; p=0.144>0.05)\);
Table(s) 39: Mann-Whitney U test for T_{12} PAI outcomes.

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>N</th>
<th>Mean Rank</th>
<th>Sum of Ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month_{12th} Er,Cr:YSGG + RFT</td>
<td>18</td>
<td>17.39</td>
<td>313.00</td>
</tr>
<tr>
<td>3% NaOCl + Ca(OH)_2</td>
<td>12</td>
<td>12.67</td>
<td>152.00</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Month_{12th}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mann-Whitney U</td>
<td>74.000</td>
</tr>
<tr>
<td>Wilcoxon W</td>
<td>152.000</td>
</tr>
<tr>
<td>Z</td>
<td>-1.615</td>
</tr>
<tr>
<td>Asymp. Sig. (2-tailed)</td>
<td>0.106</td>
</tr>
<tr>
<td>Exact Sig. [2*(1-tailed Sig.)]</td>
<td>0.156*</td>
</tr>
<tr>
<td>Exact Sig. (2-tailed)</td>
<td>0.140</td>
</tr>
<tr>
<td>Exact Sig. (1-tailed)</td>
<td>0.083</td>
</tr>
<tr>
<td>Point Probability</td>
<td>0.053</td>
</tr>
</tbody>
</table>

(*) not corrected for ties

➢ The mean PAI score at T_{12} (12 month follow up) remained not statistically different between groups

(U=74.0 ; W=152.0; p=0.106>0.05);

Table(s) 40: Wilcoxon signed ranks test – global sample.

<table>
<thead>
<tr>
<th>Month_{12th} - T0</th>
<th>Negative Ranks</th>
<th>Positive Ranks</th>
<th>Ties</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean Rank</td>
<td>Sum of Ranks</td>
<td></td>
</tr>
<tr>
<td>Negative Ranks</td>
<td>30</td>
<td>15.50</td>
<td>485.00</td>
<td></td>
</tr>
<tr>
<td>Positive Ranks</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Ties</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(ª 12 months<immediate post-operative; b 12 months>immediate post-operative; c 12 months=immediate post-operative)

<table>
<thead>
<tr>
<th></th>
<th>Month_{12th} - T0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z</td>
<td>-4.826</td>
</tr>
<tr>
<td>Asymp. Sig. (2-tailed)</td>
<td>0.000</td>
</tr>
</tbody>
</table>

(*) based on positive ranks

➢ Statistically significant differences were found between mean PAI scores from T_0 to T_{12} for the global sample

(p=0.00<0.05);
Table 41: Wilcoxon signed ranks test – sample divided by treatment group.

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>Month_12h - T0</th>
<th>N</th>
<th>Mean Rank</th>
<th>Sum of Ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>E, Cr:YSGG - RFT</td>
<td></td>
<td>18</td>
<td>0.50</td>
<td>171.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3%NaOCl + Ca(OH)</td>
<td></td>
<td>12</td>
<td>0.50</td>
<td>78.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(a 12 months<immediate post-operative; b 12 months>immediate post-operative; c 12 months=immediate post-operative)

Statistically significant differences were found between mean PAI scores from T₀ to T₁₂ for both treatment groups (p=0.000<0.05 in test groups and p=0.002<0.05 in control group);

The dichotomization of the Periapical Index (PAI) was proposed as secondary outcome measure to evaluate the proportion of teeth in each group that could be considered:

✓ Healed (PAI ≤2)
✓ Improved but failed to heal (decrease in PAI score).

Statistical significance between groups for the healed/not-healed variable was analyzed with Fisher’s Exact Test for the 12 month outcomes appraisal as follows.
Table 42: Cross tabulation between improved vs. healed conditions for each group.

<table>
<thead>
<tr>
<th>Treatment applied</th>
<th>T12_healed_fail</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healed</td>
<td>Fail</td>
</tr>
<tr>
<td>Er,Cr:YSGG - RFT</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>3%NaOCl + Ca(OH)</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 43: Chi-square tests.

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
<th>df</th>
<th>Asymptotic Sig (2-sided)</th>
<th>Exact Sig (2-sided)</th>
<th>Exact Sig (1-sided)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Chi-Square</td>
<td>1.429</td>
<td>1</td>
<td>.232</td>
<td>.503</td>
<td>.952</td>
<td>.267</td>
</tr>
<tr>
<td>Continuity Correction</td>
<td>0.201</td>
<td>1</td>
<td>.956</td>
<td>.503</td>
<td>.952</td>
<td>.267</td>
</tr>
<tr>
<td>Likelihood Ratio</td>
<td>2.188</td>
<td>1</td>
<td>.144</td>
<td>.503</td>
<td>.503</td>
<td>.267</td>
</tr>
<tr>
<td>Fisher’s Exact Test</td>
<td>1.361</td>
<td>1</td>
<td>.240</td>
<td>.503</td>
<td>.503</td>
<td>.267</td>
</tr>
<tr>
<td>Linear by Linear Association</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.267</td>
</tr>
</tbody>
</table>

➢ The preconditions for the applicability of $\chi^2$ and Monte Carlo-simulation tests were not fulfilled as 2 cells (50.0%) had expected count less than 5; Hence, the Fisher’s Exact Test was adopted and could be interpreted as follows:

➢ The observed differences of improved / healed cases, between the two groups were not statistically significant:

$$\chi^2(1)=0.201; \; n=30; \; p=0.503<0.05.$$
DISCUSSION

Errors using inadequate data are much less than those using no data at all.

Charles Babbage (1792-1871)
English mathematician, philosopher, and mechanical engineer
“The whole of medicine depends on the transparent reporting of clinical trials”(375). This clinical study included 2 groups: (1) NaOCl control-group, and (2) Er,Cr:YSGG (RFT) test-group, with sample sizes of 36 and 43 participants at the two final assessment moments. A significance level of 0.05 was adopted.

Patients were randomly assigned to treatment groups, and almost all root canal therapy was performed according to standardized endodontic procedures representing either the best and possible clinical protocols for undergraduates at the Faculdade de Medicina Dentária, University of Porto.

As most RCT reports provide adequate information about trial objectives and hypotheses we intended to follow the same procedure, stating the efficacy of a particular treatment. The hypothesis often tends to match the objectives (376); however, in practice, objectives and hypotheses are not always easy to distinguish.

Our hypothesis was the following: that necrotic teeth with CAP treated with the Er,Cr:YSGG laser and Radial Firing Tips (RFT’s) would demonstrate similar outcomes to those teeth treated with 3% NaOCl and calcium hydroxide dressing inter-appointment with a similar root canal preparation protocol. This hypothesis was far more specific than the objective: to compare radiographic evidence of periapical healing after root canal therapy, in order to assess predictable outcomes using the Er,Cr:YSGG laser for root canal disinfection devoid of any chemical substances. The hypothesis was, therefore, more suitable for explicit statistical evaluation.

While most trials assess the superiority of a new intervention, others are designed to simply measure equivalence or non-inferiority (377). Our initial hypothesis was that the treatment tested would prove to be the equivalent to that of the control group, which received a highly effective conventional treatment.
Method(s) and Trial Design

Since 1995 and increasingly in the last decade, there has been a movement towards evidence-based dentistry to scientifically evaluate the effectiveness of daily clinical treatments. This allows clinicians to use research findings as the basis for clinical decisions (378). Evidence-based dentistry was defined by the ADA (American Dental Association) as an approach to oral health care requiring the judicious integration of systematic assessments of clinically relevant scientific evidence, related to the patient’s oral and medical condition and history. It also takes into account the dentist’s clinical expertise as well as the patient’s treatment needs or preferences (379). However, many clinicians continue to base their clinical decisions on increasingly outdated primary training or over extrapolation of experiences with individual patients (380).

Since evidence-based dentistry is meant to encourage clinicians to provide the most contemporary treatment, the true benefits of applying evidence-based protocols in daily practice involve: (1) the application of the most updated treatment strategies and (2) stronger arguments to justify the treatment. With the vast amount of information accessible by way of digital technology, we can easily develop a standardized search protocol to ensure that the literature background is scientifically valid (381).

Hence, well-designed and properly conducted randomized controlled trials (RCT’s) are highly useful for providing the most reliable evidences regarding the efficacy of healthcare interventions. RCT’s should inclusively be considered the gold standard for evaluating treatment modalities due to their ability to minimize clearly identified and scrupulously reported bias (382).

RCTs normally require separating participants into one of two parallel groups in a blind randomized manner. However, fewer than 50% of published trials have reported such design. In order to be published in peer-reviewed journals, this investigation was always addressed as blind randomized clinical trial in order to carefully provide this information (383).

As alternative to RCTs, literature preconize multi-arm parallel, crossover and cluster designs as the ones to adopt (384).

Gutknecht et al. (211) reported a clinical trial evaluating the clinical effects of using Nd:YAG laser for root canal disinfection and assessing prospective endodontic outcomes; in a similar fashion, this research was intended to provide the first scientific clinical evidence addressing the effectiveness of an Er,Cr:YSGG laser assisted endodontic treatment.

However, due to its groundbreaking nature in the study of laser application in endodontics, we experienced expected as well as a few surprising obstacles.
First, it was necessary to carefully standardize the methodology and protocol plan in order to ensure future reproducibility (such as in other places, or laboratories). It is known that only through standardization (of simple but accurate methods) and transparent reporting we can judge the reliability and validity of different findings. In fact, results and conclusions depend heavily on what is called “internal validity” of the study, which pertains to the qualitative characteristics of the design and methodology of each research. Just a trial that has been conducted following appropriate guidelines is more likely to give reliable findings, improving or changing current clinical protocols.

In dental literature, a small number of studies have reported on study quality characteristics across all types of study designs, namely on randomized RCT’s in areas such as periodontics (385), prosthodontics (386, 387), implantology (386, 388, 389), orthodontics (390-392) or general dentistry (393, 394).

While in methodological analyses suggest that the report of inadequate trial designs are often associated with biased findings or excessive treatment effects (382), in a recent systematic review investigators have unfortunately shown that the reported quality of clinical trials in the latest issues of six dental journals with the highest impact factor is still relatively poor (395).

As mentioned above, depending on the type of investigation, guidelines have been compiled to aid researchers on how to report studies in biomedical literature; for conducting clinical trials, the CONSORT statement has been developed and contains key items with explanations that should be followed when reporting such studies. For other types of studies however, similar guidelines have been developed; whereas the STROBE initiative has established guidelines for conducting observational studies (including dentistry), the STARD initiative has drawn guidelines for complete reporting of studies of diagnostic accuracy (396-398).

Since 1996, the CONSORT (Consolidated Standards of Reporting Trials) statement has been adopted by hundreds of medical journals and international editorial teams, as well as five dentistry journals. Consequently, the quality of reporting trials has improved in participating journals, despite with significant variation between them (399).

The CONSORT statement was first published in 1996 and updated in 2001. The latter version consisted of a checklist and a flow diagram that authors can adopt while programming an RCT, in order to standard the critical appraisal and interpretation of all RCTs. After an expert meeting in 2007, the CONSORT statement was further revised (CONSORT 2010 Statement) to
include an explanation and elaboration document addressing topics such as selective outcome reporting bias.

Our primary concern was to adopt the CONSORT guidelines – explanation and elaboration - avoiding from the outset the adoption of inadequate methods and biased strategies which might compromise the findings of the present research; as biased results from poorly designed and reported trials can mislead decision making in health care at all levels, we have carefully scrutinized and adopted the guidelines from CONSORT 2001 followed by the CONSORT 2010 Explanation and Elaboration – updated guidelines for reporting parallel group randomized trials - to improve our investigation model while trying to eliminate as many detectable biases as possible (382, 400).

As a clinical trial, we should for instance, report the difficulty regarding the criteria adopted in order to determine the potential role of a specific bactericidal effect and its correlation to any given treatment outcome. In fact, even well-controlled clinical studies (such as other CONSORT studies) often fail to or found it difficult to appraise the real effect of a specific bactericidal agent or disinfection protocol through the evaluation of either clinical or radiologic evidences in endodontics (160, 175, 401, 402).

Probably related to the extreme difficulties in conducting controlled clinical trials in endodontics, almost all available literature demonstrating the effects of a specific agent or lasers on bacteria are performed in vitro. These studies represent an important step in scientific knowledge development, as they are easier to implement and to reproduce than clinical one’s. However, the literature demonstrates that most of in-vitro studies present bactericidal effects as a percentage of microbial deaths or log-steps reduction (208, 209, 315, 332, 333, 343, 344, 403, 404).

Such studies are useful in providing global understanding, publicity and overviews; however, they are thought to be unsuitable for scientific reports. Thus, it is interesting to point out that a killing effect of 99% when the initial bacteria population is in the order of $10^8$, will produce a remaining living population of $10^6$ bacteria, which is much more than the minimum necessary to (re)infect the root canal. Percentages should be therefore avoided when describing bacterial elimination or antibacterial effects. Percentages should only be used to compare different methods while the in vitro comparisons with an index - such as the bactericidal index – are thought to be much more appropriate and scientifically accurate (50, 217).

RCTs are scientifically recognized as the best approach for providing both safety and efficacy data. Nevertheless, they cannot or often fail to detect rare complications or adverse
effects due to the large amount of data (number of subjects/patients) needed to withdraw such assumptions (405).

We detected an adverse effect (characterized by swelling and pain related to tooth 4.5) in one single patient (M.F.F.), in the laser-treatment group one day following treatment. Although with a single amoxicillin-based antibiotic dosage and anti-inflammatory prescription all the symptoms disappeared, we do not believe that these adverse effects were related to the laser irradiation but, rather, to an immune reaction to an excess of root canal sealer which extruded slightly to periapical tissues. After remission, the patient returned to the normal follow-up schedule without further complaints. In fact, the periapical condition has continued to progress in a normal way ever since.

There is the danger that pool of participants and operators might not accurately represent the true population of patients and clinicians. Although eligibility criteria do not affect the internal validity of a trial, they must be reported as being applied prior to randomization. This is considered to be extremely important regarding the external validity of each trial.

While typical selection (inclusion) criteria relate to the nature and stage of disease under study, participants are often excluded due to concerns about potential danger to especially vulnerable patients (e.g. pregnant) or in an effort to ensure that the study satisfies both legal and ethical concerns (406). During the present investigation we carefully selected patients according to medical criteria and teeth diagnostic confirmed both clinically and radiographically. Although we could not confirm clinically the nature of the apical radiolucency (CAP versus apical cyst), its stage was set by diagnosing necrotic teeth with CAP diameter greater than 1mm. This constituted a mandatory criterion for inclusion in the study.

We excluded participants according to legal and ethical concerns (e.g. terminal stage patients, those with immune-compromising disorders, children under 12 years of age and pregnant women). Those with systemic disorder(s) or under medication that might influence either endodontic treatment outcomes or periapical healing prognosis were also eliminated from the pool of patients.

Endodontic prognostic factors may be considered subjective; they are often based on objective findings and definitely determined by the experience of the operator(407). We have excluded, patients with antibiotic use within the past month, those with antibiotic prophylaxis (e.g. bacterial endocarditis or immune-compromising disorders), as well as those suffering
from uncontrolled hypertension, diabetes mellitus, chronic renal failure, hematologic diseases, HIV, osteoporosis treated with bisphosphonates; participants receiving either steroid therapy exceeding 5mg/day of prednisolone or prior head and/or neck irradiation therapy were also ruled out.

It is possible that unknown variables, such as other systemic diseases or addiction, may have eluded our attention when deciding on inclusion/exclusion in this RCT. Deliberate exclusions included diabetic patients and those receiving radiotherapy due to the concern of possible delay in periapical bone reconstruction (408, 409). However, we did not exclude smokers despite some evidences which suggest that these patients can experience poorer outcomes; especially where lesions are found in cancellous bone (410, 411). The initial decision to include smokers was based on the potentially disappointing recruitment rate has they been excluded. Other well conducted RCTs studying periapical healing or clinical outcomes after root canal treatment have also elected to include smokers presumably for the same reasons (175, 401).

We also excluded multi-rooted teeth with apical periodontitis from both groups, due to the evidence that multi-rooted teeth have a lower probability of complete healing (compared with single-rooted teeth), representing an additional source of bias (412).

Teeth with more than 26mm in length were excluded due to the fact that the radial firing tips available for the present investigation were either 21 or 25mm in length. As irradiation was performed starting at 1mm from the working length, 26mm was set to be the maximum tooth length.

The single independent variable was the method of disinfection and respective protocol. We studied the endodontic treatment with the Er,Cr:YSGG irradiation (and radial firing tips) in the test group, while the use of both 3% sodium hypochlorite, and calcium hydroxide paste dressing inter-appointments was used in the control group.

Along with the eligibility criteria, information about settings and location are found determinant to judge the generalizability and external validity of a clinical trial. External validity refers to the question whether results can be generalizable to persons other than the population in the original study; however, the report of external validity determinants in trial publications and systematic reviews has often been found to be inadequate (413, 414).

Our study was performed at the University of Porto, Portugal and this information was clearly stated in all scientific reports, oral presentations and conference abstracts that have been published or submitted for publication (415-418) [Annex VII].
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

The environment in which the trial is conducted may differ reasonably from the one which the trial’s results are later applied, guiding clinicians in their evidence-based clinical practice. As a healthcare institution, it is arguable, but not likely, that the Faculty’s Clinic (Clínica Prof. Fernando Peres, Faculdade de Medicina Dentária, Universidade do Porto) can have a direct influence on the socio-economic and cultural characteristics of participants that might affect external validity. However, we predict that this protocol may be adopted by other clinicians in treating their own patients, generalizing the use of this laser-assisted technique regardless of the method used for root canal preparation.

The primary outcome measure is the one of greatest importance and is the one that normally used for statistical analysis and sample size calculation. Some trials have assessed more than one primary outcome, some of more interest than others. This can raise problems of interpretation associated with the range of analyses to performed and can increase the risk of false positive findings (419, 420).

We established as the primary outcome the clinical effectiveness of the Er,Cr:YSGG Laser Assisted Endodontic Treatment. In accordance, the aim of this blind randomized clinical trial (RCT) was to compare radiographic evidences of periapical healing after root canal therapy and predict potential outcomes using the Er,Cr:YSGG laser (Waterlase MD® – EndoLase Root Canal Therapy System®- Radial Firing Tips) for root canal disinfection, without the application of any chemical treatment. The PAI (Periapical Index scoring system) was used to evaluate radiographic healing such as proposed by Orstavik et al.(363). The PAI has proven to be one of the most reliable scoring systems, having become widely accepted and validated by scientific reviewers (364).

Although establishing more than one primary outcome is not recommended, researchers are widely encouraged to adopt secondary outcomes, including unanticipated effects of intervention. Nevertheless, all outcome measures should be completely defined and reported in order to allow others to use the same outcomes (421).

As secondary outcomes we looked at the proportion of teeth in each group that remained unchanged (same PAI score), improved (decreased PAI score) or healed (PAI≤2). In addition, the presence of unexpected clinical symptoms or abnormal findings during follow ups (e.g. spontaneous pain, presence of sinus tract, swelling, mobility, sensitivity to percussion or palpation) was checked through visual inspection, palpation and brief patient report. These data were registered, reported, but not submitted to analysis.
Random assignment has been successfully used to assign different treatment protocols since 1948 and has been accepted as the preferred method of assignment for more than 50 years (422).

When properly implemented, the most important advantage of randomization is to eliminate the source of bias in treatment assignment balancing both known and unknown prognostic factors. It can also serve as a blinding factor for investigators, participants, and/or evaluators. Finally, randomization enables researchers to use probability theories to express the likelihood of chance as a source for the difference between outcomes (423, 424).

Without randomization comparisons may be biased, consciously or not, through patient selection of a particular kind to receive a particular treatment (425).

Either for clinical trials or multi-group researches, participants should be assigned to comparison groups by chance, i.e. by a random process. The assignment should be therefore, unpredictable. If any restriction in the process of randomization occurs, it should be clearly stated as it can easily influence treatment outcomes. Randomization process with no restriction (i.e. a single sequence(s) of random assignment) is known as simple randomization. When the sample size is relatively large, simple randomization is expected to produce treatment groups with similar size and distribution of characteristics. Although more sophisticated and unpredictable it is superior to all restricted assignment processes regarding bias prevention (426).

Restricted randomization can be used to balance the groups in regards to characteristics such as size or demography (gender, age, ethnicity, etc.). Concomitantly, different types of restricted randomization could be implemented as follows:

1. **Blocking**: This process is often used to closely balance the numbers in each group at any time during the study. After every block, the number of participants in each group should be equal. Nevertheless, blocking tends to reduce the unpredictability of randomization (427);

2. **Stratification**: This method ensures that the numbers of participants receiving each intervention are closely balanced according to their individual characteristics. Stratified randomization can be achieved by performing a separate randomization procedure within sub-groups of participants (428); this a common restriction found in multi-centered studies (429);

3. **Minimization**: This measure is not properly randomization, but is thought to be the only acceptable alternative. Minimization can ensure balance between intervention groups for several patient factors. Randomization lists are not set up in advance. The
first patient can be truly randomly allocated and for the following patients the
treatment allocation becomes predefined (430);

For small clinical trials, restricted randomization (e.g. stratification) is considered to be
sometimes useful although it is complicated to implement and may be impossible if many
stratifying factors are adopted (382).

Methods of randomization, including stratification factors, are often poorly described
in clinical reports. In a baseline data collection, Assmman et al. reported that (1) there was
little consistency over the criteria for selecting baseline factors and (2) further baseline
adjustments were often unclear (420). Our participants were intentionally allocated in equal
numbers to each treatment group with a 1:1 ratio; only blocking restriction was applied.
Despite the fact that the credibility of the trial could have presented some weaknesses if
baseline characteristics had not been well matched (e.g. age, stage of disease, etc.) (428),
demographic characteristics were found not to be significant by chance. In fact, it is reasonable
to understand that in a randomized distribution, the statistical study of baseline characteristics
and distribution between groups are not necessarily wrong but illogical (431).

As discussed above, blocking can be used to ensure the close balance of the number of
participants allocated in each group at any time of the trial according to a pre-fixed ratio,
usually 1:1. Widely-speaking, improved balance comes at the cost of reducing sequence
unpredictability. This could introduce some bias if the person(s) running the trial were able to
deduce some of the subsequent treatments, knowing the block sizes (427).

In our study, four blocks (of 20+10+10+10 participants) were generated simply by
trying to achieve different endpoints with a close balance between the number of participants
per group. Therefore, we do not believe that knowing the block sizes would compromise the
randomized distribution. As a matter of fact, blocked randomization is the most highly
recommended form of randomization to pre-determine random group sizes when using block
randomization (432, 433).

Software’s used for random allocation usually support both simple and blocked
randomization. These software’s produce outputs as sequences of allocation based on the
selected type of blocking. Random Allocation Software v.1.0.0., Department of Anesthesia,
Isfahan University of Medical Sciences, Iran, was the Windows software used for this research;
blocks were easily generated and straightforward to implement.
Whereas only 34% of indexed PubMed trials in 2006 reported an adequate approach random sequence generation, more than 90% of these selected studies (30.6%) researchers used a computerized random generator or random table (434).

These findings appear to support the validity of the system we applied. However its importance is often neglected as random allocation of treatment was reported only in 15% of interventional studies that were published in the highest impact factor dental journals (395).

Allocation concealment defines how the sequence is applied when the participants are enrolled in the trial. Without adequate allocation, unpredictable assignment can be easily subverted. Thus, a mechanism such as a generated allocation schedule can prevent the foreknowledge of treatment assignment (435).

During this trial, no allocation schedule was applied. However, the decision to accept or reject a participant together with the signing of informed consent was made in complete ignorance of the randomly assigned treatment. This process was chosen in accordance with the CONSORT guidelines; it prevents selection bias and protects the assignment sequence until allocation (382, 436). Hence it is confusing why, in a 2005 report from Lancet, only 18% of all randomized trials indexed on PubMed reported the allocation concealment mechanism(s) and some of those were even considered inadequate (383).

As in case of other trials, non-pharmacological treatment outcomes can be assessed at several endpoints following randomization. Hence authors should specify whether any predetermined endpoint is of primary interest (405). We determined two major endpoints for follow up and, therefore, for outcome assessment and analysis: 6 months and 12 months after the treatment (postoperative radiograph). Both periods were established taking into consideration the extended duration needed for chronic apical periodontitis recovery and bone rearrangement.

In order to test both safety and efficacy, the 6 month appraisal was used to evaluate the need for any adjustment regarding treatment efficacy in the test group. Decisions about interim analysis and early interruption of clinical trials, as based on recommendations of Data Monitoring Committees (DMCs), have high consequences in the scientific validity and clinical impact of a trial (437). Hence, the 6-month outcome assessment served as interim-analysis and enabled the decision of whether to continue recruiting or not.

For organizational reasons, it is rarely desirable to stop recruitment and procedures during an interim analysis. So, new study patients were included in the trial while the interim
outcome assessment was ongoing as reported in Faldum et al. (438). We therefore never stopped recruiting and at 12 months we appraised the results.

Some researchers argue that bone healing of root filled teeth should be followed up to 7 or more years, and 4 years has been proposed as the standard. However, clinical practice and follow-up studies have demonstrated that a large proportion of cases with chronic apical periodontitis or even periapical surgeries shows signs of bone healing within 12 months of follow-up, while in some cases 2 to 4 months were enough. Thus, 12 months of follow up can be considered a determinant and consensual endpoint for evaluating the radiographic features of periapical healing (439-442).
Endodontic criteria for successful treatment and outcome evaluation

The primary objectives of root canal therapy are the removal of the pathological pulp, the cleaning and shaping of the root canal system, the disinfection of contaminated root canals, and obturation of the root canal system in three dimensions in order to prevent re-infection. The successful completion of these steps should result in the maintenance of normal radiographies, a lack of clinical symptoms and the absence of post-operative apical lesions. Similar radiographic and clinical outcomes should be expected when the same principles are applied during root canal therapy in teeth with preoperative apical lesions (chronic apical periodontitis or cysts) (443).

However, there is debate, currently, regarding different methods for evaluating outcomes in endodontics. In fact, what can be considered the “success rate” in root canal therapy? What kind of evidences can be provided? Can results be compared between similar or alternative treatment methods? All these issues can be further debated if the patient and the dentist have different expectations of ideal outcomes.

In dentistry, patients are usually satisfied as long as their teeth are functionally and aesthetical pleasing, in the absence of any clinical symptoms. By its turn, insurance companies tends to measure success by way of survival rates whereas endodontists are arguably most concerned with the absence of both clinical and radiographic symptoms.

In endodontics however, there are additional issues that can be addressed when evaluating endodontic success. Prognostic factors such as the presence of a preoperative radiolucency, the periodontal condition of the tooth, the quality of the fill and its length, and the quality of the coronal seal are often considered decisive. On the contrary, host factors such as systemic diseases appear to have little correlation with endodontic success (410, 444).

As far as back 1966, Bender and co-workers identified some of the factors that could define the success of a root canal treatment. Clinical and radiographic examinations were, and still are, the most common strategies applied to assess outcomes. If radiographic interpretation is often subject to personal bias and simple changes in angulations can provide a completely different appearance to the teeth and/or the lesion - making it appear smaller or larger -, clinical symptoms such as pain, swelling, and presence of sinus tract can occur without radiographic evidence of bone destruction. In addition, not all radiolucencies located in the periapical area are thought to be of pulpal origin (445, 446).

Most of the classic studies reported endodontic outcomes with artificially low success rates. This may be attributable to the overly strict definitions of success, such as total radiographic healing or lack of any adverse signs or symptoms (447, 448). For instance,
Metzger et al. adopted the following four categories based on radiographic assessment (449): (1) “no healing”, no reduction in the size of the lesion or enlargement of the lesion; (2) “minor healing”, a clear, but minor, decrease in the size of the lesion; (3) root canal treatment; (4) “advanced healing”, a substantial decrease in the size of the lesion but not a complete healing; and (5) “complete healing”, the lesion disappeared completely. Some residual widening of the periapical periodontal ligament could accompany as complete healing.

Following root canal therapy, there is the potential for a transient increase in radiolucency due to chemical and/or mechanical irritation that usually reverts to normal. Then, repair of periradicular tissues will consist of a complex regeneration involving bone, periodontal ligament and cementum as the area of mineral loss gradually fills with bone and radiographic intensity increases (450).

Healing after endodontic therapy is frequently monitored by radiographic studies over time, through periodic recalls. Following treatment, the contours and width of the periodontal ligament and lamina dura often return to normal but the structure of newly formed bone may differ, often being less organized. In turn, if the cortical plate becomes perforated, healing will begin with the regeneration of the external cortical plate and proceeds from the outside towards the inside of the lesion (97, 450).

Time frames for the healing of endodontically treated teeth have been, until now, poorly known but, some promising studies are slowly revealing interesting findings as regards to the periapical healing. These processes involve the activation of extracellular matrix components, growth factors, remodelling enzymes, cellular adhesion molecules, cytokines and chemokine genes. However, the molecular patterns underlying the healing process in the periapical environment remain unclear. Authors now suggest that the differential expression of wound healing markers in active and latent granulomas might account for the differences amongst the clinical outcomes for such lesions (451, 452).

Such findings suggest significant and previously unknown biases presumably associated with all clinical studies to date. In our study, all patients were, by chance, Caucasians and we therefore believe that genetic differences probably played a minor role in periapical healing when compared with other known factors such as compromising systemic disorders (e.g. osteoporosis) which were excluded from the study.

Maxillary premolars and mandibular incisors were more difficult to appraise during the radiological assessment namely while trying to adopt similar horizontal angulations during the recalls. These results are in good agreement with those of Gijbels et al. (453).
Other techniques such as cone beam tomography (CBT), ultrasound and colour power Doppler have recently been proposed to measure periapical healing more efficiently. While, on average, the periapical radiolucency measured (1 week post-operatively) on periapical radiographs was found to be approximately 10% smaller than that on CBT images, more remaining bone defects were detected after 12 months on CBT images than on periapical radiographs. However, how this information can be linked to the relation between success and failure is not yet clear (454, 455).

Despite the presumable accuracy of new imaging techniques compared to manually processed radiographs and visual assessment, the costs of eliminating this bias from the present study would be prohibitive.

**Interpretation bias and consistency**

When signs of disease are large and exacerbated, problems of interpretation hardly exist. The periapical radiolucency, with a tapered periodontal ligament into the normal areas at the root contours and an absent *lamina dura*, strongly suggest the presence of chronic apical periodontitis. Furthermore, if it is associated with clinical findings confirming pulp necrosis, it can be considered pathognomonic.

However, problems may arise in the radiographic interpretation of the transition between normal periapical/bone structures and minor signs of disease. Great variability has been repeatedly documented within and among observers of the radiographic assessment of periapical radiolucencies. There are several factors contributing to this variability although they may not be related to the apical radiographic images. For example, observer bias related to factors such as the need for treatment, root filling defects or even knowledge of the apical status of the same tooth at previous consultations may have an influence (368, 370, 456).

Some bias may result from the diagnosis of each apical radiolucency, as apical periodontitis is often regarded as a signal-detection task (371). Although it may be regarded as a very prevalent problem, the incidence of apical periodontitis in a cohort study is often difficult to determine by radiologic means (98, 457). This suggests that healing frequencies should not suggest an absolute meaning but just a relative one.

False-positive diagnoses can be minimized through the correct interpretation of anatomical structures and reviewer experience (135); not being sufficient to overcome radiographic sensitivity issues, negative reactions to vitality tests and, in some cases, histological analysis are then considered preponderant as information to act on diagnosis (458).
Although no patognomonic histological tests were used for confirmation, there appeared to have been one clinical case compatible with a periapical cyst in a patient (R.S. - teeth 2.5), within the Er,Cr:YSGG laser group. Controversy currently exists regarding the potential for heal of inflammatory apical cysts following nonsurgical endodontic therapy and how does the lining epithelium of these cysts regress after endodontic therapy.

Based on a wide-ranging molecular biology perspective, Lin et al. recently suggested that epithelial cell rests are induced to divide and proliferate by inflammatory mediators, pro-inflammatory cytokines, and growth factors released from host cells during periradicular inflammation. Thus, after endodontic therapy, epithelial cells in epithelial strands of chronic apical periodontitis and the lining epithelium of apical cysts may stop proliferating due to the reduction of inflammatory mediators, pro-inflammatory cytokines, and growth factors. Epithelial cells might also regress because of apoptosis activation, programmed cell death (through deprivation of survival factors) or even by receiving death signals during periapical wound healing (459). Our findings support Lin’s theory. After assessing the outcome of this particular clinical case treated with the laser assisted endodontic treatment, we can further suggest that this protocol may be a valuable nonsurgical treatment for periapical cysts of endodontic origin.

Although most dentists state that their colleagues would make similar decisions regarding the necessity of endodontic (re)treatment, variations between general dentists and endodontists actually differ substantially in their diagnostic and treatment decisions concerning the assessment of disease probabilities and future complications. In this case, there is evidence that endodontists are more prone to treat smaller and medium sized lesions than are general dentists (460-462). In our study all the reviewers were experienced endodontists who may not have echoed the general opinion regarding the treatment decision criteria. However, in order to ensure a diagnosis compatible with CAP, only lesions with a diameter larger than 1mm were selected. This criterion not only ensured an initial PAI score≥2 but, as it was associated with negative responses to pulpal tests it became pathognomonic of periapical disease requiring endodontic treatment.

The use of previously developed and validated scales should be used and reported in order to enhance the measurement quality and to assist the comparison between different studies (463, 464). In our study we have tried to perform accordingly to these premises and for the present clinical trial the “periapical index (PAI)” was chosen. It is primarily based on reference radiographs with verified histological diagnosis originally published by Brynolf in
1967 (465). It was subsequently described for periapical radiographs, but occasional epidemiological studies have also used it for panoramic radiographs, or even a combination of both (466-468).

The PAI provides an ordinal scale of five scores ranging from (1)“healthy” to (5)“severe periodontitis with exacerbating features”. The preoperative PAI score was not used as initial inclusion criteria for this study, although the requirement that all teeth must have a visible periapical radiolucent area at least 1.0x1.0mm assured an initial PAI score ≥2.

This scoring system can be considered an ordinal scale but there is more than one way to analyze data generated (469, 470). Some investigators have converted data extracted from the PAI into a nominal scale with a healed/not healed dichotomous outcome (142), and others have treated the PAI as interval data and analyzed findings with a parametric test (analysis of variance or t-test) (469, 471).

We adopted the PAI because it is often found in similar clinical trials and is very straightforward and simple to use. In fact, we were most interested in differences in healing between groups as measured by changes in the mean PAI score, expecting the data to exhibit a reasonably normal distribution.

Although there seems to be no standard criteria for the registration of apical periodontitis either with periapical or panoramic radiographs, the PAI has also been applied to epidemiological (472, 473) and comparative clinical studies (474, 475). Moreover, the comparison amongst studies with previously calibrated reviewers has proven the PAI system to be thorough, reliable and attractive (476).

Our calibration results are in accordance with the reliability demonstrated by previous authors (364). The intra-rater reliability value was 0.95, whereas the inter-rater agreement was 0.85 in a scale ranging from 0 to 1. According to the criteria proposed for strength of agreement by Landis et al. (372), these results were considered an “almost perfect agreement” (0.81-1.00). Hence, the blind evaluation of periapical radiolucencies can be considered appropriated and reliable between and amongst outcome assessors.

The term “blinding” is often used to refer to the withholding of information about the assignment group from people in the trial who may be influenced by this specific knowledge. Benjamin Franklin is known to be the first to implement blinding criteria in a scientific report in the 18th century (375). As the average bias associated with defects in the conduct of RCTs varies according to outcome types, blinding is mandatory to ensure protection against bias, particularly when assessing subjective measures such as pain or, in our study, the visual assessment of periapical radiolucencies (477).
Participants often respond more favourably when they receive the new therapies or just according to the treatment assignment. Unblinded investigators may introduce similar biases, and unblinded data collectors may assess outcomes differently. Data analysts may, in turn, introduce bias when selecting analytical strategies such as endpoints or when deciding whether to remove patients from the analyses (477).

During this study, patients and undergraduate clinicians were necessarily aware of the allocated group, whereas outcome assessors and data analysts were kept blinded.

As the radiographic scoring was performed by two experienced endodontists blinded to the treatment allocation, and the statistical analysis only utilized their appraisal to determine our results, we strongly believe our conclusions to be free of bias. We also believe that, despite the fact that the patients and the main investigator (who performed the laser irradiation) were aware of the treatment allocation it is unlikely that they could have had any possible influence on treatment outcomes or healing processes.

Blinding of participants and operators is often difficult or even impossible in surgical procedures but the blinding of data collectors and outcomes adjudicators is usually easy to implement. Lesions can be photographed (or radiographed) before and after treatment and during follow-up and are then assessed by a blinded (external) observer (478), as occurred in our study.

Except for the treatment providers (including the main investigator who needed to perform the laser irradiation and explain the protocol to the other participants) other investigators and staff members were kept blind to the group assignment. In fact, and regardless of the fact that they had a strict protocol plan to follow for each intervention, only the undergraduate clinicians - who were impossible to blind - could directly influence the treatment outcomes through their ability or *modus-operandis* while performing the root canal preparation, irrigation and obturation.

In recent years, endodontic therapy has undergone a marked development along with the increase in demand for endodontic treatments (479) and a wide range of long term success rates have been reported by different authors, from 70% (480) to more than 90% (34, 481).

Endodontic performance could be modified by numerous items and during recent decades all procedures and protocols used to perform successful treatments can be dependent of non-operator issues (e.g. patient health status or material related failures).

One of the most relevant findings of a 10-year retrospective evaluating the influence of different factors on the root canal filling qualities, was that there was no statistically significant
difference in operator related parameters when comparing qualified dentists and supervised undergraduate students. The concern that fillings performed by students might have poorer survival rates than those performed by qualified dentists was not confirmed. Even the comparison of the relative percentages of molars treated in the individual groups shows that the proportion treated by students (39%) was, in fact, slightly higher than that treated by qualified dentists (35%). This favorable but unexpected result may be attributed to the distribution of problematic cases, which tended to be delegated to qualified dentists after the initial clinical examinations (482). A significantly higher rate of patient satisfaction was found in patients treated by endodontists (explained by the increased skill and proficiency of specialists) (483). However, the results of treatment under quality-controlled training conditions were found no poorer than those of treatment performed by experienced dentists (482).

The clinicians in this study were undergraduate faculty-resident students, arguably less skilled than many experienced general dentists or any endodontist/specialist. Therefore, the treatment environment might not be truly representative of either general dental practice or specialty practice settings. However, all teeth treated were anterior or premolars which are often thought to present fewer treatment challenges than posterior teeth. In addition, treatment steps were always performed under supervision, with the opportunity for consultation and assistance as needed.

Data analysis can be performed in several ways, some of which may not be strictly appropriate for one particular outcome assessment or situation but an adequate sample size and increased power highly reduce the chance of false negative results (484).

The size of effect is often inversely related to the sample size necessary to detect it, which means that large samples are necessary to detect small differences. Hence, in randomized studies, the larger numbers of patients and longer follow-up times were more likely to receive positive reviews from the Health and Clinical Excellence recommendations for interventional procedures; however, the association between type and amount of clinical evidence can be considered an arguable element in an RCT. Ideally, a study should include a sample size large enough to ensure the probability (power) of detecting a clinically important difference in a statistically significant way (485, 486).

Nevertheless, it can be also considered that some information is better than no information. Thus, some methodologists have suggested that well-controlled trials should be always reported and acceptable because they can be further used either for systematic reviews or meta-analyses. It is currently under debate whether small trials can individually
present “sufficient power”. Nevertheless, independently of the estimated power or whether the trial attained its planned number of participants, authors are being encouraged to report their researches, with the accurate description of their hypothesis, methods and limitations (487, 488).

Small sample studies frequently lead to the erroneous conclusion that the intervention groups did not differ, when in fact too few patients were assessed to make such assumption (489). In reality, small but clinically meaningful differences are much more frequent than large differences; however, larger sample sizes are required to detect them (490).

In resemblance with the majority (93%) of clinical studies published within the six leading dental journals (395), we did not report the estimated sample size as we intended to recruit as many participants as possible. In fact, compared to the Journal of Clinical Periodontology, other leading dental journals such as Journal of Endodontics showed a 77% to a 94% significantly decreased odds of publishing an article reporting sample size calculation. Nevertheless, our arguably small sample size (63 participants) is, by matter of fact, consistent with the median of 54 patients in 196 trials in arthritis, 46 patients in 73 trials in dermatology or 65 patients in 2000 trials related to schizophrenia and parallel groups (491-493).

The confidence interval for the estimated effect indicates the range of uncertainty for the true treatment effect. This may be interpreted as the range of values for the treatment effect that is compatible with the observed data. Usually a 95% confidence interval is presented, which is within the acceptable range of 95 to 100 similar studies. In addition, scientific findings can also be assessed in terms of their statistical significance (p value); this represents the probability that the observed data could have transpired by chance - when the interventions did not truly differ. If relatively small p values (e.g. p=0.003) are strongly recommended, less precise thresholds of p<0.05 can be found in similar CONSORT clinical trials (175, 382, 401).

Our study reported a statistical significance of p<0.05. Within the six highest ranked dental journals, at least 8 of 10 published articles presented a statistically significant main finding; however, 2% did not provide any statistical p values, whereas only 13% presented confidence intervals. (395).

Standard methods of analysis also tend to assume data as “independent” which means that there is one observation per participant. However, treating multiple observations from one participant as independent data can be a serious error; such data are produced when outcomes can be measured on different parts of the body, as in dentistry. As a result, data
analysis should be based on counting each participant once or by using more complex statistical procedures (494, 495).

Within the present investigation we assumed data as independent when evaluating the mean reduction of PAIs for each group, at different endpoints. We assumed that, whilst comparing mean values between groups we could independently analyse the statistical significance of each treatment (from post-operative to the 6 and 12 months follow-ups) and between treatments. Therefore, we did not observe the treatment effectiveness for each patient independently rather for each group as a whole.

However, as secondary outcome assessment, we count participant as individuals when analysing the PAI changes. So, for each participant we were also interested if the PAI score remained unchanged, improved (lowered PAI score) or become considered as healed (PAI<2). We excluded from analysis teeth extracted (e.g. due to prosthetic reasons or endodontic failures) prior follow-up as these teeth were not available for the 6 and 12 month radiographic assessment.

**Results:**

The flow of participants in some RCTs is straightforward, particularly when there are no dropouts, losses or exclusions; each trial phase can be described in a few sentences. In more complex studies, however, it can be difficult to explain whether and why some participants did not receive the allocated treatment, were lost to follow up or why they were excluded from analysis (496). Therefore, the CONSORT statement strongly recommends diagrams to describe complex trial phases (382). The exact form and content of the flow diagram vary according to the trial’s specific features. We, however, used CONSORT 2010 suggested guidelines and template(s) for both endpoints, separately, as we found them to be a more thorough, understandable and reasonable way to illustrate the flow of participants without neglecting any relevant information.

The number of people assessed for eligibility is relevant for the trials’ external validity, being a useful indicator of whether the trial participants were likely to be representative of the population (433).

At the 6-month appraisal, 51 participants were assessed for eligibility and 36 were allocated (373), whereas at the 12-month analysis 62 participants were assessed for eligibility and 43 were allocated. Therefore, we consider that the majority of eligible participants were in fact included in the trial despite our strict inclusion criteria.
The “intention-to-treat” strategy in a clinical trial is not always simple to implement as participants do not always go through the full protocol as they may dropout or present characteristics making it impossible to assess their final progress. Although the exclusion of participants from the analysis can lead to erroneous conclusions, it can be restricted to the patients who fulfill the protocol in terms of eligibility, interventions and outcome assessment. This is known as an “on-treatment” or “per protocol” analysis and may be considered appropriate (374).

We adopted the “per protocol” strategy, as we did not find it reasonable to estimate either the PAI of an extracted tooth, the score of participants who failed to follow up, or assessing outcomes in patients that did not receive adequate treatment. Otherwise, these could have directly influenced the mean PAI scores.

Knowing the number of participants who did not complete the treatment might be also relevant as it can test and compare the level of compliance to the new therapy protocol and concept – laser assisted endodontic treatment- to one traditional strategy.

Hence, participants who were excluded from the study after randomization and allocation (n=6 in total after 12 months, 4 in control group and 2 in test group) were a function of protocol deviations; they were unlikely to be representative of all participants. Said protocol deviations were related to: lack of the required two-visit treatment plan not completing the endodontic treatment (n=1, control group); disrespect for the maximum interval (<24 days) allowed to complete the root canal treatment (n=3, in control group); treatment deviations regarding the working length maintenance, 1mm from the radiological apex, resulting in over-obturated canals with gutta-percha placed beyond the apical constriction (n=2, in test group).

The literature warns that a widened periodontal ligament may be associated with an excess of root filling material, leading to inflammatory changes due to either persistent toxicity or via bacterial colonization. This reaction may be considered pathological as histological analysis repeatedly demonstrates extensive granuloma formation around surplus material with a corresponding resorption of the surrounding bone (497, 498). In fact, the investigators excluded these last two participants.

Another patient was excluded from the analysis due to a prosthetic decision, determining the extraction of the treated tooth and it’s replacement with an acrylic removable prosthesis.
The literature describes reasons for the increasing dropout rates in endodontic follow-up studies (34, 499, 500). Thus, participant loss to follow-up is unavoidable and needs to be distinguished from investigator-determined exclusion (e.g. ineligibility or withdrawal from treatment) or poor adherence/deviation to/from the protocol. Otherwise, erroneous conclusions can be arrived at if participants are excluded from analysis; such omissions between groups are especially considered as indicative of bias in the treatment effect estimates (501, 502).

Missing outcome data are a common problem in randomized controlled trials, and are often inadequately handled in the statistical analysis. One of our intensive tasks was to persuade every patient to show up for the follow-up assessment. At both 6 and 12 months recalls, persistence and high motivation were needed to convince all participants that the radiographic control was part of the signed informed consent and the participant agreement. These efforts contributed to the relatively low dropout rate at the 12-month recall (n=4, 3 in control group and 1 in the test group, representing 9.3% of all allocated patients). These participants were obviously not included in the statistical analysis.

Our loss to follow-up rate is consistent with the report of Wood et al. which reviewed 71 clinical trials published in the most representative medical journals in 2001 and reported that more than 10% of randomized patients usually miss the outcome assessment (503).

Nevertheless, the adherence to our trial protocol and follow-up assessment can be considered rather surprising as no financial incentive was offered to any patient. Furthermore, schedules of participants and the investigation team were not always compatible, which could have easily compromised the adherence rate.

Other studies have reported that patients who are lost to follow-up have often experienced either an acute exacerbation of symptoms or even harms of treatment (430, 501). Interestingly, the single patient in our study who reported acute symptoms (pain and swelling) after the root canal filling never missed the recalls and was not excluded from analysis.

Following root canal therapy, the probability of periapical healing increases over time, and some authors have suggested that a period of four to five years might be necessary to adequately evaluate healing (179, 504). Yet due to practical constraints on clinical resources and the difficulty of controlling patients over time, many studies have used 12 or fewer months as an end point (469-471).

Therefore, although longer observation periods would be ideal, evidence of periapical changes in bone density associated with healing should be apparent after a few months (12) when using the PAI scoring system, and longer observation periods might not be necessary to demonstrate real treatment effectiveness (505).
Clinical outcome studies take a long time, demand substantial economic resources, and run the risk of losing patients at follow-up. Therefore, it is desirable to find simpler but accurate surrogate endpoints for such investigations. Identical conclusions were drawn after both microbiological and clinical/radiographic evaluations (506). Thus, the notion that postmicrobiologic sampling might replace radiographically-based long term studies was supported as was the assessment’s use as a surrogate endpoint (401). The idea that the absence of cultivable microbes at the time of obturation will favor healing is consistent with the notion that microorganisms are the prime reason for persistent apical periodontitis. However, at a more case-specific level, the relationship between the results of microbiologic analysis and outcomes is not yet clear.

The effectiveness of a clinical strategy must not be evaluated merely from a biological point of view but factors such as costs, patient comfort, and treatment effort should be assessed as well (507). Hence, it is important to search for one-visit treatments biologically effective as two-visit ones either with sodium hypochlorite/calcium hydroxide association or the Er,Cr:YSGG laser assisted endodontic treatment.

Despite the fact that the sample size is likely to fall below the minimum for assuring adequate data analysis power, the intention was to extend follow-up radiographic and clinical evaluation on patients in this study as longer as possible.

Early interruption of trials for reasons apparently independent of findings are unlikely to introduce bias by stopping (508). Although a large number of recruited patients achieved complete apical healing, we intend to continue following those patients whose teeth were not completely healed. Thus, due to the length of follow-up needed for further statistical analyses, this trial recruitment was temporary held independently of the outcome assessment and clinical findings.

It is our intention to continue further clinical investigations, despite the lack of financial incentives and the costs of using the laser equipment. Either we shall expand this protocol to molar teeth with more intricate root canal anatomies, or to more complex clinical cases such as, for instance, laser assisted endodontic surgeries.
CONCLUSIONS

*Science is a way of thinking much more than it is a body of knowledge.*

Carl Sagan (1934-1996)
Astrophysicist and cosmologist
The whole of medicine arguably depends on the transparent reporting of clinical trials and RCTs are recommended as the best approach for providing safe and efficacious data (375).

The only pre-defined independent variables were the disinfection mechanisms whether those of the test group applying the Er,Cr:YSGG laser irradiation with endodontic radial firing tips, or those of the control group irrigating with 3% sodium hypochlorite (plus calcium hydroxide dressing inter-appointments). The 3% NaOCl concentration was chosen for the control group not only because it is though appropriate for endodontic irrigation in necrotic tooth cases, but mainly due to the fact that it is currently used as standard protocol at the University of Porto.

In fact, one precondition for this clinical protocol regards the concern of minimum interference with the undergraduate students’ daily clinical protocols and schedules; so, it seemed a valid strategy not to change previous routines. Using students with little experience as operators relied on the fact that they could easily be blinded to treatment allocation and, in the second place, we could easily justify the need for calcium hydroxide dressing between appointments as it could be seen as a complement for bacterial eradication in cases of rather poorer instrumentation and irrigation techniques.

The need for two appointments would also be easily understood as both efficient and predictable in disinfecting necrotic root canals. Despite the scientific debate about the need for two appointments, this pattern was maintained throughout the investigation in order to ensure reliable operating conditions and sufficient time to fulfill both protocols.

Due to the fact that all operators were undergraduate students of the same university, with similar training conditions and background knowledge, we must assume that all endodontic procedures were more or less accomplished in both groups, in a randomized situation. Although our success rates should not be discussed or compared with other external sources, in terms of external validity, it can always be extrapolated, as our protocol seems to be very simple to reproduce or even possibly upgrade.

Following the present methodology and restricted criteria, we aimed for enough outcomes to obtain a non-inferiority approach between the two treatment plans (to achieve at least similar clinical outcomes). Moreover, with the increase in the number of participants and longer follow-ups we also thought that faster healing processes of periapical chronic periodontitis in the laser assisted endodontic treatment group might be observed.

This particular expectation is related to the interpretation of previous in vitro studies, which state that the Er,Cr:YSGG has considerably deeper dentinal penetration - and therefore...
bactericidal efficiency - than sodium hypochlorite, regardless of its concentration or irrigation volume. The biophysical interpretation of having scattered light penetrating into the surrounding tissues, also raises the possibility of having biostimulation effects, which could help to increase the scarring process, decreasing periapical radiolucency faster than in the control group.

The sample size might not accurately represent the true population of patients and treatment effects may be underestimated by this limitation. For this RCT, the sample size was restricted by the inclusion/exclusion criteria at the time of the volunteer enrollment. The sample size was also determined by the limited time for recruitment, the restricted undergraduate clinical schedules and the long follow-up assessments combined with the need for reporting outcomes in peer-reviewed publications.

To date there is strong scientific evidence suggesting factors that might affect endodontic outcomes and that could be discussed from the theoretical (microbial nature, host response or epithelium surrounding CAP) and clinical (preoperative factors, intra-operative factors and miscellaneous factors) points of view. In addition, there are numerous prognosticators more strongly implicated than the simple absence or presence of pre-operative radiolucency; periodontal tooth conditions, filling quality, and coronal seal adaptation should also be considered as key factors when analyzing endodontic success rates along long periods of follow-up.

Therefore, many of the classic endodontic studies show artificially low rates of success because of stringent definitions of positive outcomes, such as total radiographic healing or a complete lack of signs or symptoms which may be very difficult to obtain in short post-operative evaluations.

It is debatable whether it is reasonable to proclaim “endodontic success” within 6 months of treatment. Instead, a 12-month assessment is assumed to be mandatory.

It is remarkable that there was not a single endodontic failure in the laser group within the first year of follow-up. The two failures belonged to the control group. However, despite these two treatment failures - which were excluded from statistical analyses - both groups presented successful endodontic disinfection strategies as measured by significant reductions in the mean PAI scores.

Evaluating the reduction of PAI mean scores over time, we observed that this mean reduction was higher in the laser group than in the control group at the two endpoint evaluations. However, for all appraised teeth to date, these inter-group differences have not been statistically relevant. Hence, it is possible to suggest that if we had included those treatment failures in the statistical analysis or in future findings we might better appraise
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outcomes with the laser assisted endodontic treatment rather than with the conventional approach.

Future research is intended to broaden this RCT and its data collection as much as possible, increasing the impact of this new laser assisted disinfection concept along with its external validity. In conformity, the impact of this protocol – using the Er,Cr:YSGG laser with Radial Firing Tips – was additionally applied in specific compromised endodontic scenarios. The outcomes of the reported methodology could be attested while analyzing both Annexes XIX and X.

Such preliminary clinical results should definitely encourage researchers and clinicians to either pursue or adopt innovative (e.g. laser-assisted) techniques, not only for extended endodontic clinical trials but also for their own daily practice.

Analysis of Cost-Effectiveness

New technologies and therapies must meet several criteria before becoming routine, including improved clinical outcomes, protocol convenience and patient acceptance. Still, for the implementation of an optimized treatment, one should also combine the maximum effect with a reasonable cost. Otherwise, the external validity and practitioner’s acceptance of a determined evidence-based or scientifically promising treatment may be excluded due to primarily financial concerns.

Thus, evidence-based dentistry should guide us to scrutinize which innovative methods would be ideal in a particular context, in order to achieve the best results with a reasonable, affordable investment.

The Er,Cr:YSGG laser (Waterlase MD®, Biolase, CA) may be found to be far too expensive for private clinics given the current Portuguese economical context, namely if used merely for endodontics.

Still, we should point out that this is the dental laser with the widest range of applications, as it can perform both hard/soft tissue surgeries and minimally invasive conservative dentistry and it is also considered extremely effective for implantology and periodontics. Currently, with these recently developed radial firing tips for endodontics, a new breakthrough has been achieved in terms of deep uniform disinfection and effective debridement. This can represent not only an advantage over conventional therapies but also relative to other lasers (having straight-forward emitting tips), surpassing their inherent limitations and hazard risks.
Complementing the possibility of performing an Er,Cr:YSGG laser-assisted dentistry, the healing process following any surgical procedure is considerably faster, as both per- and post-operative pain and oedema are substantially reduced (several procedures do not even require anaesthesia) and truly minimally invasive dentistry can actually be accomplished. All these applications and advantages suggest the opportunity of making investments more profitable if used on a daily basis for all dental indications.

On the other hand, it is common sense to accept that costs may have to be subsidized until the outstanding results and dental excellence in laser treatments make this state of the art wide spread. Ultimately, the patients’ socio-economic status, needs and expectations must constitute the routine gauge for dentistry practice; they must be able to justify, or not, our standards of oral health care.

To a certain extent it is understandable that in socio-economic contexts, some conventional treatments or old protocols may be neither ideal nor the most cost effective, but may be the ones that our patients can afford and therefore accept.

A clinical interpretation may be more or less sceptical regarding new evidence-based approaches but, from a discerning point of view, it can never represent a sufficient excuse for the continual quest for self-development and the quest for knowledge.

Nevertheless, other traditional therapies represent nothing more than historical dogmas without any logical rationale for their application. For those, Adam Smith a prominent social philosopher from the eighteenth century once stated that “science is the great antidote to the poison of enthusiasm and superstition”. In this particular case, it is just a matter of adjustment to reality, and then laser assisted reality will be...now!

Accept the challenges so that you can feel the exhilaration of victory!

George S. Patton (1885-1945)
US Army General
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

SOURCES OF FUNDING

- RWTH International Academy, Aachen University – Germany.
  (Photographed by: Marc Smith)

- AALZ – Aachen Dental Laser Institute, Aachen University - Germany.
  (www.aalz.de)

- Faculdade de Medicina Dentária da Universidade do Porto - Portugal.
  (www.fmd.up.pt)

- Biolase™, CA, USA.
  (www.biolase.com)
ANNEXES

An expert is a man who has made all the mistakes
which can be made in a very narrow field.

Niels H. Bohr (1885-1962)
Danish physicist
ANNEX I


510(k) Summary of Safety and Effectiveness Information

Regulatory Authority: Safe Medical Devices Act of 1990, 21 CFR 807.92

Company: Biolase Technology, Inc.
4 Cromwell
Irvine, CA 92618

Contact: Ms. Isana M. Rizoiu
Biolase Technology, Inc.
4 Cromwell
Irvine, CA 92618
Tel: (949) 226-8144
Fax: (949) 273-6680

Trade Name: Waterlase® and Waterlase® MD

Common Name: Er,Cr:YSGG laser

Classification Name: Surgical laser instrument

Classification Code: 79 GEX, MXF, DZI a Class II device

Equivalent Devices:
Biolase Technology, Inc.
EndoPur™ Root Canal Cleanser

Deatyspll Inst, Inc.
BioPure® MTADs Root Canal Cleanser

Device Description:

The Waterlase®/Waterlase® MD dental laser system is a device used to perform a variety of dental soft and hard tissue indications. For hard tissue procedures the Waterlase®/Waterlase® MD uses the Erbium, Chromium: Yttrium, Scandium, Gallium Garnet (Er,Cr:YSGG) laser in combination with advanced water atomization spray technology to cut, remove, shape, contour, roughen and etch tissues. Soft tissue procedures are performed using a different mode of operation where direct Er,Cr:YSGG laser energy is applied to incise, excise or ablate these tissues. For soft tissue procedures the water spray is applied for hydration, cooling or to keep tissues and the field of view clean. For hard tissue applications the spray is part of the tissue removing process as well as hydration, cooling and keeping tissues and field of view clean.

Page 1 of 3

A flexible fiberoptic terminated into the handpiece delivers the Waterlase®/Waterlase™ MD laser energy to the end fiber tip and target. A visible aiming light emitted from the handpiece's distal end pinpoints the area of treatment.

Three fiber optic ports provide illumination from the handpiece to the tissue site in addition to the center beam emitting source. In both hard and soft tissue applications the power output, pulse duration, repetition rate (frequency) and air and water flow rates are adjustable to specific user requirements. The spot size and spot geometry can also be varied by changing tips which include different diameters and end configurations.

Indications for Use:

Root Canal Disinfection
Laser root canal disinfection after endodontic instrumentation

Contraindications:

All clinical procedures performed with Waterlase®, Waterlase™ MD must be subject to the same clinical judgment and care as with traditional techniques. Patient risk must always be considered and fully understood before clinical treatment. The clinician must completely understand the patient's medical history prior to treatment. Exercise caution for general medical conditions, which might contraindicate a local procedure. Such conditions may include, but are not limited to, allergy to local or topical anesthetics, heart disease, lung disease, bleeding disorders, sleep apnea or an immune system deficiency. Medical clearance from patient's physician is advisable when doubt exists regarding treatment.

Substantial Equivalence:

The purpose of the 510(k) is to expand the Waterlase®, and Waterlase™ MD indications for use to include root canal disinfection, an indication that has already been cleared by the FDA for equivalent medical devices. The Waterlase®, and Waterlase™ MD have already been cleared by the FDA for indications which relate to root canal procedures and surgical endodontic procedures related to root end amputation. Other indications cleared for this device include cavity preparations class I, II, III, IV and V, cutting, shaving, contouring and resection of osseous tissue, osteotomy, hard tissue roughening or etching, enucleoplasty, soft tissue procedures and periodontal procedures related to surgery and the periodontal pocket. The Waterlase®, and Waterlase™ MD indications enumerated above have been cleared by the FDA as part of the 510(k) submissions K031140, K019908, K035923, K022803, K0110741, K012511, K990098, and K990219. For the indication on root canal disinfection requested with this submission, the Waterlase®, and Waterlase™ MD are equivalent to the following products: BIOPURE MTAD Root Canal Cleanser K035167, and EndoPure Root Canal Cleanser K032361. Comparison between Waterlase®, and Waterlase™ MD and the predicate devices is included in Table 1. Based on the comparison, the Waterlase®, and Waterlase™ MD are substantially equivalent in relation to previous clearances.
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Equivalency was further substantiated through performance data, including in-vitro evaluation of anti-microbial efficacy of the Er,Cr:YSGG (Waterlase®) and the evaluation of the temperature rise which related to the safety of these devices during root canal disinfection. Copies of the study reports are included under section 8, titled Performance Data.
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

ANNEX II

F.M.D.U.P. Ethics Committee Approval (s).

Exmo. Senhor
Dr. Miguel Rui Antunes Rodrigues Martins
Faculdade de Medicina Dentária da
Universidade do Porto

000068
17 JAN. 2011

Assunto: Avaliação pela Comissão de Ética do projecto de investigação subordinado ao tema: “Estudo clínico sobre a eficácia do laser Er, Cr: YSGG, no tratamento endodontico assistido por laser”.

Serve a presente para comunicar a V. Exa. que o seu projecto se encontra:

- Aprovado.

Sem outro assunto de momento, subscrevemo-nos com a mais alta estima e consideração.

Com os melhores cumprimentos,

O Presidente da Comissão de Ética

[Signature]

Prof. Doutor Fernando Morais Branco

Rua Dr. Marcelo Pontes da Silva, 4400-387 Porto - Portugal
Tel/fax: 22 299 11 40 - Fax: 22 299 11 40
www.fmd.up.pt
Exmo. Senhor
Dr. Miguel Martins

c/c ao Senhor Prof. Doutor Manoel Fontes de Carvalho e
Senhora Prof. Doutora Irene Pina Vaz

22 JUN. 2009

00682

A Comissão de Ética da Faculdade de Medicina Dentária da Universidade do Porto,
em reunião tida a 08 de Junho de 2009 para apreciação do Projecto que V. Exa. se
dignou enviar, a fim de obter parecer da referida Comissão e cujo título é: “Estudo
clínico sobre a eficácia do Laser Er, Cr: YSGG, no tratamento endodôntico assistido por
laser”, achou por bem que:

- Assegure a existência de um seguro que cubra eventuais danos a terceiros no
decurso da investigação.

Fica assim a Comissão de Ética a aguardar a recepção do elemento citado para poder
aprová-lo que V. Exa. se propõe vir a fazer.

Com os melhores cumprimentos,

O Presidente da Comissão de Ética

Prof. Doutor Fernando Moreira Branco
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

ANNEX III
Main investigator liability insurance.

DECLARAÇÃO

RAMO: RESPONSABILIDADE CIVIL
ACTIVIDADE: Dentistas – O.M.D
APÓLICE: 008410045513
CAPITAL: 300.000.000
TOMADOR: MIGUEL RUI ANTUNES RODRIGUES MARTINS
INÍCIO: 04-10-2007
VALIDADE: Ano e Sequintes

Entre a AXA PORTUGAL COMPI. SEGUROS SA, e os Exmos. Srs. MIGUEL RUI ANTUNES RODRIGUES MARTINS, existe celebrado contrato de seguro nos moldes acima referidos.

Para os devidos efeitos se declara que nos termos das Condições Gerais da Apólice de Responsabilidade Civil e da Condição Especial 21 – Profissões de Saúde, estão garantidos os danos corporais causados pela utilização de aparelho Laser no decorrer de tratamentos no âmbito da actividade profissional do Tomador, garantida pela presente apólice.

Porto, 04-11-2009

Pela AXA PORTUGAL, Companhia de Seguros, SA
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

ANNEX IV

Informed consent for trial participants – page 1.

AUTORIZAÇÃO – DECLARAÇÃO DE CONSENTIMENTO INFORMADO
Considerando a “Declaração de Helsinki” – Associação Médica Mundial
(Helsinqui 1964; Edição 1975; Verona 1983; Hong Kong 1989; Somerset West 1996 e Edimburgo 2000)

DESIGNAÇÃO DO ESTUDO: PROJECTO DE INVESTIGAÇÃO CLÍNICA SOBRE A EFICÁCIA DO LASER DE Er,Cr:YSGG NO TRATAMENTO ENDODONTICO ASSISTIDO POR LASER.

Eu, abaixo assinado (nome completo). .................................................................

Pai, mãe ou responsável pelo paciente (nome completo). .................................

Compreendi a explicação que me foi fornecida, por escrito e verbalmente, acerca da investigação conduzida pelo Dr. Miguel Rui Martins com o apoio da Faculdade de Medicina Dentária da Universidade do Porto, para qual é pedida a sua participação. Foi-me dada oportunidade de fazer as perguntas que julguei necessárias e, para todas obtive resposta satisfatória.

Objetivo do estudo: comparação da eficácia clínica entre o laser Er,Cr:YSGG e o Hipoclorito de Sódio (3%) em dentes necrosados. A relativa ineficácia bactericida deste último e os riscos ao seu uso associados, justificam a importância do tema e a obtenção da posterior análise estatística dessa informação.

Métodos: Preenchimento da ficha médico-dentária individual, exame clínico da cavidade oral com preenchimento dos dados clínicos, eventual necessidade de registo radiográfico e fotográfico (sem custos adicionais para o paciente); colheita de uma amostra bacteriológica; uso de óculos de protecção durante a irradiiação; necessidade de compatibilidade para controlo ao final de 1, 3 e 6 meses.


Eventual desconforto: Inerente ao exame clínico e registo radiológico/fotográfico. Uso de óculos de protecção obrigatório durante a irradiiação.

Tomei conhecimento de que, de acordo com as recomendações da Declaração de Helsinki, a informação que me foi prestada versou sobre os objectivos, os métodos, os benefícios previstos, os riscos potenciais e o eventual desconforto. Além disso, foi-me afirmado que tenho o direito de decidir livremente aceitar ou recusar a todo o tempo a participação no estudo. Sei que posso abandonar o estudo e que não terei que suportar qualquer penalização, nem quaisquer despesas pela participação neste estudo.

Foi-me dado todo o tempo de que necessitei para reflectir sobre esta proposta de participação.

Nestas circunstâncias, consinto que a/o minha/meu filho(s) participe neste projecto de investigação, tal como me foi apresentado pelo investigador responsável sabendo que a confidencialidade dos participantes e dos dados a eles referentes se encontra assegurada.
Mais autorizo que os dados deste estudo sejam utilizados para outros trabalhos científicos, desde que irreversivelmente anonimizados.

Porto, __/__/______
Assinatura do responsável pelo paciente:

O investigador:
Nome:________________________
Assinatura:______________________

Dados de contacto do investigador: Dr. Miguel Rui Martins
miguel.ar.martins@gmail.com
Tel: 914610046

Faculdade de Medicina Dentária
Universidade do Porto
Rua Dr. Manuel Pereira da Silva
4200-392 Porto
Tel: 229011100

O co-orientador da investigação:
Nome:________________________
Assinatura:______________________
### Effect of Er,Cr:YSGG Laser in Endodontic Treatment

**Miguel Rui A. R. Martins**

#### Table with participant's data included/excluded in the trial.

| N° | Name      | Gender | Age | C/E | Tooth | M/ (Temp) | Initial A-Ray | Settings | Final A-Ray | Lab Month | Std Month | Lab Month | Std Month | END | Cause (A) |
|----|-----------|--------|-----|-----|-------|-----------|---------------|----------|-------------|------------|-----------|-----------|-----------|-----------|-----|----------|
| 1  | M. W. C. | F      | 42  | C   | 32    | 20        | 19.09.2002    | 4.00     | 11.00.2002  | EXCLUDED | EXCLUDED | EXCLUDED | EXCLUDED |     |          |
| 2  | S. J. R. | M      | 33  | C   | 34    | 21        | 19.09.2002    | 4.00     | 11.00.2002  | EXCLUDED | EXCLUDED | EXCLUDED | EXCLUDED |     |          |
| 4  | M. P. A. | F      | 19  | C   | 15    | 19        | 19.09.2002    | 4.00     | 11.00.2002  | EXCLUDED | EXCLUDED | EXCLUDED | EXCLUDED |     |          |
| 5  | J. M. C. | F      | 24  | C   | 12    | 21        | 19.09.2002    | 4.00     | 11.00.2002  | EXCLUDED | EXCLUDED | EXCLUDED | EXCLUDED |     |          |
| 6  | S. M. P. | F      | 56  | C   | 12    | 20        | 19.09.2002    | 4.00     | 11.00.2002  | EXCLUDED | EXCLUDED | EXCLUDED | EXCLUDED |     |          |
| 7  | C. L. F. | F      | 28  | C   | 22    | 19        | 19.09.2002    | 4.00     | 11.00.2002  | EXCLUDED | EXCLUDED | EXCLUDED | EXCLUDED |     |          |
| 8  | P. H. G. | F      | 32  | C   | 15    | 19        | 19.09.2002    | 4.00     | 11.00.2002  | EXCLUDED | EXCLUDED | EXCLUDED | EXCLUDED |     |          |
| 9  | R. M. S. | F      | 39  | C   | 15    | 19        | 19.09.2002    | 4.00     | 11.00.2002  | EXCLUDED | EXCLUDED | EXCLUDED | EXCLUDED |     |          |
| 10 | M. S. M. | F      | 53  | C   | 12    | 19        | 19.09.2002    | 4.00     | 11.00.2002  | EXCLUDED | EXCLUDED | EXCLUDED | EXCLUDED |     |          |
| 11 | M. M. C. | F      | 67  | C   | 44    | 21        | 19.09.2002    | 4.00     | 11.00.2002  | EXCLUDED | EXCLUDED | EXCLUDED | EXCLUDED |     |          |
| 13 | A. M. C. | F      | 56  | C   | 42    | 21        | 19.09.2002    | 4.00     | 11.00.2002  | EXCLUDED | EXCLUDED | EXCLUDED | EXCLUDED |     |          |
| 14 | J. M. C. | M      | 52  | C   | 25    | 20        | 19.09.2002    | 4.00     | 11.00.2002  | EXCLUDED | EXCLUDED | EXCLUDED | EXCLUDED |     |          |
| 15 | P. C. C. | F      | 36  | C   | 15    | 19        | 19.09.2002    | 4.00     | 11.00.2002  | EXCLUDED | EXCLUDED | EXCLUDED | EXCLUDED |     |          |
| 16 | L. H. P. | F      | 37  | C   | 35    | 21        | 19.09.2002    | 4.00     | 11.00.2002  | EXCLUDED | EXCLUDED | EXCLUDED | EXCLUDED |     |          |
| 17 | A. F. B. | F      | 37  | C   | 42    | 20        | 19.09.2002    | 4.00     | 11.00.2002  | EXCLUDED | EXCLUDED | EXCLUDED | EXCLUDED |     |          |
| 18 | H. R. M. | M      | 61  | C   | 13    | 19        | 19.09.2002    | 4.00     | 11.00.2002  | EXCLUDED | EXCLUDED | EXCLUDED | EXCLUDED |     |          |
| 19 | M. G. M. | F      | 56  | C   | 31    | 20        | 19.09.2002    | 4.00     | 11.00.2002  | EXCLUDED | EXCLUDED | EXCLUDED | EXCLUDED |     |          |
| 20 | A. C. M. | F      | 46  | C   | 22    | 20        | 19.09.2002    | 4.00     | 11.00.2002  | EXCLUDED | EXCLUDED | EXCLUDED | EXCLUDED |     |          |

**Note:** Cause (A) column indicates the reasons for exclusion or follow-up.
ANNEX VI

Examples of participant’s radiographic data for blind outcome evaluation.

A.M.V. » Tooth 4.2

Immediate Post-Operative  6 Month Follow-up  12 Month Follow-up

M.G.M. » Tooth 12

Immediate Post-Operative  6 Month Follow-up  12 Month Follow-up
Examples of participant’s radiographic data for blind outcome evaluation (cont.).

M.F.F. » Tooth 4.5

Immediate Post-Operative 6 Month Follow-up 12 Month Follow-up

J.S.T. » Tooth 4.2

Immediate Post-Operative 6 Month Follow-up 12 Month Follow-up
Examples of participant’s radiographic data for blind outcome evaluation (cont.).

**J.C.S. » Tooth 2.2 T**

Immediate Post-Operative  
6 Month Follow-up  
12 Month Follow-up

**M.A.C. » Tooth 3.4 C**

Immediate Post-Operative  
6 Month Follow-up  
12 Month Follow-up
ANNEX VII


Efficacy of Er,Cr:YSGG laser with endodontical radial firing tips on the outcome of endodontic treatment: blind randomized controlled clinical trial with six-month evaluation

M.R. Martins • M.F. Carvalho • L.P. Vaz • J.A. Capelas • M.A. Martins • N. Gutknecht

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Abstract Clinical reports stating the efficacy of novel root canal disinfection protocols are an important focus in endodontic research. This blind randomized clinical trial assessed the clinical efficacy of the erbium, chromium:yttrium–scandium–gallium–garnet (Er,Cr:YSGG) laser radial firing tips (RFT) versus the concomitant use of 3% sodium hypochlorite and interim calcium hydroxide paste in necrotic teeth with chronic apical periodontitis. We hypothesized to find similar or improved bone healing in the laser-assisted endodontic treatment. Thirty-six anterior and premolar teeth were randomly assigned. In group 1, teeth were prepared with 3% sodium hypochlorite for irrigation and calcium hydroxide as inter-appointment dressing; in group 2, teeth were prepared with saline solution and irradiated with Er,Cr: YSGG laser using RFT2 (140 μs, 37.5 mJ, 20 Hz) and RFT3 (140 μs, 62.5 mJ, 20 Hz) in the first and second appointment, respectively, four times each, moving at 2 mm s⁻¹ from apical to coronal. The primary outcome measure was changed in apical bone density at 6 months, using the periapical index (PAI) for blind radiographic evaluation. Twenty-nine patients were examined and subjected to statistical analysis, 12 in group 1 and 17 in group 2. There was one treatment failure in group 1. Both groups gave similar outcomes exhibiting statistically significant decreases in PAI scores.

Keywords Er,Cr:YSGG laser · Randomized clinical trial · Sodium hypochlorite · Calcium hydroxide · Apical periodontitis · Endodontics

Introduction

Bacterial presence and maintenance within the root canal system can be considered the principal etiological factor for the development of pulp and periapical lesions [1–3]. Moreover, the idea that absence of cultivable bacteria at the time of obturation will favor healing is consistent with the one that microorganisms could be responsible for chronic apical periodontitis (CAP) [4]. Chemomechanical preparation using sodium hypochlorite (NaOCl) as irrigant can be conventionally accepted for effective root canal disinfection [5]. To supplement these procedures and eliminate persistent bacteria, calcium hydroxide is arguably the most used intracanal medication [6, 7]. However, the effectiveness of these conventional strategies against microorganisms commonly associated with CAP remains under debate [7–10].

Despite the fact that currently used intracanal medicaments have limited antibacterial spectrum and low ability to diffuse into the dentine [11, 12], during mechanical preparation, a smear layer is produced. The smear layer can cover the root canal walls, serve as a reservoir of bacteria, and also prevent antibacterial solutions from penetrating into the dentinal tubules [13]. Thus, clinicians should ideally adopt a treatment protocol that has been shown to be effective in well-controlled studies so that a predictable outcome can be

achieved. These must include strategies that could penetrate deeper into dentinal tubules and eliminate microorganisms located beyond the host defense mechanisms [14].

Lasers have been suggested to assist endodontic treatments, being a suitable method to remove the smear layer [15, 16] and to achieve deep root canal system disinfection [17, 18]. Although laser effectiveness may depend on several factors such as wavelength, pulse power, pulse duration, and light distribution through the end of the optical fiber [15], endodontic clinical research to date has been promising but limited [19].

The erbium, chromium: yttrium–scandium–gallium–garnet (Er:Cr:YSGG) laser, at a wavelength of 2,780 nm, has been shown capable of removing the smear layer [20–22] and improving root canal system disinfection [23–25] without being hazardous to surrounding structures [26–27]. Recently, in order to improve light distribution inside root canals, modified radial firing tips (RFTs) were developed and have been showing to be a valuable tool for smear layer removal using water concomitantly to root canal disinfection in dry conditions [28–30]. However, there is still no clinical evidence stating the efficacy of the Er:Cr:YSGG laser-assisted endodontic treatment while using RFTs for both purposes within the same protocol.

We hypothesized that necrotic teeth with CAP treated with Er:Cr:YSGG laser and RFTs would demonstrate similar outcomes using the periapical index (PAI) [31], when compared with teeth treated with 3 % NaOCl and calcium hydroxide dressing. In accordance, the aim of this blind randomized clinical trial was to compare radiographic evidence of periapical healing after root canal therapy, suggesting the possibility of achieving predictable outcomes using the Er:Cr:YSGG laser without the aid of any chemical substances.

Material and methods

Subject enrollment Approval for the project was obtained by the University of Porto Ethical Committee. Participants were recruited from referrals made to the university dental clinic and then referred to endodontics for initial nonsurgical root canal treatment between September 2009 and May 2010.

Patients with asymptomatic teeth with necrotic pulps and CAP verified radiographically (minimum size, ≥1.0 x 1.0 mm) were consecutively enrolled in the study. Diagnosis was confirmed by negative response to thermal pulp tests. Pulp testing was performed by undergraduate students, and radiographic interpretation was verified by independent faculty members. Only anterior or premolars with mature, fully formed apex teeth were selected. Rubber dam isolation technique was mandatory. Within-person design was allowed (two patients contributed with more than one tooth).

Patients were excluded if they were younger than 12 years old, pregnant, had a positive history of antibiotic use within the past month, needed antibiotic premedication for dental treatment (for infective endocarditis or immunocompromising disorders), suffering from uncontrolled hypertension, uncontrolled diabetes mellitus, chronic renal failure, hematoletic diseases, HIV, osteoporosis treated with bisphosphonates, steroid therapy in excess of 5 mg/day of prednisolone, or head and neck irradiation therapy. No compulsion was allowed (e.g., terminal stages, prisoners). Teeth with abnormal root canal anatomy, larger than 26 mm in length, non restorable teeth, and teeth with advanced periodontal disease were not included in the study.

Once eligibility was confirmed, the study was explained to the patient by one endodontic resident, and the patient was invited to participate. Treatments were not subsidized, and no financial incentive was offered (i.e., patients were responsible for the usual root canal treatment fees). It was advised that root canal treatment would be performed regardless of participation in the study. After informed consent (Helsinki Declaration 1973, revised in Edinburgh 2000) was acquired, participants were randomly assigned to either test or control group using block sequences from a randomized computer generator program—generated by an independent investigator (M.A.M.)—resulting in a 1:1 ratio between the groups.

Allocation concealment and participants Neither the undergraduate clinician nor the patient was aware of the group assignment before agreeing to participate in the study. Standard patient (control) treatment allocation was done randomly according to the sequence given by the computer-randomized generated tables. When more than one tooth was allocated in the same patient (within person), randomization was done by assigning the right or more mesial tooth to the control treatment group, whereas the left or more distal tooth was allocated to the laser-assisted group.

All endodontic procedures were performed by enrolled undergraduate (4th and 5th graduation year) students always supervised by graduated, trained, and experienced professors who were not aware of the patient group assignment. Laser irradiation was provided by the main investigator (M.R.M.). Radiographic evaluation was done by experienced, previously calibrated, endodontic specialists (M.F.C., I.P.V., and J.A.C.).

Clinical procedures All treatment sessions were approximately 3 h in length and were performed by undergraduate residents following a standardized treatment protocol for each intervention. Local anesthesia (2 % lidocaine with
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment


1:00,000 epinephrine) was administered as needed for patient comfort.

Initial carious excavation was performed and previous restorations removed. Rubber dam isolation was obtained and standard access cavity was prepared.

Initial canal working length (WL) was established at 1 mm short of the biological apex of the root, established radiographically with a size ISO #15 stainless steel file. WL was confirmed and adjusted using straight and angled radiographs.

Both groups were subjected to root canal treatments in two appointments. Finishing the first appointment, a sterile cotton pellet imbiber on Cresophène® (Septodont) solution was placed in the pulp chamber, and the access cavity was sealed with a reinforced zinc-oxide eugenol intermediate restorative material (IRM®, Dentsply).

During the second appointment, which took place 7 to 24 days after the first visit, every patient was inquired for symptom history such as pain, sensitivity to percussion, or swelling.

A minimum apical file size of #30 ISO was required for all teeth. If canals were initially large, then the master apical file size was set at least three sizes larger than the first file to bind at the WL.

**Group 1—control** On the first appointment, root canal instrumentation was performed using the manual step-back technique and irrigated with 5.0 mL of 3 % sodium hypochlorite after each cycle until reaching the minimum enlargement of #30 ISO K-file (Zipperer CC®, VDW GmbH, Munich, Germany). Root canals were dried with sterile paper points and dressed with calcium hydroxide paste. At the second appointment, all of the calcium hydroxide paste was removed using K-files (Zipperer, VDW GmbH, Munich, Germany) and copious irrigation with 3 % sodium hypochlorite. Complete removal of the calcium hydroxide paste was confirmed by visual inspection, and manual instrumentation was completed. After final irrigation with 5.0 mL 3 % sodium hypochlorite, all canals were dried with sterile paper points, checking for the absence of suppurative or exudate.

Finally, root canals were filled with gutta-percha cones (Gutta-Percha Points, ISO Color Coded—Dentsply, Maillefer) using cold lateral condensation technique including zinc oxide eugenol handmacle paste as sealer. The access cavity was sealed with a reinforced zinc oxide eugenol IRM (Dentsply) followed by taking a postoperative radiograph.

**Group 2—test** On the first appointment, root canal instrumentation was performed in similarity with the protocol described for group 1, while irrigation was performed with 2.0 mL of saline solution between files. After reaching the #30 ISO K-file (Zipperer CC®, VDW GmbH, Munich, Germany), the main canal was filled with distilled water, and laser irradiation was performed with the 2,700 nm Er,Cr: YSGG laser (Waterlase MD, Biolase Technology, Inc, San Clemente, CA) and a 270-μm-diameter radial firing tip (RFT2 Endolase, Biolase Technology, Inc; irradiation power of 0.55) with a pulse setting of 0.75 W, 20 Hz (37.5 mJ), 140 μs pulse, 0 % water, and air. The tip was placed at the working length, and irradiation was performed approximately at the speed of 2 mm s⁻¹ until reaching the most coronal part of the canal. This irradiation procedure was repeated four times (two with the canal filled with distilled water and two in dry conditions), resting approximately 15 s between each irradiation.

On the second appointment, canal preparation was completed with saline solution as irrigant. The main canal was filled with distilled water, and laser irradiation was performed with a 320-μm radial firing tip (RFT3 Endolase, Biolase Technology, Inc; irradiation power of 0.85) with a pulse setting of 1.25 W, 20 Hz (62.5 mJ), 140 μs pulse, 0 % water, and air. The irradiation protocol was identical to the first appointment.

After irradiation, canals were irrigated with 5.0 mL of saline solution during approximately 1 min as final rinsing and dried with sterile paper points, checking for the absence of any suppuration or exudate. Root canals were filled with the technique described for group 1. All teeth must have been permanently restored by the referring undergraduates within a 30-day period.

**Outcome classification and data analysis**

All radiographic assessments were carried by one operator (M.R.M.). The long-cone paralleling technique, with one film holder, was used for the immediate postoperative and follow-up radiographs. Radiographic exposure settings were recorded for each tooth. Radiographs were all made under the same condition and exposure settings. Radiographic images were coded and stored by two investigators (V.I.P. and C.J.A.).

The primary outcome measure for this study was change in apical bone density at 6 months. The PAI [31] was used to radiographically evaluate the proportion of teeth that could be considered improved (decreased PAI score) or healed (PAI ≤ 2) in each group.

Radiographic evaluation was blind and independently performed by two experienced, previously calibrated endodontists (C.M.F. and V.I.P.). Instructions for grading images with the PAI scoring system were adapted from Oenzi et al. [31]. All images were scored in a random order in a darkened room using an illuminated viewer.

box whilst mounted in a cardboard slit to block off ambient light.

For calibration, each examiner graded a series of 30 radiographic images not associated with the study sample and representing a wide range of periapical bone densities. To access intra-exam agreement, 1 week after the first session, the examiners scored the same images. This method generated four PAI scores for each image, two from each of the two examiners. The examiners then met as a group to reach consensus on cases that did not receive unanimous agreement and reviewed all scores to enhance calibration and inter-exam agreement. Consensus was reached on images that were not initially scored the same. The identifying code for each image was not broken until after consensus score was determined. Consensus score for each image was considered the true score and used for statistical analysis. Agreement between and within examiners was determined using the interclass correlation coefficient (ICC). Intra-exam reliability was measured with the single-measure ICC (SPSS 17 for Windows; SPSS Inc, Chicago, IL), and inter-exam agreement was measured with the average measure ICC (also known as the inter-exam reliability coefficient). The criteria proposed for strength of agreement by Landis and Koch [32] were used: 0.00–0.20, slight agreement; 0.21–0.40, fair agreement; 0.41–0.60, moderate agreement; 0.61–0.80, substantial agreement; and 0.81–1.00, almost perfect agreement.

One week following the final calibration session, both examiners (C.M.F. and V.I.P.) randomly scored the assembled study images, without knowing the treatment protocol used for each patient. If they disagreed, another independent specialist (C.J.A.) decided the final scoring.

To evaluate differences between groups, the Mann–Whitney U test was applied for both baseline (immediate postoperative) and 6-month follow-up evaluation. Change in PAI score for each group from baseline to 6-month follow-up evaluation was tested with the Wilcoxon signed rank test. The proportion of teeth in each group that could be considered improved (decreased PAI score) or healed (PAI ≤2) was assessed with the χ² Monte Carlo simulation test.

Presence of clinical symptoms or abnormal findings at 6 months (spontaneous pain, swelling, mobility, and sensitivity to percussion or palpation) were recorded but not subjected to statistical analysis.

Results

Thirty-six patients met the inclusion criteria and consented to participate in the trial. Due to deviation from protocol (no compliance), four patients were excluded from statistical analysis. Twenty-nine patients were examined and subjected to statistical analysis at the 6-month follow-up, 12 in the control group (group 1) and 17 in the test group (group 2) (Fig.1).
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Miguel Rui A. R. Martins

Six-month results published in Lasers in Medical Science – Page 5 of 7.

No adverse effects were found. As abnormal clinical finding, one patient (group 2) complained about swelling and sensitivity immediately after abruption which has disappeared in approximately 1 week with antibiotic and anti-inflammatory prescription.

We defined failure as the need for any additional treatment. The single failure (group 1) was related to the presence of swelling, sensitivity to percussion, and sinus tract after 3 months. In group 2, one tooth was extracted due to prosthetic reasons before the 6-month examination and was considered lost to follow-up. One additional patient was lost to follow-up. Treatment failures were not included in the primary data analysis.

Before the consensus scoring meeting, intra-rater reliability score was 0.95, and the overall inter-rater agreement was 0.85, considered as almost perfect agreement.

The mean PAI score for group 1 was 3.83 (SD=1.19) at the immediate postoperative examination and 2.17 (SD=1.47) at the 6-month follow-up, a decrease of 1.66; the mean PAI score for group 2 was 4.49 (SD=1.05) at the immediate postoperative examination and 2.47 (SD=1.23) at the 6-month follow-up, a decrease of 2.02; Both groups exhibited a statistically significant decrease in PAI score ($P<0.05$). There was no statistically significant difference between groups at either the immediate postoperative examination ($P=0.28$) or the 6-month evaluation ($P=0.38$).

In group 1, 66.67 % of teeth were considered healed (PAI ≤2) at 6 months, 83.33 % improved (lower PAI score), and 16.67 % were unchanged (same PAI score). There was one treatment failure which was not accessed to follow-up. In group 2, 58.82 % of the teeth were considered healed at 6 months, 82.35 % improved, and 17.65 % were unchanged. There was no record of increased PAI score. There was no statistically significant difference between groups ($P=0.69$).

Discussion

The limited effectiveness of sodium hypochlorite and calcium hydroxide paste against several common endodontic pathogens has caused some investigators to question its optimal concentration and use [5]. Moreover, there is still no consistent evidence that a single appointment would result in improved healing when compared to multiple-appointment root canal therapy performed with calcium hydroxide dressing [7, 33]. However, the expectation that teeth treated in two appointments without the use of chemical solutions or dressing paste would result, at least, in similar outcomes using this laser-assisted endodontic treatment protocol was confirmed.

The sample size and operators might not accurately represent the true population of patients and clinicians. The sample size can be considered typical when compared to other high-quality clinical trials [6, 34]. Thirty-six from patients from an original sample of 51 were examined at the 6-month follow-up, 12 in group 1 and 17 in group 2.

Patients were randomly assigned to treatment groups, and root canal treatments were performed by undergraduates according to a standardized protocol that may represent the University of Porto consensus for both best and possible clinical practice. As residents (fourth- and fifth-year graduation students) can arguably be considered less skilled than general dentists or experienced endodontists all treatments were performed under supervision with the opportunity for consultation and assistance as needed. Interestingly, results of treatment under quality-controlled training conditions were found similar to those performed by experienced professionals [35].

The PAI score was not used as initial inclusion criteria for this study, although the requirement that all teeth must have a visible periradicular radiolucency area at least 1.0–1.0 mm assured an initial PAI score ≤2. There was an approximately equal distribution of immediate postoperative PAI score between the two groups so that difference between disinfec tion protocols was the only independent variable.

Results can be influenced by many unknown and uncontrolled variables (e.g., inclusion of smokers) that may suggest that these patients could experience poorer treatment outcomes. In contrast, it was decided to exclude multi-rooted teeth from the study because they appear to have lower probability of complete healing when compared to single-rooted teeth [36]. Both groups were similar regarding basic demographic characteristics (Table 1) even if small baseline differences can be considered the result of chance rather than source of bias in a randomized trial.

<table>
<thead>
<tr>
<th>Table 1 Demographic characteristics for each group (age, gender, and tooth type)</th>
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<tr>
<td><strong>Male</strong></td>
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<tr>
<td>Control group (n=12)</td>
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<tr>
<td>Test group (n=17)</td>
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<tr>
<td>Treatment failure</td>
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<td>Totals</td>
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Radiographic diagnosis of apical periodontitis may be regarded as a signal task, and its prevalence in a cohort study is considered difficult to access by radiographic means [35]. Some authors have also suggested that 4 or 5 years might be necessary to adequately evaluate periapical healing after root canal therapy. However, considering the risk of losing patients during follow-ups, some studies have used shorter periods as an endpoint while others suggested simpler but accurate surrogate endpoints [9, 33].

The ICC was used to determine the agreement between and within examiners. A score of 0 represents no agreement, beyond the level of agreement expected by random chance whereas 1.0 signifies perfect agreement. The inter-examiner reliability score of 0.85 in this study represents an almost perfect level of agreement between examiners, even before meeting to establish the consensus scores for each image. Despite being able to support the PAI reliability to measure radiographic changes in apical bone density, there is more than one way to analyze data generated by the PAI considering that this is an ordinal scale [31]. For this study, we were most interested in differences in healing between groups by measuring changes in the mean PAI score. We considered that early evidences of periapical change in bone density should be apparent even if longer observation periods might be recommended. Such studies will soon be in progress. These may verify whether predictable outcomes can be achieved with the Er:Cr:YSGG laser-assisted endodontic treatment.

At the present stage, with a follow-up period of 6 months, in necrotic teeth with apical periodontitis and root canal therapy, there were no significant differences in terms of periapical healing between groups. Nevertheless, taking into consideration that both groups exhibited significant differences between the postoperative and final healing score, it could support the idea that the Er:Cr:YSGG laser-assisted endodontic treatment (using RFTs in wet and dry conditions) can be non-inferiority approach to perform endodontic treatments with less restrictions and adverse effects.

Acknowledgments
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References
ANNEX VIII


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<td>Martins, Miguel; Faculty of Dental Medicine - Universidade do Porto, Department of Endodontics</td>
</tr>
<tr>
<td></td>
<td>Carvalho, Manuel; Faculty of Dental Medicine - Universidade do Porto, Department of Endodontics</td>
</tr>
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<td>Capelas, José; Faculty of Dental Medicine - Universidade do Porto, Department of Endodontics</td>
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<td>Martins, Miguel; Universidade Católica Portuguesa - CRB, Department of Endodontics</td>
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<tr>
<td></td>
<td>Gutincht, Norbert; RWTH Aachen University, Department of Conservative Dentistry</td>
</tr>
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</table>

Keyword: Endodontics, YSGG laser, Bone Healing, Clinical Research, Lasers in Dentistry
Outcome of Er,Cr:YSGG Laser Assisted Treatment of Teeth with Apical Periodontitis: A Blind Randomized Clinical Trial

Miguel Rui A. R. Martins, DDS, MS*, Carvalho, M.F., DDS, PhD*, Pina-Vaz, I., DDS, PhD*, Capelas, J.A., DDS, PhD*, Martins, M.A., DDS**, Gutknecht, N., DDS, PhD***.

*Department of Endodontics, Faculdade de Medicina Dentária, Universidade do Porto, Portugal.  
**Department of Endodontics, Universidade Católica Portuguesa-CEG, Portugal  
***Department of Conservative Dentistry, RWTH Academy, Aachen University, Germany.

Running Title: Er,Cr:YSGG Laser on Apical Periodontitis - Blind RCT

Corresponding author:  
Miguel Rodrigues Martins  
Departamento de Endodontia, Faculdade de Medicina Dentária, Universidade do Porto  
E-mail address: miguel.r.martins@gmail.com

Contact  
Manuel Fontes Carvalho  
Departamento de Endodontia, Faculdade de Medicina Dentária, Universidade do Porto  
E-mail address: mfontescarvalho@gmail.com

Irene Pina Vaz  
Departamento de Endodontia, Faculdade de Medicina Dentária, Universidade do Porto  
E-mail address: ipinavaz@gmail.com

João António Capelas  
Departamento de Endodontia, Faculdade de Medicina Dentária, Universidade do Porto  
E-mail address: jadecnr@gmail.com

Miguel Andre Martins  
Departamento de Endodontia, Centro Regional das Beiras, Universidade Católica Portuguesa  
E-mail address: mgal.martins@uc.pt

Norbert Gutknecht  
Department of Conservative Dentistry, RWTH Aachen University  
E-mail address: ngutknecht@dr-az.de

Mary Ann Liebert, Inc., 140 Huguenot Street, New Rochelle, NY 10801
Abstract

Introduction: As clinical studies conducted to explore the safety and efficacy of new procedures is considered an important focus in endodontic research, the aim of this controlled clinical study was to compare a laser-assisted endodontic treatment using an Er,Cr:YSGG laser and Radial Firing Tips (RFT) versus the conventional use of 3% sodium hypochlorite and interim calcium hydroxide paste, in teeth with chronic apical periodontitis. Methods: 43 single-rooted and premolar teeth were randomly assigned. In group 1 teeth were prepared and irrigated with 3% sodium hypochlorite and calcium hydroxide inter-appointment dressing was applied; in group 2 teeth were prepared with saline solution and irradiated with Er,Cr:YSGG laser using the RFT2 (140µs, 37.5mJ, 20Hz) and the RFT3 (140µs, 62.5mJ, 20Hz) at the first and second appointment respectively, four times each, moving at 2mm/s from apical to coronal. The primary outcome measure was change in apical bone density at 12 months, using the periapical index (PAI) for blind radiographic assessment. Results: 30 teeth were examined and subjected to statistical analysis, 12 in Control group and 18 in Test group. There were two treatment failures in Control group that were not included for analysis, both groups exhibited statistically significant decreases in PAI scores. Conclusion: The present findings suggest that for single-rooted and premolar teeth this laser-assisted protocol can achieve predictable endodontic outcomes, comparable to conventional strategies in one year of follow up.

Keywords: Er,Cr:YSGG laser, radial firing tips, randomized clinical trial, sodium hypochlorite, apical periodontitis, endodontics.
Introduction

Microbial presence and maintenance within the root canal system can be considered the main etiologic factor for the development and persistence of chronic apical periodontitis [CAP]. Thus, the fundamental aim of endodontic therapy is considered to be the disinfection of the root canal and its complex tubular network. For this purpose, sodium hypochlorite (NaOCl) is frequently employed for effective root canal disinfection while calcium hydroxide (Ca(OH)₂) paste is often proposed as intracanal medication to eliminate persistent microflora. However, both substances have shown limited antibacterial spectrum and low ability to diffuse into the dentinal tubules; in consequence, as they require direct contact, their effectiveness is an ongoing source of controversy.

Thus, clinicians should ideally find and adopt treatment strategies that could allow deeper penetration into dental tubules and eliminate microorganisms located beyond the host defense mechanisms.

The Erbium, Chromium:Yttrium-Scandium-Gallium-Garnet (Er:Cr:YSGG) laser, operating at a wavelength of 2780nm, has been demonstrating to be a valuable tool to assist endodontic treatment without being hazardous to surrounding periodontal tissues based on the high absorption coefficients in hydroxyapatite (OH) groups and water molecules and with consequent biophysical interactions.

To overcome concerns related to the energy emission in axial direction and not towards the canal wall, the Radial Firing Tip (RFT) unique emission profile has been playing a significant improvement as the beam expansion by the tip geometry not only reduces emissions in the forward direction but favours homogeneous energy distribution along the root canal wall.

In fact, Erbium lasers have been demonstrating to induce shock waves in aqueous solutions inside root canals and radial firing tips positively influence their configuration. Hence, through the activation of aqueous solutions (e.g., water, EDTA) the Er:Cr:YSGG laser induce primary and secondary cavitation effects, useful for debris and smear layer removal.

However, the best results in terms of thorough root canal disinfection with the Er:Cr:YSGG laser are achieved while operating in dry conditions, relying on the fact that - without water inside the main root canal - the ability of such wavelength to penetrate into the dentinal tubules is increased.

Both features - in the presence and absence of water - could justify this laser assisted endodontic protocol as straightforward method to achieve increased access and bactericidal effects into

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formerly unreachable parts of the tubular network. In addition, despite all in-vitro reported applications, there is limited literature addressing the clinical outcomes of endodontic therapy using RFTs without the aid of chemical substances.25

Therefore, the aim of this blind randomized clinical trial (RCT) was to evaluate the outcome of a Er,Cr:YSGG laser assisted endodontic treatment compared to a traditional protocol in teeth with chronic apical periodontitis. The null hypothesis was chosen to demonstrate that the Er,Cr:YSGG laser could demonstrate similar healing at 12 months when compared to 3% NaOCl irrigation and Ca(OH)2 dressing, using the periapical index (PAI) as outcome measure.26

Material and Methods

Subject Enrollment: Approval for the study protocol (NV682/068) was obtained from University of Porto Ethics Committee. Eligible participants were recruited from October 2009 until April 2011 from among patients who attended the Faculdade de Medicina Dentária Dental Clinic, at Universidade do Porto, Portugal, for initial nonsurgical root canal treatments. The main inclusion criteria were radiographic evidence of apical periodontitis (minimum size 1.0mm in diameter) and a diagnosis of pulpless infected root canals confirmed by a negative response to sensibility pulp tests were consecutively enrolled in the study. Whereas undergraduate students performed diagnosis, clinical and radiographic interpretations were verified by supervising faculty members. Single-rooted and premolar teeth with mature, fully formed apices were selected. Within-person assignment was allowed (2 patients contributed with more than one tooth).

Patients were excluded if they were younger than 12 years of age, pregnant, had a positive history of antibiotic use within the past month, indication for antibiotic prophylaxis (bacterial endocarditis or immunocompromising disorders), suffering from uncontrolled hypertension or diabetes mellitus, chronic renal failure, hematologic diseases, HIV, osteoporosis treated with bisphosphonates, steroid therapy exceeding 5mg/day of prednisolone and prior to head and neck irradiation therapy. No compulsion was permitted (e.g. prisoners). Non-restorable teeth due to decay, teeth with abnormal root canal anatomy, more than 26mm in length and teeth with advanced periodontal disease were also excluded from the study. Effective rubber dam isolation technique was considered mandatory for inclusion in the trial.

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Once eligibility was confirmed, one endodontic resident explained the study to the patient, and the patient was invited to participate. No financial incentive was offered (i.e., patients were responsible for the usual root canal treatment fees). All patients were advised that root canal treatment would be performed regardless of participation in the study. After verbal and written informed consent (Helsinki Declaration 1973, revised in Edinburgh 2000) was acquired, participants were randomly assigned to either control (CON) or test (LAS) group using block sequences generated from a randomization computer program with a 1:1 ratio between groups. The flow of participants during the phases of the trial is detailed in Fig. 1.

Allocation concealment and Participants: Neither the undergraduate clinician or the patient were aware of the group assignment before agreeing to participate in the study. Patient allocation was performed accordingly to the sequence provided by the randomization tables and all assigned interventions started immediately. If for the same patient more than one tooth was included in the study (within person), the right or more mesial tooth was allocated to the control group. In consequence, the left or more distal tooth was allocated to the laser group. Laser irradiation was performed by the main investigator (M.R.M.). All other endodontic procedures were performed by enrolled 4th and 5th year students who were always supervised by staff who were not aware of group assignment. Two blinded and previously calibrated endodontic specialists (M.F.K., J.P.V.) independently scored the radiographs.

Standardized Clinical Procedures: Teeth in both groups were subjected to a common two-visit root canal treatment. All treatment sessions were performed by undergraduate students and each appointment was approximately 3 hours long. Local supraperiosteal anesthesia (2% lidocaine with 1:10000 epinephrine, Otoacaine) was administered as needed for patient comfort.

During the first visit, all carious lesions or previous restorations were removed. For each tooth the access cavity was prepared and isolated with rubber dam. Working length (WL) was established at 1mm short of the biological apex of the root, with an ISO #15 K stainless steel file, being confirmed and adjusted using straight and angled radiographs. At the end of the first visit a sterile cotton pellet soaked on Cynosphére (Septodont) was placed in the pulp chamber, and the access cavity was sealed with a reinforced zinc-oxide-eugenol temporary cement (IRM®, Dentsply, Germany).

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To complete the root canal preparation, the second visit was scheduled between 7 to 24 days after the first visit and every patient was questioned for symptoms such as pain, sensitivity to percussion, or swelling. The tooth was then isolated with rubber dam and the temporary filling removed and the master apical file for each canal was set at least 3 sizes larger than the first file to bind at the WL. The minimum apical file of ISO #35 was required for all teeth. Subsequently the canals were obturated using cold lateral compaction with hand made zinc oxide eugenol as sealer (Zinc Oxide, Prodes Dentaires SA, Switzerland) and gutta-percha (Gutta Percha Points, ISO Color Coded –Dentsply, Maillefer). The access cavity was sealed with a reinforced zinc oxide eugenol temporary cement (AM – intermediate restorative material, Dentsply) and a post-operative radiograph was taken. All teeth were restored permanently by the referring dentists within 30 days.

**Control Group (CON):** During the first visit, root canal preparation was performed using the manual step-back technique with 1mm increments using K-File instruments (Zimmerer CC, VDW GmbH, Munich, Germany), and irrigated with 5.0mL of 3% NaOCl after each increment until reach the minimum enlargement of ISO #30 K file. Root canals were dried with sterile paper points and dressed with CH paste. On the second visit, Ca(OH)2 paste was removed by using Hedstrom-type files (Zimmerer, VDW GmbH, Munich, Germany) and copious irrigation with 3% NaOCl. Complete removal of the Ca(OH)2 paste was confirmed by visual inspection and manual preparation was completed. After final irrigation with 5.0mL of 3% NaOCl the root canal was checked for the absence of suppuration or exudate, dried with sterile paper points and obturated according to the previously described technique.

**Test Group (LAS):** During the first visit, root canal instrumentation was performed as described for the Control group. However, irrigation was performed with 2.0mL of sterile saline solution between files. After reaching the ISO #30 K file (Zimmerer CC, VDW GmbH, Munich, Germany), the main canal was filled with distilled water and laser irradiation was performed with the 2780nm Er,Cr:YSGG laser (Wattrolase MD; Biolase Technology, Inc., San Clemente, CA) and a 270μm diameter radial firing tip (LF12 Endolase; Biolase Technology, Inc; calibration factor of 0.55) with panel settings of 0.75W, 20Hz, 37.5μJ per pulse, 14.0 J/cm² energy density, 140μs pulse duration, 0% water and air. The tip was placed at the working length and irradiation was performed at an approximate speed of 2mm/s as the tip was withdrawn.

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from the canal, until the most coronal part of the canal was reached. This procedure was repeated four
times (two with the main canal filled with distilled water and the following two with the canal dry), with
approximately 15 seconds between each laser irradiation. On the second visit, canal instrumentation
was completed with saline solution as irrigant. The minimum apical file of ISO #35 was required for all
teeth. The main canal was filled with distilled water and laser irradiation was performed with a 320μm
radial firing tip (8F73 Endoless, Biolase Technology, Inc; calibration factor of 0.85) with panel settings of
1.25W, 20Hz, 62.5μJ per pulse, 23.6 μJ/cm², 140μs pulse duration, 0% water and air. The laser protocol
was identical to the first visit.
Following final irrigation with 5.0μl of saline solution, the root canal was dried with sterile paper points
checking for the absence of any suppuration or exudate and filled according to the previous obturation
protocol.

Outcome Classification and Data Analysis

The radiologic technique was carried by one operator (M.R.M.). The long-cone paralleling
technique coupled to a film holder was used for both immediate postoperative and follow-up
radiographs. Radiographic exposure settings (Trophy 70 X, USA Inc.) were recorded for each tooth and
follow-ups were reproduced under similar conditions. Radiographic images were coded and stored by 2
of the investigators (I.P.V. and J.A.C.).

The primary outcome measure for this study was change in apical bone density after 12
months. Presence of clinical symptoms or abnormal findings (e.g. spontaneous pain, swelling, mobility
and sensitivity to percussion or palpation) were recorded but not subjected to statistical analysis.

Follow-up radiographs were compared with those taken immediately after treatment and the
PAI was adopted to evaluate radiographic healing.17 Radiographic evaluation was blinded and
independently performed by 2 experienced, previously calibrated reviewers (M.F.C. and I.P.V.).
Instructions for grading images with the PAI scoring system were adapted from Ostadav et al.17 as
follows: 1 (normal periapical structures); 2 (small changes in bone structure), 3 (changes in bone
structure with some mineral loss), 4 (periodontitis with well-defined radiolucent area) and 5 (severe
periodontitis with exuberating features). All images were scored in a random order in a darkened room
using an illuminated viewer box whilst mounted in a cardboard slit to block ambient light.

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All reviewers (endodontists) were initially calibrated by evaluating 3D radiographic images not associated with the study sample and representing a wide range of periapical bone densities. To assess intra-rater agreement, 1 week after the first session the examiners scored the same images. This method generated 4 PAI scores for each image, 2 from each of the 2 examiners. The examiners then met as a group to reach a consensus on cases that did not receive unanimous agreement and reviewed all scores to enhance calibration and inter-rater agreement. A consensus score for each image was considered the definitive score to be used for statistical analysis.

Agreement between and within examiners was determined using the interclass correlation coefficient (ICC). Intra-rater reliability was measured with the single measure ICC (SPSS 17 for Windows; SPSS Inc, Chicago, IL), and inter-rater agreement was measured with the average measure ICC (also known as the inter-rater reliability coefficient). The adopted criteria regarding strength of agreement was: 0.00-0.20, slight agreement; 0.21-0.40, fair agreement; 0.41-0.60, moderate agreement; 0.61-0.80, substantial agreement; and 0.81-1.00, almost perfect agreement.

One week following the final calibration session, both reviewers randomly scored the study images, blinded to the treatment protocol used for each patient. When both reviewers agreed, the score was registered. When disagreement occurred, another reviewer (J.A.C.) decided the final score.

Sample size was determined to be the maximum number of participants who could be considered eligible during the recruitment period. To evaluate changes in PAI scores between groups and for each group from immediate postoperative to 12 months follow-up radiographs, the Mann-Whitney U and the Wilcoxon signed rank tests were respectively applied. Proportion of teeth in each group that could be considered unchanged, improved (decreased PAI score) or healed (PAI ≤2), was assessed with the \( \chi^2 \) Fisher's Exact Test. All hypotheses were conducted at 0.05 level of significance.

Results

Sixty-two patients were assessed for eligibility. Forty-three patients met the inclusion criteria and consented to participate in the trial. At the 12 months follow-up, thirty teeth were examined and subjected to statistical analysis, 12 in the CON group and 18 in the LAS group. Demographic characteristics for each group are listed in Table 1.

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Failures were defined as the need for any additional treatment and were not included in data analysis. There were 2 treatment failures before the 12 months examination (2 in CON group related to the presence of swelling and abnormal symptoms). In LAS group one tooth was extracted due to prosthetic reasons before the 12 month examination and was considered lost to follow-up. 5 additional patients were lost to follow-up (Table 2). No adverse effects were found.

Before the consensus scoring meeting, intra-rater reliability score was 0.95, and the overall inter-rater agreement was 0.85, considered as almost perfect agreement.

For the CON group, two treatment failures were reported and could not be assessed at the 12 months follow-up. The mean PAI score for the CON group was 3.83 (SD=0.89) at the immediate postoperative examination and 1.33 (SD=0.14) at the 12 month follow-up, a decrease of 2.50; the mean PAI score for the LAS group was 4.33 (SD=0.24) at the immediate postoperative examination and 1.72 (SD=0.16) at the 12 month follow-up, a decrease of 2.61; whereas both groups exhibited a statistically significant decrease in PAI score (p<0.05) after 12 months of follow up, no statistically significant difference was found between groups at either the immediate postoperative examination (p=0.14) or the 12 month evaluation (p=0.11) (Table 3).

Despite the two treatment failures detected in the CON group that could not be assessed, there were no records of teeth with unchanged or increased PAI scores. In CON group all assessed teeth were considered healed (PAI ≤2); in the LAS group no treatment failures were detected, 88.90% of the teeth were considered healed, and 11.15% have improved (lower PAI scores). These differences were also not found statistically significant (p=0.50). Characteristic examples of differences between immediate postoperative and 12 months of follow-up radiographs can be found in Table 4.

Discussion

To adopt a single wavelength treatment protocol that more reliably renders root canals free of smear layer and bacteria before filling seems interesting. Our findings confirmed the hypothesis that teeth treated with this novel laser assisted protocol without the use of any chemical solutions would result in equal or superior outcomes when compared to conventional treatment.

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In fact, the limited dentin penetration and consequent effectiveness of irrigating solutions such as sodium hypochlorite, against common endodontic pathogens has caused some investigators to question its optimal concentration and use. There is also no consistent evidence showing that two visit root canal therapy with calcium hydroxide dressing would result in improved outcomes. In addition, given its low solubility, direct contact cannot always be achieved to effectively destroy bacteria harbored on untouched canal walls, dentinal tubules, and other anatomic variations.

Our findings are consistent with other well-designed endodontic clinical studies that found difficult to report statistically significant differences while assessing conventional treatment outcomes. Thirty patients from an original sample of 43 were randomly assigned and examined at the 12 months follow-up, 12 in control group and 18 in test group. Although the participants and operators might not accurately represent the general population of patients and clinicians, this sample size can be considered arguably small but typical comparing to similar trials.

All root canal treatments were performed by undergraduate students (according to a standardized protocol that represents the University of Porto consensus for best endodontic clinical practice) under close supervision and opportunity for assistance. Although undergraduates (4th and 5th year) can arguably be considered less skilled than general dentists, results of treatment under quality-controlled training conditions were found similar to those performed by experienced professionals.

In this study, the PAI score was not used as initial inclusion criteria. However, the requirement that all teeth must have a periapical radiolucent area at least 1.0 x 1.0mm assured an initial PAI score ≥2.

The mean immediate postoperative PAI scores were not significantly different between the two groups so that difference between protocols was the only independent variable. Even though results can be influenced by unknown and uncontrolled variables (e.g. inclusion of smokers) that may predict poorer treatment outcomes, it was decided to exclude multi-rooted teeth from the study as they appear to have lower probability of complete healing when compared to single-rooted teeth.

Although both groups were similar regarding basic demographic characteristics, in a randomized trial small baseline differences can be considered the result of chance rather than source of bias (Fig. 1). In contrast, the mean dropout age was approximately 10 years younger than the overall mean age, which can be found similar to findings obtained in another clinical trial.

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While clinical as well as radiographic data can be used to access treatment outcomes, the relative absence of clinical symptoms in CAP makes the assessment primarily a radiographic one. As a consequence, in endodontic controlled clinical studies data generated by radiographic means are often used. Radiographic diagnosis of apical periodontitis may be regarded as a complex task being difficult to assess. However, systems for training and calibration of observers may improve diagnostic performance namely in the "periapical index" approach, which provides an ordinal scale ranging from "healthy" to "severe periodontitis with exacerbating features". To validate and support the PAI reliability to measure radiographic changes in teeth with CAP, the intra-class correlation coefficient (ICC) was used to determine the agreement between and within examiners. A score of 0 represents no agreement beyond the level of agreement expected by random chance whereas 1.0 means perfect agreement. In this study, the intra-rater reliability and inter-rater agreement score of 0.95 and 0.85 respectively, represents a very high level of agreement within and between examiners.

For this study we were most interested in differences in apical healing between groups by measuring changes in the mean PAI score after 12 months. Results may verify whether predictable outcomes can be achieved with this protocol for the Er,Cr:YSGG laser assisted endodontic treatment.

After 12 months of follow-up and with a 0.05 level of significance, there were no significant differences found between the two groups. In addition, both CON and LAS groups have shown to be effective as independent treatment strategies, exhibiting significant differences between immediate postoperative and follow-up score.

Conclusion

After one year of follow up, and in single rooted and premolar teeth, the Er,Cr:YSGG laser has shown to be at least equally effective as a conventional irrigation/medication regimen, reducing Chronic Apical Periodontitis significantly. Within the same clinical protocol, it can also confirm previous in vitro findings suggesting that the Radial Firing Tips could (1) remove smear layer in wet conditions and (2) achieve deep disinfection in dry conditions.

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These results should be considered preliminary but relevant to clinically appraise the possible benefits of using the Radial Firing Tips physical characteristics either to achieve predictable outcomes or even to overcome conventional irrigation solutions’ limitations. However, further studies should be performed to evaluate its efficacy with a larger sample size and in teeth that present additional challenges such as intricate root canal morphologies and complex curvatures.

Acknowledgments

This research was not supported by any grants or manufacturers. The authors thank the AALZ Institute (Aschen, Germany) for their personal motivation and Ms. Daniele Abreu for the statistical evaluation done specifically for this study.

Author Disclosure Statement

All authors deny any conflict of interests or commercial association with the companies involved in this research. No competing financial interests exist.

References


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Address requests for reprints to:
Miguel R. Martins, DDS, MS:
Departamento de Endodontia, Faculdade de Medicina Dentária, Universidade do Porto, E. Dr. Manuel Pereira da Silveira 4200;
3035 Porto, Portugal
Tel: +351/914651846
E-mail address: migueljr.martins@gmail.com

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### Table 1 - Demographic characteristics for each group (age, gender, and tooth type).

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Age</th>
<th>Single-Rooted</th>
<th>Premolar</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control Group</strong> <em>(n=12)</em></td>
<td>6</td>
<td>6</td>
<td>mean=51 (range: 12 to 76 y/o)</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td><strong>Laser Group</strong> <em>(n=18)</em></td>
<td>7</td>
<td>11</td>
<td>mean=43 (range: 24 to 57 y/o)</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td><strong>Treatment failures</strong> <em>(n=2)</em></td>
<td>0</td>
<td>2</td>
<td>41 &amp; 45 y/o</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Lost to Follow-up</strong> <em>(n=5)</em></td>
<td>2</td>
<td>3</td>
<td>mean=38 (range: 26 to 36 y/o)</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td><strong>Analysed</strong> <em>(n=30)</em></td>
<td>13</td>
<td>17</td>
<td>mean=46 (range: 12 to 76 y/o)</td>
<td>19</td>
<td>11</td>
</tr>
</tbody>
</table>
Table 2. CONSORT (Consolidated Standards of Reporting Trials) flow diagram.

Enrollment

Assessed for eligibility (n=62)
- Excluded (n=19)
  - Not meeting inclusion criteria (n=9)
  - Declined to participate (n=1)
  - Unavailable for follow-up (n=9)

Randomized (n=43)

Group 1 - Control
- Allocated to intervention (n=21)
  - Received allocated intervention (n=17)
  - Did not receive allocated intervention (protocol deviation) (n=4)

Group 2 - Test
- Allocated to intervention (n=22)
  - Received allocated intervention (n=20)
  - Did not receive allocated intervention (protocol deviation) (n=2)

Allocation

Lost to follow-up (n=5)
- Unable to contact (n=3)

Analysis

Analysed (n=12)
- Treatment failure exclusion (n=2)

Analysed (n=18)
- None excluded
Table 3. Results and mean PAI scores (SD=Standard Deviations) along with statistical significances within and between groups. Statistically significant differences are marked (*).

<table>
<thead>
<tr>
<th></th>
<th>INITIAL MEAN PAI SCORE</th>
<th>12 MONTHS MEAN PAI SCORE</th>
<th>MEAN PAI DIFFERENCE</th>
<th>TREATMENT FAILURES</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL GROUP</td>
<td>n=52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(M=3.83, SD=0.89)</td>
<td>(M=1.33, SD=0.14)</td>
<td>2.50* (p&lt;0.05)</td>
<td>2</td>
</tr>
<tr>
<td>TEST GROUP</td>
<td>n=18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(M=4.33, SD=0.24)</td>
<td>(M=1.72, SD=0.16)</td>
<td>2.61* (p&lt;0.05)</td>
<td>0</td>
</tr>
<tr>
<td>STATISTICAL SIGNIFICANCE</td>
<td>p=0.14</td>
<td>p=0.11</td>
<td>p&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

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### Table 4. Characteristic examples of clinical radiographs for both Test and Control Groups.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>IMMEDIATE POST-OPERATIVE</th>
<th>12 MONTHS FOLLOW UP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Group (2.2)</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>Test Group (1.2)</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>Control Group (1.5)</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>Control Group (1.1)</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
<tr>
<td>Control Group (4.4)</td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
</tr>
</tbody>
</table>

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ANNEX IX

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**Er,Cr:YSGG laser and radial firing tips in highly compromised endodontic scenarios**

*Authors*: Miguel Rodrigues Martins, Manuel Fontes Carvalho, Irene Pinho-Vaz, José Capelas, Miguel André Martins, Norbert Guldricht

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**Introduction**

A bacterial contamination is considered the primary etiological factor for the development of pulpal and periapical lesions, and the root canal system must be free of irritants to allow complete healing.

The idea that a visible smear layer is a normal and healthy healing process is consistent with the notion that microorganisms are the primary cause of persistent apical periodontitis. According to the OPT [Ozone Pulse Therapy] theory, the presence of microorganisms at the time of obturation will favor the disease and compromise healing.

The bactericidal effect of conventional irrigation strategies using sodium hypochlorite (NaOCl) has been studied by numerous investigators. The concentration and temperature of NaOCl in root canal irrigation remains controversial and is not well understood.

In fact, it is known that the bactericidal effectiveness of NaOCl is limited to a depth of 100μm. However, heavy E. coli and E. faecalis infection was found 600μm deep in the canal lumen and other bacterial propagation into the dentinal tubules may reach up to 1100μm in depth.

During canal enlargement procedures, a smear layer is mechanically produced, covering the instrumented walls of the root canal. Together with the possibility that the smear layer itself may be infected, it can also protect the bacteria harbored in the dentinal tubules by reducing root dentin permeability from 25% to 60%. Hence, it is generally accepted that the complete removal of the smear layer would be consistent with the elimination of irritants from the root canal system.

Whether adequate microbial control can be obtained in one appointment is an ongoing source of controversy. Although there are multiple scientific arguments to prefer multiple appointments in root canal therapy of infected teeth with apical periodontitis, clinical research to date has been equivocal.

Although calcium hydroxide paste is one of the most commonly used intracanal medications for multiple appointments, root canal therapy, it’s effectiveness against several microorganisms commonly associated with persistent apical periodontitis remains questionable.

Though, newer treatment strategies designed to eliminate microorganisms from the root canal system should be considered, in order to penetrate the dentinal tubules and destroy the microorganisms beyond the root-canal mechanism. Alternative possibilities such...
as a case treatment, ultrasonic and laser-assisted treatments are being suggested as suitable methods to achieve endodontic disinfection, possibly overcoming the limitations of commonly used chemical solutions as well as any hazardous effects. 

The goals for the adjunctive application of erbium lasers in root canal therapy are the ability of infrared light to interact with water and efficiently remove the inner layer of debris from the root canal walls, together with the ability of light to propagate into the dentinal tubules further than any chemical solution, providing deep disinfection. 

The goal of EOE/ESR99 laser-assisted endodontic treatment (LAET) is to provide successful long-term outcomes, namely in cases of persistent infections or periapical abscesses, insufficiency, post-operative obstacles (e.g. isthmus, recurrent canals, retained root fragments, root canal perforations, or wide apical foramina) which are often associated to either lower or compromised clinical expectations.

Chronic apical periodontitis and apical cysts

Following the formation of a periapical inflammatory lesion secondary to pulp necrosis, chronic apical periodontitis (granuloma) is considered the next step in the progression of these inflammatory events showing the replacement of adjacent tissue by inflammatory cells, typically containing fibrous tissue and cholesterol crystals.  

Over time, due to inflammatory stimulation and proliferation of the epithelial rests of Malassez, an inflammatory cyst can develop around the root apex and through the bone. If the lumen of the cyst is continuous with the infection source at the pulp apistry, it may not be self-sustained ("pocket" cyst), this will heal following infection source elimination. On the other hand, if the cyst is completely encased by epithelium and removed from the source of infection, it may be self-sustained ("true" cyst) and become refractory to treatment except by surgical excision.

Cysts mostly appear as round or pear-shaped, unilocular radiolucid lesions in the periapical region. They are usually classified when they become bigger than 1 cm in diameter, being bordered by a thin rim of cortical bone. Cysts may displace adjacent teeth or cause root resorption. 

The differentiation between cystic and granulomatous inflammation is impossible by traditional radiographic techniques, even if several radiographic features have been proposed to make this distinction. These may include the lesion size and the presence of a radiopaque rim lining the cystic lesion. While the probability of a lesion being a cyst may increase with its size, a reliable diagnosis still remains based on histology.

Although being very prevalent, the location of periapical lesions in the oral cavity was found quite similar in different populations. The majority is found in the anterior maxilla (44.5–47.3%), followed by the posterior maxilla (30.7–35.2%), posterior mandible (15.3–16.3%), and anterior mandible (6.1–14.9%).

The stages in development and healing of chronic apical periodontitis, granulomas, and cysts are depending on several circumstances, reflected by changes in the radiographic appearance of periapical areas.}

Miguel Rui A. R. Martins
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

The role of Er,Cr:YSGG laser in endodontics

The rapid development of laser technology as well as a better understanding of laser interaction with biological tissues has broadened the spectrum of possible laser applications in endodontics. The development of new delivery systems, including thin and flexible fibers as well as newly designed endodontic tips, has enabled the use of this technology in a wide range of endodontic procedures.

According to the wavelength and tip configuration, they are applied to disinfect strongly curved root canals and those susceptible to canal enlargement. Due to the absorption or transmission properties in dentin, laser energy was found to be still effective in deep dentinal layers adjacent to the canal lumen as well as in periradicular regions."

Generally, lasers may be especially recommended in the following situations: teeth with purulent pulps, or pulp necrosis, pathological lesions, absence of lateral canals, resorption of the apex caused by inflammation or trauma and for tooth retreated with low prognosis of success.

The Er:YSGG laser shows high absorption coefficients in hydroxyapatite and water, so that germ reduction would be practically impossible. Furthermore, however, apart from being useful to remove organic tissue and smear layer through its mechanical effects, researchers have reported that dental tubules may act as light optical conductors and therefore erbium lasers could still be considered effective for root canal disinfection up to a depth of 500 μm.

Although in-vitro investigations may support the use of Er:YSGG laser in endodontics, few clinical trials have been reported regarding the potential benefits and long-term outcomes after such treatments.

Radial firing tips

Up to now, endodontic fibers have bare tips, so the energy is transmitted forward with a relatively small divergence. This limitation requires the clinician to move the fiber in a withdrawing and rotating action in order to obtain a uniform coverage of the canal walls. Thus, with bare fibers, it was found almost impossible to obtain uniform coverage of the canal surface along with reproducing results.

The delivery of the laser from the tip of the optical fiber near the root end may also result in the transmission of the irradiation beyond the apical foramen, leading to undesirable effects on either teeth in close proximity to the mental foramen or the mental bular nerve. Most lasers have been commonly reported disadvantages: (1) Most of the laser energy is redirected only in the axial direction, and little energy can be obtained perpendicular to the fiber; and (2) many wavelengths cannot penetrate the smear layer and the bacteria in the root canal, making the use of lasers less applicable.

To overcome concerns related to the energy emission in axial direction and root foramen, the unique emission profile obtained for the Er:YSGG laser radial firing tips (RFI) may have a significant role in...
creasing the efficiency of laser delivery for endodontic applications. Not only does the beam expansion by the tip geometry reduce emissions in the forward direction, but it favours homogeneous energy distribution along the root canal wall.228

The debridging action of a laser in endodontics has shown to be better when delivered through conical fibers than with bare fibers as the divergent laser energy will interact with the canal walls, causing direct and indirect ablation through photomechanical effects. In fact, erbium lasers have been demonstrating to induce shock waves in aqueous solutions inside root canals and radial firing tip positively influence their configuration. Hence, through the activation of aqueous solutions (e.g., water, EDTA), the Er:YSGG laser induces primary and secondary cavitation effects, useful for debris and smear layer removal.138

However, the best results in terms of thorough root canal dissection with the Er:YSGG laser are achieved while operating in dry conditions, relying on the fact that – without water inside the main root canal – the ability of such wavelength to penetrate into the dentinal tubules is increased.108 Today, limited literature addresses the clinical outcome of endodontic therapy using RFTS without the aid of any chemical substances.228 This clinical case study aims to represent an interesting proof of concept for the benefits of using radial firing tips in highly compromised teeth with apical pathology.

Clinical case

A female patient (E.F.), 33 years old, presented a history of recurrent sinusitis and multiple antibiotic administrations. A previous endodontic treatment was performed within the past two years. At this time, she was referred by her dentist to an oral surgeon for cyst ablation and tooth extraction under general anaesthesia. An active fistula on the apical buccal area of the tooth 1.6 was detected. Vertical percussion was also found positive (Fig. 1). A non-surgical laser-assisted endodontic retreatment was proposed prior to surgery for cyst ablation. Written informed consent was previously signed. The endodontic retreatment was performed in two appointments according to the protocol described in Martins et al.228 During the first appointment, initial carious excision was performed and the root filling was removed. Rubber dam isolation was obtained and the access cavity prepared. The working length (WL) was electronically established as 1 mm short of the biological apex of the root.
Discussion and conclusion

To adopt a single wavelength treatment protocol that more reliably renders root canals free of smear layer and bacteria before filling seems interesting. This could be an additional clinical evidence to suggest that the Er,Cr:YSGG laser with radial firing tips could be a valuable strategy to (1) remove smear layer in wet conditions and (2) achieve deep disinfection in dry conditions, as in the same protocol.

While clinical as well as radiographic data can be used to access treatment outcomes, the relative absence of clinical symptoms in CP patients makes the assessment primarily a radiographic one. As a consequence, in endodontic controlled clinical studies, data generated by radiographic means are often used. An important step forward in this regard is the clinical report that has shown that, presumably, even apical cysts could be successfully treated by endodontic means by using a 2,780 nm wavelength and radial firing tips. The Er,Cr:YSGG laser should be considered a predictable tool to assist endodontic treatments overcoming possible limitations commonly associated to conventional strategies. However, few clinical trials and single-case reports have been reported. This clinical case should stimulate other researchers to conduct additional blind randomized trials or clinicians to report their clinical findings in order to provide an evidence-based concept for the use of radial firing tips in endodontics.

Miguel Rodrigues Martins
DDE, DDS, MS
Endodontic Department, Faculty of Dental Medicine, University of Porto - Portugal
Rua Dr. Manuel Pereira de Silva, 4200-393 Porto PORTUGAL
Tel: +351914010046
miguelar.martins@gmail.com
ANNEX X

Further clinical cases.

Patient: M.B.P. (Male; 55y.o.)

Clinical Case (Tooth): Suspect of Root Fracture (1.4)

Clinical History:
- History of vocal cords surgery 1.5 years ago;
- Extensive Chronic Apical Periodontitis;
- Negative reply to thermal tests;
- Vertical Percussion (+) / Lateral Percussion (++);
- Suspect of root micro fracture, as no further plausible etiologic factor could be detected.

Treatment:
- Root Canal Preparation ProTaper® (Maillefer, Dentsply) up to F3;
- SL removal and Disinfection: Er,Cr:YSGG Laser (2780nm) RFT2+RFT3;
- Obturation: Tapered Gutta-Percha, Vertical Compaction & OZE cement.

(During LAET)  
(Post-Operative)

(12 Months Follow-up)  
(2 Years Follow-up)
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

Patient: C.N. (Female; 37y.o.)

Clinical Case (Tooth): Endodontic Retreatment with Apical Resorption (1.2)

Clinical History:
- Endodontic Treatment performed “several” years ago;
- Extensive Chronic Apical Periodontitis;
- Active Fistula (vestibular);
- Vertical Percussion (+);
- History of regular antibiotic usage over the past 2 years.

Treatment:
- Root Canal Preparation: ProTaper® (Maillefer, Dentsply)
  - Retreatment Files D1-D3; Apical calibration to ISO#80.
- SL removal and Disinfection: Er,Cr:YSGG Laser (2780nm) RFT2+RFT3;
- Obturation: Tapered Gutta-Percha, Vertical Compaction & TopSeal® (Dentsply).

(Fistula Tractus)  (Pre-LAET)  (During LAET)

(Post-LAET)  (6 Months Follow-Up)  (2 Years Follow-Up)
(2 Years Follow-Up & Clinical Picture)
**Patient:** H.P. (Male; 24y.o.)

**Clinical Case (Tooth):** Auto-Transplant (3.8 to 3.6)

**Clinical History:**
- 3.6 extraction with immediate 3.8 auto-transplant performed by Oral Surgery Department, FMDUP;
- Following 6 months after surgery, vertical percussion (++);
- Chronic Apical Periodontitis associated to the transplanted tooth.

**Treatment:**
- Root Canal Preparation: ProTaper® *(Maillefer, Dentsply)*
  - MB+ML#F2 & D#F3
- SL removal and Disinfection: Er,Cr:YSGG Laser (2780nm) RFT2+RFT3;
- Obturation: Tapered Gutta-Percha, Vertical Compaction & TopSeal *(Dentsply)*.

(Auto-Transplant Surgical Sequence)
(6 Months after transplant - Before LAET)

(After LAET)

(7 Months Follow-Up)

(13 Months Follow-Up)

(2,2 Years Follow-Up)
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

(2,2 Years Follow-Up Panoramic Radiography)


**Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment**

**Patient:** L.P. (Female; 31y.o.)

**Clinical Case (Tooth):** Invasive Cervical Root Resorption (1.1)

**Clinical History:**
- History of trauma >10 years ago;
- Recurrent swelling and occasional pain;
- Multiple antibiotic prescriptions;
- Economical constraint to perform Crown/Implant Rehabilitation

**Treatment:**
- Root Canal Preparation: ProTaper® (*Maillefer, Dentsply*) up to #F4;
- SL removal and Disinfection: Er,Cr:YSGG Laser (2780nm) RFT2+RFT3;
- Obturation: Continuous Wave of Obturation - Calamus® & TopSeal (*Dentsply*)

![Pre-Operative Picture and Intra-oral Film](image)

![During LAET](image)

![Post-LAET](image)

![6 Months Follow-Up](image)
(12 Months Follow-Up)  (2 Years Follow-Up)

(2 Years Follow-Up Panoramic Radiography)

(2 Years Follow-Up Clinical Pictures)
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

**Patient:** I.C. (Female, 22 y.o.)

**Clinical Case (Tooth):** Iatrogenic Necrosis (3.7)

**Clinical History:**
- Assymptomatic necrosis;
- Extensive Chronic Apical Periodontitis;
- Vertical Percussion +

**Treatment:**
- Root Canal Preparation: ProTaper® (Maillefer, Dentsply)
  - MB+ML#F3, D#F4
- SL removal and Disinfection: Er,Cr:YSGG Laser (2780nm) RFT2+RFT3;
- Obturation: Tapered Gutta-Percha, Vertical Compaction & TopSeal (Dentsply).

(Pre-LAET)  
(During LAET)  
(Post-LAET)

(Post-LAET)  
(6 Months Follow-Up)  
(12 Months Follow-Up)
(Post-LAET Panoramic Radiography)

(12 Months Follow-Up Panoramic Radiography)

(Post-Operative)  (2,2 Years Follow-Up)
**Patient:** A.M. (female, 48y.o.)

**Clinical Case (Tooth):** Endodontic Re-Treatment (3.1)

**Clinical History:**
- Endodontic Treatment failure and with intermittent pain;
- Chronic Apical Periodontitis with absence of apical constriction.

**Treatment:**
- Root Canal Preparation: ProTaper® *(Maillefer, Dentsply)* up to #F4;
- SL removal and Disinfection: Er,Cr:YSGG Laser (2780nm) RFT2+RFT3;
- Obturation: Tapered Gutta-Percha, Vertical Compaction & TopSeal (Dentsply)

![Initial Panoramic Radiography](image1)

![During LAET](image2)

![Post-LAET](image3)
(12 Months Follow-Up) (2,5 Years Follow-Up)

(2,5 Years Follow-Up)

(2,5 Years Follow-Up Panoramic Radiography)
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

Patient: M.M. (Female, 32y.o.)

Clinical Case (Tooth):
- Endodontic Re-Treatment (1.1 + 2.2)
- Endodontic Re-Treatment + Metal Post Removal (2.1)

Clinical History:
- Assymptomatic necrosis;
- Extensive Chronic Apical Periodontitis;
- Aim: Pre-Prosthetic Crown Rehabilitation.

Treatment:
- Root Canal Preparation: ProTaper® (Maillefer, Dentsply) 1.1+2.1#F3, 2.2#F4
- SL removal and Disinfection: Er,Cr:YSGG Laser (2780nm) RFT2+RFT3
- Obturation: Tapered Gutta-Percha, Vertical Compaction & TopSeal (Dentsply).

(Initial Clinical Picture)

(During LAET 1.1+2.1) (Metal-Post Removal 2.1)
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

(6 Months Follow-Up)  (12 Months Follow-Up)  (2 Years Follow-Up)

(Pre-LAET Panoramic Radiography)

(2 years Follow-Up Panoramic Radiography)
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

Patient: R.M. (Male, 23y.o.)

Clinical Case (Tooth):
- Endodontic Re-Treatment (2.1) & Endodontic Treatment (2.2)

Clinical History:
- (2.1) Endodontic failure with extensive Chronic Apical Periodontitis;
- (2.2) with negative reply to thermal tests and positive vertical percussion;
- Recurrent localized swelling and fistula tractus;
- History of several antibiotic administration in the past years.

Treatment:
- Root Canal Preparation: ProTaper® (Maillefer, Dentsply)
  - 2.1#D1,D2,D3. Apical Preparation/Calibration #F4
  - 2.2#F3
- SL removal and Disinfection: Er,Cr:YSGG Laser (2780nm) RFT2+RFT3;
- Obturation: Tapered Gutta-Percha, Vertical Compaction & TopSeal (Dentsply).

(Initial Panoramic Radiography)

(Pre-LAET X-Rays)
(During LAET 2.1+2.2)  (Post-LAET 2.1)  (Post-LAET 2.2)

(6 Months Follow-Up 2.1 + 2.2)

(12 Months Follow-Up 2.1 + 2.2)
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

(18 Months Follow-Up)          (2 Years Follow-up)

(2 Years Follow-Up Panoramic Radiography)

(2 Years Follow-Up Clinical Picture)
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

**Patient:** C.F. (Female, 21y.o.)

**Clinical Case (Tooth):**
Endodontic Treatment (1.1 + 1.2) with Palatine Abscess

**Clinical History:**
- Extensive Chronic Apical Periodontitis;
- Recurrent localized palatine abscess & intermittent fistula tractus;
- History of several antibiotic administration in the past 2 years.

**Treatment:**
- Root Canal Preparation: ProTaper® *(Maillefer, Dentsply)*
  - 1.1#F5, 1.2#F3
- SL removal and Disinfection: Er,Cr:YSGG Laser (2780nm) RFT2+RFT3;
- Obturation: Tapered Gutta-Percha, Vertical Compaction& TopSeal *(Dentsply).*

(Initial Panoramic Radiography)

(Pre-LAET 1.1+1.2)  (Palatine Abscess: Pre-LAET)
Palatine Abscess: Pre-LAET

2nd appointment – 7 days interval

(During LAET)

(Post-LAET 1.1+1.2)

(6 Months Follow-up)
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

(12 Months Follow-Up Clinical Pictures)

(12 Months Follow-Up)
(2 Years Follow-Up)

(2 Years Follow-Up Panoramic Radiography)

(2 Years Follow-Up)
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

Patient: B.A.S. (Male, 16y.o.)

Clinical Case (Tooth):
Endodontic Treatment (3.1+4.1+4.2) & Extra-Oral Fistula

Clinical History:
- Extensive Chronic Apical Periodontitis;
- Persistent Extra-Oral Fistula;
- Dermatologist Diagnosis: sebaceous/follicular glands obstruction;
- History of several antibiotic administration.

Treatment:
- Root Canal Preparation: ProTaper® (Maillefer, Dentsply) / 3.1, 4.1 & 4.2 #F2
  - 3.1 & 4.1 Er,Cr:YSGG Laser (2780nm) only with RFT2;
  - 4.2 Disinfection with 3%NaOCl & Ca(OH)₂ inter-appointments
Obturation: Tapered Gutta-Percha, Vertical Compaction & TopSeal (Dentsply)

(Initial Pictures: Extra-Oral Fistula)
(Initial Panoramic Radiography)

(Initial Picture and intra-oral film 3.1 + 4.1 + 4.2)

(During LAET)
Efficacy of the Er, Cr: YSGG Laser in the Laser Assisted Endodontic Treatment

(Post-LAET: fistula cicatrization)

[6 Months Follow-Up (inverted Intra-oral Films)]
(12 Months Follow-Up)

(12 Months Follow-Up Panoramic Radiography)

(2 Years Follow-Up: Panoramic Radiography)
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

(2 Years Follow-Up)

(2 Years Follow-Up: Clinical Pictures)
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

**Patient:** D.P. (Male, 27y.o.)

**Clinical Case (Tooth):**
- Extensive Chronic Apical Periodontitis (4.6) with apical resorption

**Clinical History:**
- Extensive mesio-buccal decay with absence of clinical symptoms;
- Tooth necrosis confirmed by vitality thermal tests and positive vertical percussion.

**Treatment:**
- Root Canal Preparation: ProTaper™ (*Maillefer, Dentsply*)
  - MB+ML #F3 ; D#F5
- SL removal and Disinfection: Er,Cr:YSGG Laser (2780nm) RFT2+RFT3
- Obturation: Tapered Gutta-Percha, Vertical Compaction & TopSeal (*Dentsply*)

(Initial Panoramic View)

(Pre-LAET)  (During LAET)  (Post-LAET: missing)
(12 Months follow-up)  (2 Years Follow-Up)
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

Patient: A.F. (Male, 30y.o.)

Clinical Case (Tooth):
- Endodontic Re-Treatment (1.5)

Clinical History:
- Chronic Apical Periodontitis with absence of clinical symptoms;
- Previous endodontic treatment performed >1year ago;

Treatment:
- Endodontic Retreatment in single appointment;
- Root Canal Preparation: ProTaper® (Maillefer, Dentsply)
  - V+P #F3
- SL removal and Disinfection: Er,Cr:YSGG Laser (2780nm) RFT2+RFT3
- Obturation: Tapered Gutta-Percha, Vertical Compaction & TopSeal (Dentsply)

(Initial Panoramic Radiography)

(Pre-LAET)  (Post-LAET)
(8 Months Follow-Up)  (18 Months Follow-Up)

(18 Months Follow-Up Clinical Picture)
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

Patient: M.L.C (Female, 41y.o.)

Clinical Case (Tooth):
- Endodontic Re-Treatment (2.2)

Clinical History:
- Previous endodontic treatment performed >3 years ago;
- Extensive Chronic Apical Periodontitis with recurrent swelling;
- History of several re-treatment appointments, using NaOCl and Ca(OH)$_2$ inter-appointments;
- Association with antibiotics; still revealing incapacity to contain suppuration;
- Apical resorption / widened apical constriction.

Treatment:
- Endodontic Retreatment in single appointment;
- Root Canal Preparation: ProTaper\textsuperscript{a} (Maillefer, Dentsply) #F5 + ISO#60
- SL removal and Disinfection: Er,Cr:YSGG Laser (2780nm) RFT2+RFT3
- Obturation: Tapered Gutta-Percha, Vertical Compaction & TopSeal (Dentsply)

(Initial Clinical Pictures)
(During LAET)

(6 Months Follow-Up)

(9 Months Follow-Up)

(18 Months Follow-Up and Clinical Picture)
Efficacy of the Er,Cr:YSGG Laser in the Laser Assisted Endodontic Treatment

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