v. 3, n. 96, 2023

International Journal of Health Science

EVALUATION OF THE EFFECT OF PHOTODYNAMIC THERAPY MEDIATED BY METHYLENE BLUE CARRIED IN SURFACTANT MEDIUM AS AN ADJUVANT IN THE TREATMENT OF PARTS WITH APICAL PERIODONTITIS AND PRESENCE OF FISTULA RANDOMIZED, CONTROLLED, DOUBLE-BLIND CLINICAL TRIAL

Carolina Wince González

Prof. Dr. Renato Araujo Prates

International Journal of Health Science ISSN 2764-0159 DOI 10.22533/at.ed.1593962321116

Abstract: In the present study, the effect of photodynamic therapy (aPDT) as an adjuvant in conventional endodontic treatment in teeth with apical periodontitis and presence of fistula will be evaluated. Referring to the fact that the moment in which the presence of a fistula is evident, it is generally the product of an injury. A fistula is a small canal that forms from the infected area of the tooth, which usually coincides with the apex of the root, to the outer surface of the gum. Once the pathology is established, it is necessary to treat it with endodontic treatment, which consists of disinfecting, cleaning and sealing the root canals. It is based on the premise that photodynamic therapy appears as an adjuvant, being a non-invasive treatment that has a photosensitizer and a light source for the formation of reactive oxygen species that cause bacterial death. The methodology to be applied will consist of the selection of 30 teeth with a diagnosis of apical periodontitis and the presence of a fistula. Patients will be randomly assigned to two groups as follows. Group I, patients undergoing conventional endodontic treatment (n=15) and Group II, patients undergoing conventional endodontic treatment combined with antimicrobial photodynamic therapy (n=15). The clinical findings will be counted due to the absence of symptoms, presence of fistula and radiographic parameters.
Keywords: Periodontitis, Fistula, Endodontic Treatment, Photodynamic Therapy, Methylene Blue.

CONTEXTUALIZATION

When talking about Endodontics, reference is made to the science and art that covers the etiology, prevention, diagnosis and treatment of pathological changes in the dental pulp and its repercussions in the periapical region, therefore, in the body. As one of the pillars, infection control is applied, if there is no prevention and treatment when it exists. When the dental disease is already established, affecting the periapical tissues, producing inflammation, reference is then made to apical periodontitis.

It is from this moment that inflammation begins and, therefore, the destruction of the periapical tissue as a consequence of the agents responsible for pulp infection. When referring to the presence of a fistula, it is usually the product of an injury. Infections or inflammation can also cause the formation of a fistula, also called intraoral sinus tract. A dental fistula is a small canal that forms from the infected area of the tooth, which usually coincides with the apex of the root, to the outer surface of the gum. The passage acts as a reservoir for microorganisms and their products, and as it fills, a small lump forms on the gums.

At the time that the pathology is established and must be treated, we speak of endodontic treatment, either through manual, mechanized or combined treatments; as well as the use of irrigators, intracanal medication and canal sealers. All procedures take time and supplies and are often unsatisfactory. It is for this reason that photodynamic therapy (PDT) appears as a complement in endodontic treatment.

Now, since it is a non-invasive treatment, it has a photosensitizer and a light source for the formation of reactive oxygen species that cause bacterial death. The main limitation of this technique is the formation of attenuators that reduce the effectiveness of the therapy. On the other hand, sodium dodecyl sulfate (SDS) showed the ability to reduce this effect of dummer formation.

Therefore, the reason for this study is to validate the photodynamic effect of methylene blue transmitted in 0.25% SDS for the treatment of patients with apical periodontitis and presence of fistula, in order to eradicate persistent microorganisms in root canals. evolution of these treatments. The methodology will consist of the selection of 30 dental parts with a diagnosis of apical periodontitis and presence of fistula. Patients will be randomly divided into two groups: Group I, patients undergoing conventional endodontic treatment (n = 15) and Group II, patients undergoing conventional endodontic treatment combined with photodynamic antimicrobial therapy (n = 15).

Clinical findings will be recorded due to absence of symptoms, presence of fistula and radiographic parameters. From the moment non-vital parts are evident and when the disease is already established, the microbial factor will begin to be considered. The microbes in the lumen of the duct are plankton. Biofilm is one that adheres to the walls of the canal, as well as to the teeth, it adheres to the walls. When there is a periradicular lesion, there is a large amount of biofilm (Hargreves, Berman, & Kenneth, 2016).

Evidence shows the polymicrobial etiology of endodontic infections, in which bacteria and their products are the main agents for the development, progression and spread of apical periodontitis (Singh, Nagpal, Manuja, & Tyagi, 2015). Microbial factors in root canals are those that extend to the apical tissue causing chronic inflammatory load. Therefore, apical periodontitis is the result of the complex interaction between microbial factors and host defense (Siqueira, 2002).

When endodontic infection becomes chronic, it can drain to the gingival surface through the intraoral communication known as the sinus tract or fistula (Hargreves, Berman, & Kenneth, 2016). This pathway, sometimes covered by epithelium, extends to a shallow hole or stoma in the attached gum. In general, a periapical infection with the associated sinus tract is generally not painful, although sometimes there is associated discomfort before the development of the sinus tract (Garcez, Nuñez, Hamblin, & Ribeiro, Antimicrobial effects of photodynamic therapy on patients with necrotic pulps and periapical injury, 2008). In addition to providing a conduit for the release of infectious exudate with subsequent pain relief, the presence of the fistula can also help us identify the origin of a specific infection.

The fistula may be located directly near or far from the source of the infection. Therefore, the trajectory of the sinus tract is what will provide us with objective data to locate the tooth causing the disease (Hargreves, Berman, & Kenneth, 2016) (Garcez, Nuñez, Hamblin, & Ribeiro, Antimicrobial effects of photodynamic therapy on patients with necrotic pulps and periapical lesion, 2008).To trace this path, what we call fistulography is performed, which consists of the introduction of a c-caliber gutta-percha cone through the orifice of the tract until resistance is felt, a radiographic image and after revealing what the image will give us, following the path that the cone has traveled to find out which tooth is causing the infection.

The mechanism of action of bacteria is that they are found at the edges of the root canal, not in the bone, so it is important to clean it. It is from this moment that importance begins to be given to the cleaning and conformation of the piping system (Baumagartner, Piquete, & Muller, 1984).

The most recommended treatment to eliminate a fistula is endodontics to drain the area and prescribe prior medication. Endodontics consists of carrying out a deep cleaning treatment of the affected area to complete the elimination of any tissue that may have been affected (Hargreves, Berman, & Kenneth, 2016) (Garcez, Nuñez, Hamblin, & Ribeiro, Antimicrobial effects of photodynamic therapy on patients with necrotic pulps and periapical lesion, 2008).

Any successful endodontic therapy

requires the removal of microorganisms and biofilms through root canal disinfection methods. Irrigation is defined as flushing a cavity with a liquid. The objectives of irrigation in endodontics are mechanical and chemical (Baumagartner, Piquete, & Muller, 1984). Sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA) and chlorhexidine (CHX) are the most common effective irrigators to eliminate these microorganisms from the coronal and middle thirds of the dental canals, but are greatly reduced towards the apical third (Basrani, Ghanem, & Tjaderhane, 2004).

The intracanal medication of choice is chlorhexidine mixed with calcium hydroxide, since studies have shown the good properties of both, enhanced when used in combination. In recent years, new alternative treatment modalities have been proposed, including high-power lasers and antimicrobial photodynamic therapy (PDT) (Asnaashari, Kooshki, Salehi, Azari-Marhabi, & Moghadassi, 2020).

Photodynamic therapy (PDT) has been proposed as a new complementary method for additional disinfection of the root canal system with the possibility of improving treatment outcomes (Soukos, et al., 2006). This technique is based on the use of photo-sensitizing substances that bind to cells and will be activated by light with an appropriate wavelength that will promote the death of microorganisms.

The photosensitizer is a chemical sensitive to light (Plotino, Grande, & Mercade, 2019). The use of photosensitizer for photosensitization of infected tissues can allow irradiation capture of bacterial cells to the tissues, which can result in the destruction of both bacteria and infected tissues (Prates, Yamada, Hashimoto, & Ribeiro, 2010). For this study, the use of PDT applied in endodontic treatment was validated using a photosensitizer such as methylene blue at a concentration of 10 mg/ml and irradiation with a diode laser with a wavelength of 665 nm and a power of 100 mW (Tortamano, et al., 2020).

The use of methylene blue in photodynamic therapy has shown very good results. The hydrophilic characteristic of methylene blue, its low molecular weight and its positive charge allow its passage through the walls of microorganisms (Karaoglu, Ugugr, Erdonmez, Gol, & Durmus, 2020). Based on the use of photo-sensitizing substances that bind to cells and will be activated by light with an appropriate wavelength, which will promote the death of microorganisms due to the formation of reactive oxygen species. Phenotic dyes are the most used in dentistry, with methylene blue being the most common.

It is important to note that the antimicrobial effect of PDT only occurs when the absorption spectrum of the photosensitizer and the emitted radiation are compatible. The wavelength, light intensity, exposure time and absorption capacity of the photosensitizer determine the results (Prates, Suzuki, & Claudio, 2012). Therefore, the effectiveness of the treatment depends on the improvement of this large number of parameters.

Another very important factor for the effectiveness of PDT is the aggregation of the photosensitizer, which loses part of its effect at higher concentrations. This effect known as dimerization can be controlled with the presence of surfactant substances, so the use of sodium dodecyl sulfate (SDS) can favor photochemical conditions to obtain the greatest therapeutic potential of the photodynamic effect (Mozayeni, et al., 2020).

After investigating many previous studies, it is possible to verify that PDT must not replace endodontic treatment procedures, but must be considered as an aid in conventional treatment, because low-level laser was found to be safe, easy to handle and widely used. accepted by patients, in addition to promoting antimicrobial activity when associated with a photo-sensitizing dye.

It is indisputable that correct endodontic treatment, with its correct conformation, irrigation and intracanal medication are essential for the success of the treatment. However, the improvement of the technique is always due to providing better quality treatment (Alvarenga, et al., 2015).

This study aims to evaluate the reduction of bacterial load after conventional endodontic treatment with and without antimicrobial photodynamic therapy in teeth with apical periodontitis and presence of fistula. Diode laser equipment (DMC, THERAPY EC, São Carlos, Brazil) will be used, with a wavelength of 660nm and power of 100 mV for 3 minutes.

ANTIMICROBIAL PHOTODYNAMIC THERAPY

Antimicrobial photodynamic therapy (aPDT) is a non-invasive treatment modality and can be used as an adjunct to conventional periodontitis treatment. It is a form of phototherapy with the goal of eliminating microbial cells through the mass production of reactive oxygen species. This technique eliminates bacteria and fungi, including those that are resistant to antimicrobials. APDT acts through the appropriate combination of light and a photo-sensitizing agent (PS), in the presence of oxygen, promoting the death of microorganisms through the formation of ROS (reactive oxygen species) and singlet oxygen (Mozayeni, et al., 2020). (Alvarenga, et al., 2015).

After the disorganization of the biofilm through endodontic treatment of the tooth, the periodontal pockets may contain residual bacteria inside, so it is necessary to supplement antimicrobials. APDT is a non-invasive treatment modality, and can be used as a complementary treatment for periodontal diseases, avoiding the excessive use of systemic antimicrobials, and thus reducing the risk of developing bacterial resistance in the use of these medications. This therapy consists of the topical administration of a SF that is distributed within the root canal.

With activation by light exposure using laser or LED with the appropriate dosimetric parameters, the absorption of FS causes photochemical changes that cause the destruction of the target tissue (Baumagartner, Piquete, & Muller, 1984) (Firmino, et al., 2016). The mechanism of action can be described by the Jablonski diagram (illustration 1), the FS absorbs visible light at an appropriate wavelength passing from the ground singlet state (S0) to the first singlet excited state (S1), whose useful life It's short. In this state, FS can lose energy through radioactive processes (fluorescence) or convert to the triplet state (T1) through intersystem crossing.

Starting from the triplet state, presenting a longer useful life compared to the singlet state, FS can lose energy through radioactive processes (phosphorescence) or participate in the formation of reactive oxygen species (ROS) by two different mechanisms: the reaction type I, which is carried out by electron transfer, forming free radicals or type II reaction, which is the transfer of energy to molecular oxygen generating singlet oxygen. The occurrence of type I and type II reactions depends on several factors, such as the physicochemical characteristics of the SF and the microenvironment in which the SF is inserted (Asnaashari, Homayuni, & Pymanpour, The antibacterial effect of additional photodynamic therapy in failed endodontically treated teeth: a pilot study, 2016).

The latter affects its state of aggregation and the amount of oxygen and other substrates present (Mozayeni, et al., 2020) (Alvarenga, et al., 2015) (Anagnostaki, Mylona, Parker, Lynch, & Grootveld, 2020). APDT, unlike antibiotics, has low toxicity to humans and there are no reports of bacterial resistance (Soukos, et al., 2006).

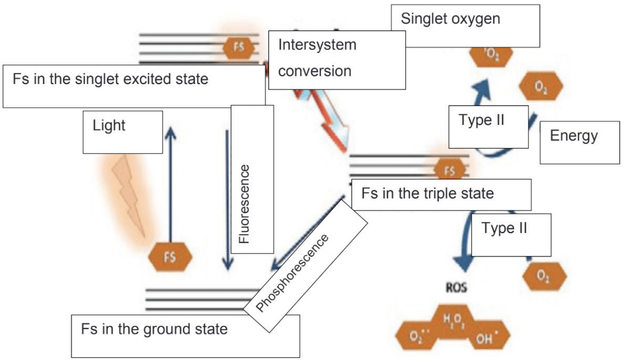

Illustration 1. Representation of the Jablonski diagram

The photosensitizer (FS) absorbs a photon of light and goes from the singlet ground state to the excited singlet state. In this state, the FS can return to the ground state by fluorescence emission or transition to the excited triplet state. In this state, FS can regress to the ground state by fluorescence emission or interact with molecular oxygen resulting in a type I reaction (radical formation) or a type II reaction (triplet oxygen formation).

METHYLENE BLUE IN THE SURFACTANT VEHICLE

Phenothiazines are planar amphipathic tricyclic molecules that contain a quaternary intrinsic nitrogen atom and have phototoxic efficiency against bacteria. The class of photosensitizers (PS) frequently used in dentistry are phenothiazines, represented by methylene blue (AM) and toluidine blue (TA). They are heterocyclic dyes formed by two benzene rings linked to a nitrogen atom and a sulfur atom. They present intense absorption when combined with visible light around 600-660 nm, causing effective penetration of light into the tissues (Asnaashari, Homayuni, & Pymanpour, The antibacterial effect of additional photodynamic therapy in endodontically treated teeth with failure: a pilot study, 2016) (Anagnostaki, Mylona, Parker, Lynch, & Grootveld, 2020) (Garcez, Nuñez, Hamblin, & Ribeiro, Antimicrobial effects of photodynamic therapy on patients with necrotic pulps and periapical lesion, 2008).

Metachromatic dyes are capable of controlling their aggregation by changing their color. The most used in dentistry are basic or cationic dyes, which are the most common. Metachromasia is a phenomenon whereby a dye can absorb light at different wavelengths, depending on its concentration and environment. The dye, which exhibits this phenomenon without changing its structure here.

Some authors have shown that the use of methylene blue (MA) in an ethanol or urea solution is more effective in microbial death than when dispersed in water. It was found that the use of other molecules such as sodium dodecyl sulfate can control the aggregation of AM, resulting in an increase in the amount of monomer formation and a decrease in attenuators in the solution, through an increase in the electrostatic and hydrophobic interaction between its molecules (Bonsor, Nichol, Reid, & Pearson, 2006) (Komerik & MacRobert, 2006).

The application of AM in an SDS solution (surfactant vehicle) at a concentration of 0.25% showed greater microbial reduction when used in PACT against Candida albicans compared to the use of AM without SDS. This study found that in the form of monomers, AM was more effective as a photosensitizer and that type II reactions (via singlet oxygen) were the photochemical mechanisms that most efficiently induced C. albicans to die (Alvarenga, and others, 2015).

In an in vitro study carried out; SDS

linked to methylene blue photosensitizer was used to increase the microbial killing of A. actinomycetemcomitans and P. gingivalis compared to methylene blue in PBS, because this vehicle has a bacterial wall dissolving effect and is able to decrease the molecular aggregation of the photosensitizer, increasing the amount of monomers and decreasing the amount of dyes, where the best efficacy of aPDT was found only in P. gingivalis, when it was associated with FS to the Surfactant Vehicle SDS, raising the hypothesis that the presence of 0.25% SDS can lead to different responses, depending on the metabolic behavior of each microbial species (Soukos, et al., 2006).

In view of these findings, it is necessary to develop formulations for clinical applications that can control AM aggregation and, consequently, improve clinical protocols by making them more efficient (Soukos, et al., 2006) (Alvarenga, et al., 2015).

GOAL

GENERAL GOAL

To evaluate the action of PDT as an adjuvant in the endodontic treatment of parts with apical periodontitis and presence of fistula.

METHODOLOGY

After submitting this research project to the approval of the UCU Research Ethics Committee, this clinical trial will be carried out in patients with apical periodontitis and presence of fistula in single-rooted teeth who must present the following characteristics; Initial periapical radiograph showing the presence of apical radiolucency and fistulography to confirm its presence.

Participants will be recruited at `` Clínica Universitaria de Salud de la Universidad Católica del Uruguay``, where all clinical procedures for the study will be performed. Participants must read, understand and sign the Free and Informed Consent Form approved by the UCU Research Ethics Committee (Annex A).

The clinical parameters analyzed will be the evaluation of periapical radiographs for the evaluation of apical radiolucency and the presence of fistula.

30 patients will be selected and randomly divided into two experimental groups:

Group 1:

Mechanized endodontic treatment and medication with CAOH (calcium hydroxide) between sessions. This medication is the most used in all conventional endodontic treatments.

Group 2:

Mechanized endodontic treatment with CAOH (calcium hydroxide) medication after the application of photodynamic therapy with methylene blue. Methylene blue is a substance that has many studies that demonstrate its effectiveness in the oral cavity and its active ingredient is already used in endodontic treatments.

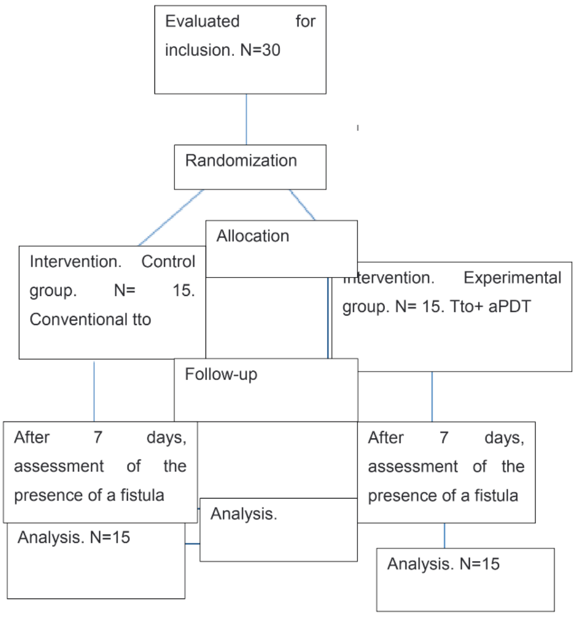

Illustration 2. Fluxogram

PREPARATION OF THE PHOTOSENSITIZER

Methylene blue will be prepared under aseptic conditions in 10cc amber glass bottles from 1% 10cc methylene blue, forming a 0.005% aqueous solution by pharmaceutical chemist Analía Techera.

IRRADIATION SOURCE

Diode laser equipment (DMC, THERAPY EC, São Carlos, Brazil, with a wavelength of 660nm and a power of 100 mV for 3 min. parameters mentioned in the following table (Table 1).

Wavelength	660
Direction	Continuous
Power	Mw
Irradiated area	Duct entry and fistula
Number of irradiated points	02
Number of sessios	01
Energy	4 J
Photosensitizer	Methylene blue 0.005

Table 1. Dosimetry of the laser light source to be used in the experiments

TREATMENT PROTOCOL

Current periapical radiographs will be required for all participants. All patients will be treated by an experienced endodontist, who will be responsible for data collection and procedures.

For the treatments, an endodontic rotary motor (Denstply) will be used to prepare the root canals.

Diode laser (DMC, THERAPY EC, São Carlos, Brazil) with a wavelength of 660 nm and a power of 100 mV for 3 min will be used as equipment.

INCLUSION CRITERIA

Patients must have a single-rooted tooth with apical periodontitis and the presence of a fistula.

All the selected pieces cannot present the presence of periodontal disease, which means not having periodontal pockets more than 4 mm deep.

Patients must be over 18 years of age.

EXCLUSION CRITERIA

Patients with comorbidities such as cancer, diabetes, coagulation diseases, anemia.

Patients undergoing orthodontic treatment.

Pregnant or lactating patients.

Patients with parts that make it impossible to perform absolute isolation.

ENDODONTIC CLINICAL EXAMINATION

Patients will be pre-examined for evaluation of endodontic parameters through periapical radiographs using radiographic positioners to determine the presence of apical radiolucency, as well as clinical verification of the presence of fistula.

PHOTODYNAMIC THERAPY - PDT

After endodontic treatment, pdT will be applied using diode laser equipment (DMC, THERAPY EC, São Carlos, Brazil) with a wavelength of 660nm and power of 100mV for 3min.

EVALUATION

The evaluation will be carried out 15 days after treatment to verify the healing of the fistula and a photograph and x-ray will be taken.

Then a control will be carried out after 30 days to compare these same data again.

REFERENCES

Alvarenga, L., Prates, R., Yoshimura, T., Kato, I., Suzuki, L., & Ribeiro, M. (marzo de 2015). Aggregatibacter actinomyceremcomitans biofilm puede ser inactivado por terapia fotodinámica mediada por azul de metileno. *Fotodiagnóstico Photdyn Ther, 12*(1).

Anagnostaki, E., Mylona, V., Parker, S., Lynch, E., & Grootveld, M. (2020). Systematic review on the role of lasers in endodontic therapy: valuable adjunct treatment. *Dentistry Journal, 8*(3).

Asnaashari, M., Homayuni, H., & Pymanpour, P. (2016). El efecto antibacteriano de la terapia fotodinámica adicional en los dientes tratados endodónticamente con fracaso: un estudio piloto. *Journal Lasers MEd Sci, 7*(4).

Asnaashari, M., Kooshki, N., Salehi, M., Azari-Marhabi, S., & Moghadassi, H. (2020). Asnaashharim et al Comparación de los efectos antibacterianos de la terapia fotodinámica y un sistema de activación de irrigación en conductos radiculares infectados con enterococus faecalis: un estudio in vitro. *Journal of Lasers in Medical Sciences, 11*(3).

Basrani, B., Ghanem, A., & Tjaderhane, L. (2004). Propiedades físicas y químicas de los medicamentos que contienen clorhexidina e hidróxido de calcio. *Revista de Endodoncia, 30*(413).

Baumagartner, J., Piquete, A., & Muller, J. (1984). Examen microscópico de los senos orales y sus lesiones periápicas asociadas. *Revista de Endodoncia, 10*.

Bonsor, S., Nichol, R., Reid, T., & Pearson, G. (2006). An alternative regimen for root canal disinfection. *British dental journal, 201*(2).

Firmino, R., Brandt, L., Ribeiro, G., Dos Santos, K., De Vasconccelos, M., & Gomes, D. (2016). Endodontic treatment associated with photodynamic therapy: Case report. *Photodiagnosis and photodynamic therapy,, 15*.

Garcez, A., Nuñez, S., Hamblin, M., & Ribeiro, M. (2008). Antimicrobial effects of photodynamic therapy on patients with necrotic pulps and periapical lesion. *Journal of endodontics, 34*(2).

Garcez, A., Nuñez, S., Hamblin, M., & Ribeiro, M. (2008). Antimicrobial effects of photodynamic therapy on patients with necrotic pulps and periapical lesion. *Journal of endodontics, 34*(2).

Hargreves, L., Berman, H., & Kenneth, M. (2016). *Cohens Pathways of the pulp.* Brasil: Décima Primeira Edição. Cap 14 e 15.

Karaoglu, G., Ugugr, Z., Erdonmez, D., Gol, C., & Durmus, M. (diciembre de 2020). Eficacia de la terapia fotodinámica antimicrobiana administrada con azul de metileno, azul toloides y ftalocianina de zinc reemplazada por tetra 2-mercatopridina en conductos radiculares contaminad. *Fotodiagnóstico Photodyn Ther*.

Komerik, N., & MacRobert, A. (2006). Photodynamic therapy as an alternative antimicrobial modality for oral infections. *Journal of environmental pathology, toxicology and oncology, 25*((1-2)).

Mozayeni, M., Vatandoost, F., Asnaashari, M., Shokri, M., Azari-Marhabi, S., & Asnaashari, N. (2020). Comparación de la eficacia del azul de toloudina, el azul de metileno y el cúrcuma en la terapia fotodinámica contra Enterooccus faecalis. *Revista Lasers Med Sci* .

Plotino, G., Grande, N., & Mercade, M. (2019). Photodynamic therapy in endodontics. *International endodontic journal, 52*(6).

Prates, R., Suzuki, L., & Claudio, K. (2012). *Terapia Laser de Baixa Potencia na Periodontia In: Laser de baixa potencia Principios basicos e aplicacoes Clinicas na odontologia.* Rio de Janeiro: lsevier.

Prates, R., Yamada, J., Hashimoto, M., & Ribeiro, M. (2010). Terapia fotodinamica: mecaniscos e aplicacoes. En G. Koogan, *Fundamentos de Odontologia Lasers em Odontologia.* (págs. p. 36-42). Rio de Janeiro:: 1ed.

Singh, S., Nagpal, R., Manuja, N., & Tyagi, S. (2015). Photodynamic therapy: An adjunct to conventional root canal disinfection strategies. *Australian Endodontic Journal,, 41*(2).

Siqueira, J. (2002). Endodontic infections: concepts, paradigms, and perspectives. Oral Surgery, Oral Medicine, Oral Pathology. *Oral Radiology, and Endodontology, 94*(3).

Soukos, N., Chen, P., Morris, J., Ruggiero, K., Abern, A., Som, S., & Stashenko, P. (2006). Photodynamic therapy for endodontic disinfection. *Journal of endodontics, 32*(10).

Tortamano, A., Anselmo, G., Kassa, C., Godoy-Miranda, B., Pavani, C., & Kato, I. (mayo de 2020). Terapia fotodinámica antimicrobiana mediada por azul de metileno en vehículo surfactante en periodontopatógenos. *Fotodiagnóstico Photodyn Ther*.

ANNEXES

Research ethics committee

UCU

Definitive Approval

Dr. Wince

Dear researcher:

The Committee on Ethics in Research with Human Beings of the ``Universidad Catolica de Uruguay," after studying all the documentation related to the Research Protocol, presented by you in your capacity as responsible, under the title: EVALUATION OF THE EFFECT OF THE PHOTODYNAMIC THERAPY MEDIATED BY METHYLENE BLUE CARRIED IN A SURFACTANT MEDIUM AS AN ADJUVANT IN THE TREATMENT OF PARTS WITH APICAL PERIODONTITIS AND PRESENCE OF FISTULA AND A RANDOMIZED, CONTROLLED DOUBLE-BLIND CLINICAL TRIALS, considers that said protocol: - meets the main requirements necessary for the approval and there are no substantial objections that prevent its implementation.

Yours sincerely,

Dr. Ana Fascioli (President)

Thomas Roche (Secretary)

Montevideo, november 3, 2021

Annex A: Free and Informed Consent Form