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XIMENA TRÉVIA PRADO DE VASCONCELOS

EFEITOS DA TERAPIA FOTODINÂMICA ANTIMICROBIANA NA DESCONTAMINAÇÃO DE ALVÉOLOS COM LESÃO PERIAPICAL IMEDIATAMENTE PÓS-EXODONTIA

FORTALEZA
2014

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Dissertação apresentada à comissão examinadora aprovada pela coordenação do Programa de Pós-graduação em Odontologia, como pré-requisito para obtenção do título de Mestre em Clínica Odontológica.

Orientadora: Profa. Dra. Karina Matthes de Freitas Pontes.

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RESUMO

Os avanços da Odontologia, com especial atenção ao uso dos implantes imediatos, despertou o interesse em revisar os aspectos relacionados ao tema, como taxa de sucesso e sobrevivência, protocolos de tratamento para sítios contaminados e microbiota encontrada. O presente trabalho contém 2 capítulos. No primeiro capítulo foi realizada uma revisão sistemática de artigos científicos na língua inglesa, utilizando os termos *immediate implant placement, fresh socket, dental implants,* datados entre 2003 e 2013, nas bases de dados Bireme, Medline e The Cochrane Library. Foram encontrados 131 títulos, dos quais 23 resumos foram selecionados e 6 artigos preencheram os critérios de inclusão, que foram: estudos clínicos randomizados prospectivos, com amostragem mínima de 10 implantes imediatos sobre alvéolos contaminados e mínimo de 12 meses de acompanhamento. Foi verificado que a taxa de sucesso dos implantes imediatos em sítios contaminados fica em torno de 90 a 100% e o protocolo clínico inclui o debridamento do alvéolo após exodontia e antibioticoterapia sistêmica pré e pós operatória. O segundo capítulo corresponde a um estudo clínico controlado, em que foi avaliado o efeito da terapia fotodinâmica antimicrobiana (TFA) mediada por azul de metileno 0,005% (AM) associado à irradiação por 90 segundos com laser de baixa potência, 40 mW, de 660 nm, na descontaminação de alvéolos dentários com lesão periapical, após exodontia. Foram selecionados 16 pacientes com indicação de extração de 2 elementos unirradiculares. Após a exodontia, foram coletadas amostras de sangue, inserindo-se cones de papel absorvente estéreis em cada alvéolo, que foram armazenados em tubos contendo *Reduced Transport Fluid*. Um dos alvéolos foi preenchido com a solução AM e, após 5 minutos, irradiado (P+L+). O outro alvéolo foi preenchido com solução AM por 5 minutos, sem irradiação (P+L-). Após a intervenção, nova coleta de sangue foi realizada. As amostras foram diluídas e semeadas em triplicata em placas de Petri contendo Ágar Sangue e BHI enriquecido. As placas com Ágar Sangue foram incubadas em aerobiose por 2 dias e as com Brain Heart Infusion (BHI) em anaerobiose por 7 dias, a 37°C. Realizou-se a contagem das unidades formadoras de colônias e a conversão dos dados em logaritmos de base 10, que foram submetidos ao teste t de Student pareado (α=0,05). Os resultados obtidos mostraram, nos grupos P+L+, redução na contagem de UFC/mL para bactérias aeróbias (2,5 log) e anaeróbias (1,5 log), porém sem diferença estatisticamente significante em relação aos grupos P+L- ($p>0,05$). A TFA mostrou uma tendência em reduzir a contaminação em alvéolos com lesão periapical, assim como o AM demonstrou ter um potencial antimicrobiano nestas lesões, independentemente da irradiação com laser. Mais estudos são necessários com diferentes concentrações de AM e diferentes potências e tempos de irradiação da fonte de luz, a fim de se obter maior eficácia para um protocolo de descontaminação alternativo à necessidade de antibioticoterapia relatada na literatura.

DESCRITORES: Fotoquimioterapia, Implantação dentária, Azul de Metileno, Microbiologia

ABSTRACT

Advances in dentistry, specially the use of immediate implants, have aroused interest in reviewing aspects related to this topic, such as success and survival rate, treatment protocols for contaminated sites and also found microbiota. This study consists of two chapters. In the first chapter it was conducted a systematic review of scientific literature in English language using BIREME, MEDLINE and The Cochrane Library databases. The search included studies dated between 2003 and 2013 and used the following terms: immediate implant placement, fresh socket, dental implants. A total of 131 titles were found, out of which 23 abstracts were selected and six articles met the inclusion criteria, which were: prospective randomized clinical trials with minimum sample of 10 immediate implants in contaminated sockets and a minimum of a 12-month follow-up. It was found that the success rate of immediate implants in contaminated sites is around 90-100% and the clinical protocol includes debridement of the socket after tooth extraction, as well as pre- and postoperative systemic antibiotic therapy. The second chapter corresponds to a controlled clinical study in which it was evaluated the effect of photodynamic therapy (PDT) mediated by methylene blue 0.005 % (MB) associated with irradiation for 90 seconds with low power laser, 40 mW, 660 nm, in the decontamination of dental sockets with periapical lesion after tooth extraction. There was the selection of 16 patients with indication for extraction of two single-rooted elements. After extraction, blood samples were collected by inserting sterile absorbent paper cones in each socket, which were stored in tubes containing Reduced Transport Fluid. One of the sockets was filled with MB solution and after five minutes, it was irradiated (P+L+). The other socket was filled with MB solution for five minutes and had no irradiation (P+L-). New blood collection was performed after the intervention. The samples were diluted and plated in triplicate in petri dishes containing blood agar and enriched BHI. The blood agar plates were incubated for two days under aerobic conditions whereas the ones with BHI were under anaerobic conditions for seven days at 37 ° C. There was the counting of colony forming units and the conversion of data into logarithms of base 10 , which were submitted to paired Student's t test ($\alpha = 0.05$). The results showed reduction in number of CFU/mL for aerobic bacteria (2.5 log) and anaerobic ones (1.5 log) in groups P+L+ although with no statistically significant difference in the groups P+L- ($p > 0.05$). The PTD revealed a tendency to reduce contamination in sockets with periapical lesion, as well as MB has shown to have an antimicrobial potential in these lesions, regardless laser irradiation. Further studies are needed with different concentrations of MB and different powers and irradiation times of the light source in order to obtain a more effective protocol for decontamination alternative to the antibiotic therapy reported in the literature.

DESCRIPTORS: Photochemotherapy, Dental Implantation, Methylene Blue, Microbiology

LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

%	Porcentagem
n	Número
mL	Mililitros
°C	Graus Celsius
p	Significância
>	Maior que
<	Menor que
Ltda	Limitada
nm	Nanômetro
J/cm^2	Joules por centímetro quadrado (fluência de energia)
J	Joules (energia)
mW	Miliwatts
AM	Azul de Metileno
BHI	Brain Heart Infusion
UFC	Unidade Formadora de Colônia
min	Minutos
TFA	Terapia Fotodinâmica Antimicrobiana
λ	Comprimento de onda
TBO	Azul de Toluidina O

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1 INTRODUÇÃO GERAL

A reabilitação de áreas edêntulas tem sido buscada desde a antiguidade, quando os primitivos usavam dentes de marfim para confeccionar unidades dentárias. Com o passar dos anos, frente a todos os avanços tecnológicos da Odontologia e associado às recentes pesquisas sobre o uso dos biomateriais, os implantes tem se mostrado uma alternativa viável e segura para tal (ORTEGA-MARTINEZ et al, 2012). Agenesias, fraturas dentárias, reabsorções radiculares, impossibilidade de recuperação restauradora da raiz, comprometimento periodontal e falha do tratamento endodôntico são algumas das indicações para o uso de implantes (MISCH, 2009).

Os protocolos usados são os mais diversos e envolvem desde a instalação imediata, mediata e tardia, classificação esta desenvolvida para estabelecer o tempo de instalação do implante: imediato, quando o implante é instalado no mesmo momento cirúrgico da exodontia; mediato, quando instalado em período até 8 semanas; e tardio, quando instalado após 8 semanas (ESPOSITO et al, 2006). As indicações para se estabelecer o período ideal para a instalação pode variar. Preservação das paredes alveolares, redução do tempo cirúrgico, preservação do tecidos moles circunjacentes e presença ou não de lesão apical e /ou periapical são fatores a serem considerados (MISCH, 2009).

Alguns autores consideram a presença de lesão periapical como uma contraindicação absoluta aos implantes imediatos (TOLMAN et al, 1991; BARZILAY et al ,1993; LUNDGREEN et al, 1991), entretanto, outros estudos, mostram que este aspecto é relativo (NOVAES et al,1998). O uso de antibioticoterapia profilática e o debridamento dos tecidos alveolares fazem parte do protocolo estabelecido em algumas pesquisas envolvendo alvéolos contaminados, buscando reduzir esta contaminação e favorecer o sucesso da osseointegração (SIEGENTHALER et al, 2007; CASAP et al, 2007; CORNELINI et al, 2005; LINDEBOOM et al, 2006; CRESPI et al, 2010; TRIPODAKIS & NAKOU, 2011). Apenas o debridamento mecânico dos tecidos alveolares tem sido descrito como técnica adicional para a descontaminação bacteriana (SIEGENTHALER et al, 2007; CASAP et al, 2007; CORNELINI et al, 2005; LINDEBOOM et al, 2006; CRESPI et al, 2010; TRIPODAKIS & NAKOU, 2011).

A microbiota relacionada a estes sítios contaminados fazem parte

daquelas responsáveis pelas lesões apicais e periodontais e são em sua maioria anaeróbias estritas ou facultativas, logo, o simples contato com o ar torna sua viabilidade reduzida (ROCHA et al, 1998; HAAPASALO, 1993; FERREIRA et al, 1988). Assim, alguma redução bacteriana já seria promovida pela remoção do elemento dental envolvido e pela curetagem dos tecidos circundantes, como demostrado por Tripodakis & Nakou (2011), em estudo que referiu mudança de uma microbiota periodontal para uma microbiota periimplantar composta, em sua maioria, de micro-organismos benéficos, após instalação de implantes, mas ainda contando com micro-organismos patogênicos em menor quantidade, quando avaliados em período de 1 ano.

A observação das aplicações da terapia fotodinâmica antimicrobiana (TFA) com sucesso em Endodontia e Periodontia, desperta a curiosidade quanto ao seu possível comportamento na descontaminação de alvéolos imediatamente após a exodontias, podendo proporcionar condições para instalação imediata de implantes. A TFA é baseada no conceito de que um agente fotossensível ou cromóforo pode ser preferencialmente absorvido por bactérias e depois ativado por fótons de luz de comprimento de onda complementar, para gerar radicais livres e oxigênio singleto, os quais são citotóxicos para os micro-organismos (FINK-PUCHES et al, 1997). Podem ocorrer dois tipos de reações: a reação tipo I, que envolve transferência de elétrons do fotossensibilizador para moléculas vizinhas ou átomos de hidrogênio, produzindo radicais que podem reagir com o oxigênio para produzir espécies citotóxicas, tais como superperóxido, radicais hidroxila e radicais livres (ATHAR et al, 1988); a reação tipo II, que envolve transferência de energia do fotossensibilizador no estado tríplice ao oxigênio molecular no estado fundamental, para produzir oxigênio singleto. Juntos, todos estes produtos, oxidam muitas moléculas biológicas, como proteínas mitocondriais, alterando sua estrutura e atividade, desnaturam proteínas e lipídios da membrana e modificam a estrutura do DNA celular (MITRA, 2004). O oxigênio singleto ainda pode ter efeito direto nas moléculas extracelulares devido a sua alta reatividade química, de modo que polissacarídeos presentes na matriz extracelular de biofilmes orais também sejam susceptíveis ao fotodano (KONOPKA; GOLINSKI, 2007).

Há vários fatores que influenciam o fotodano, incluindo o tipo, a dose, o tempo de incubação e localização do fotossensibilizador, a disponibilidade de

oxigênio, o comprimento de onda da luz (nm), a densidade de potência de luz (mW/cm²) e a densidade de energia da luz (J/cm²) (ROLIM et al, 2012).

Os fotossensibilizadores usados frequentemente na TFA são compostos fenotiazínicos como o azul de metileno ou azul de toluidina O, havendo também as porfirinas e a clorofilas (AGHAHOSSEINI, 2006) em diferentes concentrações e combinados a lasers ou LEDs (diodos emissores de luz) de comprimento de onda compatíveis para ativá-los (ZANIN et al, 2003, MACHADO, 2000). A toxicidade destes tem sido bastante questionada, mas experimentos com cultura de fibroblastos tem referido não haver citotoxicidade associada, referindo o uso como seguro (KASHEF et al, 2012).

A aplicação desta terapia é bem difundida e utilizada nas áreas de Periodontia e Endodontia. Na primeira, sua utilização está relacionada ao tratamento adjuvante na descontaminação de bolsas profundas ou lesões de furca. Em estudos, como de Campos et al (2013), foi observado redução estatisticamente significativa da profundidade de sondagem e sangramento a sondagem para o grupo onde a TFA foi aplicada como auxiliar ao tratamento convencional. Na segunda, o emprego está relacionado a descontaminação residual dos canais radiculares, como estudado por Garcez (2010), onde se observou uma maior eficiência da TFA na redução da carga microbiana e demonstrou ser eficiente para microrganismos resistentes a múltiplas drogas.

Em Implantodontia, os estudos referem o uso na área periimplantar, visando a descontaminação bacteriana imediatamente após a instalação do implante e prevenindo a infecção bacteriana (FRANCO et al, 2010) ou ainda no tratamento da periodontite. Dörtbudak et al (2001) encontraram em seu estudo *in vivo* que a aplicação de laser diodo de 690 nm de comprimento de onda, associado ao fotossensibilizador TBO (azul de toluidina O) como tratamento adjuvante da periimplantite, mostrou redução bacteriana de 2 log para todas as espécies avaliadas, referindo uma redução significante dos valores iniciais nos 3 grupos bacterianos analisados.

Assim, a utilização da TFA após exodontias de elementos com lesão periapical, pensando-se na possibilidade de instalação de implantes imediatos, pode ser de grande utilidade clínica. Sua atuação pode servir como forma e redução da contaminação do leito cirúrgico, reduzindo a necessidade de utilização de

antimicrobianos sistêmicos, promovendo maior eficácia da descontaminação local desses alvéolos e viabilizando a osseointegração.

2 PROPOSIÇÃO

A presente dissertação de Mestrado será apresentada em capítulos, tendo os seguintes objetivos:

Capítulo 1: Realizar uma revisão de literatura sistemática sobre os implantes dentários imediatos em alvéolos infectados, suas indicações, contraindicações, limitações, taxa de sucesso, taxa de sobrevivência e protocolo de execução.

Capítulo 2: Avaliar *in vivo* a influência da terapia fotodinâmica na descontaminação de alvéolos com lesão apical ou periodontal imediatamente pós-exodontia.

3 CAPÍTULOS

REGIMENTO INTERNO

Esta dissertação baseia-se no Artigo 46 do Regimento Interno da Pós-Graduação em Odontologia da Universidade Federal do Ceará que regulamenta o formato alternativo para dissertações de Mestrado e teses de Doutorado, permitindo a inserção de artigos científicos de autoria ou co-autoria do candidato. Uma vez que a pesquisa aqui tratada envolve seres humanos, o projeto de pesquisa do trabalho relatado no capítulo 2 foi cadastrado na Platafoma Brasil (CAAE: 12308313.2.0000.5054) em janeiro de 2013, tendo sido aprovado pelo Comitê de Ética em Pesquisa da Universidade Federal do Ceará (parecer 255-653, de 04/04/2013). Desta forma, esta dissertação é composta de dois capítulos contendo artigos a serem submetidos para publicação em revistas científicas, conforme descrito abaixo:

Capítulo 1

"CLINICAL USE OF OSSEOINTEGRATED IMMEDIATE LOADING IMPLANTS IN INFECTED DENTAL SOCKETS - A SYSTEMATIC REVIEW OF THE LITERATURE"

Capítulo 2

"***EFFECTS OF ANTIMICROBIAL PHOTODYNAMIC THERAPY IN THE DECONTAMINATION OF SOCKETS WITH PERIAPICAL LESION – A CONTROLLED CLINICAL STUDY"***

3.1 CAPITULO 1

"CLINICAL USE OF OSSEOINTEGRATED IMMEDIATE IMPLANTS IN INFECTED DENTAL SOCKETS - A SYSTEMATIC REVIEW OF THE LITERATURE"

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ABSTRACT

Dental implants are an alternative for the rehabilitative treatment. Technological advances in this area allowed the reassessment of the concepts of osseointegration, and the replacement of a tooth by means of implant becomes a successful proven and predictable treatment. The immediate, early or conventional implant placement is based on the healing of soft and hard tissues, and due to the reduction of surgical time as well as socket preservation, immediate implants have received special attention and their installation in contaminated sites is focus of discussion. The aspects discussed in this systematic review are: the use of immediate implants in infected sockets, indication, contraindication, success and survival rates, and also protocol implementation. Articles in English were searched from January 2003 to August 2013 in BIREME, MEDLINE and The Cochrane Library databases, including randomized controlled trials with at least 10 implants and initial follow-up of one year. Six articles that met the proposed inclusion criteria were selected. Immediate implants in contaminated sites showed success rates similar to implants in healthy sites or conventional implants, associating the use of systemic antibiotic therapy and debridement to control bacterial contamination. The species associated with apical lesions, such as *Fusobacterium nucleatum* and as *Peptostreptococcus micros*, were predominant. Due to the reduced number of articles found, further randomized clinical trials with longitudinal follow-up are needed in order to ensure the possibility of more frequent indication of immediate implants in contaminated sites.

DESCRIPTORS: Immediate implant placement, fresh sockets, dental implants.

INTRODUCTION

The advent of implants brought an innovative alternative for prosthetic and rehabilitation treatments. In the past three decades, advances in biomaterials technology and clinical research have been a tool for the progress of such treatment, even leading to revising some of the prerequisites for osseointegration.

The replacement of a tooth using implants has been a proven successful and predictable treatment. Different installation protocols were developed aiming at minimizing the surgical time and providing patients with comfort and satisfaction. [2]

Immediate, early and conventional loading is the classification for implant installation proposed by Esposito et al, in 2006, which is based on healing of soft and hard tissues as well as treatment duration. When the implant is placed in fresh sockets, it is considered immediate load. Early loading is the one installed after less than an eight-week period, whereas conventional load is carried out longer than eight weeks. [3]

Immediate implant placement in post-extraction sockets was first described over 30 years ago. [4] The advantages described for this technique include reduction in the number of surgical interventions, reduction in treatment time, ideal three-dimensional position of the implant, preservation of alveolar bone around the tooth and also favorable positioning of the soft tissue around the implant. [5,6,7]

Retained deciduous teeth replacement, vertical or horizontal root fracture, lost teeth due to the impossibility of restoration, periodontal disease, endodontic failure and poor aesthetics are indications for immediately loaded implants. [8] The presence of pathologies involving the dento-alveolar region can be a limiter to be considered for the recommendation on immediate implants, therefore seeking alternatives and service protocols, making this situation contraindication to the technique. [9,10,11,12]

The focus of this study is to review the current literature on immediate load implants in contaminated sockets in order to find out not only the indications and contraindications, limitations, protocol used for infection control, but also alternative treatments and success rate.

Thus, this review aims at answering the following questions:

- Does the presence of apical lesion impair the success of implants? What is the success /survival rate of implants in contaminated sites? Is there any difference when

compared to healthy sites?

- What is the treatment protocol for contaminated sockets? What are the strategies used in order to minimize contamination?
- What is the microbiota found in contaminated sockets?

METHODS AND MATERIALS

Bibliographic research was carried out involving articles in English published in MEDLINE (PubMED) and *The Cochrane Library* databases from January 2003 to August 2013. The used keywords were: immediate implant placement, fresh socket, dental implants.

Twenty-three papers were selected out of 131 titles found in the initial search and then full texts were obtained. Only randomized prospective clinical studies were included. The inclusion and exclusion criteria of this study are listed in Tables 1 and 2, respectively.

Table 1: LIST OF INCLUSION CRITERIA

INCLUSION CRITERIA
RANDOMIZED PROSPECTIVE CLINICAL TRIALS
MINIMUM OF A 12-MONTH FOLLOW-UP
MINIMUM SAMPLE OF 10 IMPLANTS IN MAXILLA OR MANDIBLE
PRESENCE OF INFECTION/ DENTO-ALVEOLAR CONTAMINATION

Table 2: LIST OF EXCLUSION CRITERIA

EXCLUSION CRITERIA
INCOMPLETE OR CONFUSED INFORMATION ABOUT THE SAMPLE
STUDY IN ANIMALS
CLINICAL CASE REPORT

At the end, six articles met all inclusion criteria proposed for this proposed study. Such articles were taken from the following journals: *Clinical Oral Implants Research, J Periodontology, Oral Surgery Oral Medicine Oral Pathology, Oral Radiology and Endodontology, International Journal Periodontics Restorative Dentistry* e *Journal Oral Maxillofacial Surgery.*

RESULTS

In the initial search a total of 131 citations were found using different combinations of descriptors. Twenty-three abstracts were selected and based on the inclusion criteria 11 articles were selected and their full texts obtained (Fig. 1). Five studies were excluded after being read as they were retrospective studies and did not meet all inclusion criteria proposed (Table 1). The articles included in this study are shown in Table 2.

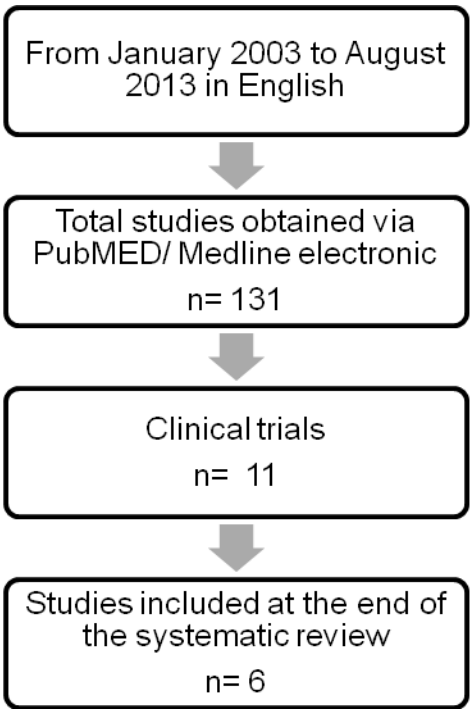

Figure 1: Diagram of the study

Article	**Year**	**Reason for exclusion**
Bell et al	2011	Retrospective study
Diago et al	2012	Retrospective study
Soydan et al	2012	Contaminated sites were not included
Covani et al	2012	Contaminated sites were not included
Botticelli et al	2008	Contaminated sites were not included

Table 1: List of articles excluded at the end of the selection.

TAuthor	Year	Journal	Country	Follow-up	N° of patients	N° of implants	Success/ survival rate	Contaminated sites	Immediate/conventional Implant placement	Protocol of treatment	Microbiota
Siegenthaler et al	2007	*Clin Oral Impl Res*	USA	12 months	34	34	100%	Yes	Immediate	Debridement and antibiotic therapy (Amoxicilline before and for 5 days + mouthwash of chlorhexidine 0,2% for 15 days)	Not specified
Casap et al	2007	*J Oral Maxillofac Surg*	USA	72 months	20	30	96.67%	Yes	Immediate	Debridement, antibiotic therapy (Amoxicilline for 4 days before and keeping it up to 10 days) and guided bone regeneration.	Not specified
Cornelini et al	2005	*Int J Periodontics Rest Dent*	USA	12 months	22	22	---	Yes	Immediate	Debridement and antibiotic therapy (Amoxicilline for 5 days + mouthwash of chlorhexidine 0,2% - duration is not determined)	Not specified
Lindeboom et al	2006	*Oral Surg Oral Med Oral Pathol Oral Radiol Endod*	USA	12 months	50	50	92 – 100%	Yes	Immediate	Debridement and antibiotic therapy (Clindamycin / 1h before) + mouthwash of chlorhexidine for 7 days.	*Fusobacterium nucleatum* and *Peptostreptococcus micros*
Crespi et al	2010	*J Periodontol*	USA	24 months	30	30	100%	Yes	Immediate	Debridement and antibiotic therapy (Amoxicilline before procedure and for 1 week) + Mouthwash of chlorhexidine for 15 days.	Not specified
Tripodakis & Nakou	2011	*Int J Periodontics Restorative Dent*	USA	12 months	10	20	100%	Yes	Immediate	Debridement and antibiotic therapy (not specified for 1 day).	Gram (+) and Gram (-) anaerobic and facultative

Table 2: Articles included in the systematic review

The questions proposed in this review were answered throughout this study and compared considering the included articles.

Does the presence of periodontal or periapical lesion/contamination impair the success of implants? What is the success/survival rate of implants in contaminated sites? Is there any difference when compared to healthy sites?

Crespi (2010) conducted a study involving 30 patients divided into test group and control group in which the test group included patients with apical or periapical lesion; however, with no sign of pain, suppuration or fistula. The control group consisted of patients with fracture or root caries without periapical pathology. A total of 30 teeth were extracted and immediate implant installed whose load was placed in both groups after three months. After 24 months, a survival rate of 100% was reported for all implants, stating that immediate implants placed in contaminated sites showed positive integration of hard and soft tissues, therefore determining a favorable outcome for the group teste. [13]

In a randomized prospective clinical trial involving 50 patients, in which all radiographically evaluated teeth showed chronic periapical lesion, Lindeboom (2006) randomly allocated subjects to immediate or conventional installation protocol. The success rate of the implants ranged from 92 to 100% for the immediate implants and the conventional ones, respectively. A success rate of 100% was achieved after one year following-up both groups. [14]

A prospective study involving 34 patients started aiming at comparing the placement of implants immediately after tooth extraction in sites with and without the presence of periapical pathologies. Seventeen patients formed the study group, who were initial and consecutively recruited, showing signs and symptoms such as pain, periapical radiolucency, fistula, suppuration or combination of these findings. The control group included patients with no apical lesions, but who met all the inclusion criteria. There was no statistically significant difference between test and control groups in all evaluated standards. [15]

The immediate installation of 30 implants in 20 patients with infected sites was the focus a study conducted by Casap in 2007. Dento-alveolar pathologies included subacute periodontal infection, endo-perio infection, chronic periodontal infection, chronic periodontal lesions and periodontal cyst. There was no division between the test or control group and at the end, after the removal of a mobile implant found after the restoration, a success rate of 96.67% was achieved. [6]

Therefore, according to the referred literature, the presence of periapical pathologies has not impaired the success rate of implants installed in contaminated sites, besides there is no difference between immediate loading in infected or healthy sites.

What is the treatment protocol for contaminated sites? What are the strategies used in order to minimize contamination?

The antibiotic therapy has been used to reduce contamination before and after surgery [1,6,13,14] , in some cases, or only after such procedure. [15] This protocol includes 1 g of amoxicillin before the procedure and 1 g amoxicillin per day for no longer than one week[1,6,13,15,16] or 600 mg of clindamycin 1h before surgery. [14] Mouthwash with chlorhexidine 0.2% has been used during postoperative period for seven to fifteen days to control secondary infection [1,6,13,14]. As local infection control strategy, tissue debridement has been used in all cases. [1,6,13,14,15,16]

What is the microbiota found in contaminated sites?

Only two of the studies analyzed in this review refer to the microbiota of contaminated sites. In one study, nine out of 50 extracted teeth had no bacterial growth resulting from the material collected in the debridement of the socket. In 21 cases of immediate implant and 20 conventional implants there was culture resulting from the blood collected in the alveoli after debridement and prior to implant placement. The most prevalent bacteria were *Fusobacterium nucleatum* (70%) and *Peptostreptococcus micros* (42%). In most cases (62%) there was polymicrobial flora, and in only 20% of cases one single microorganism was isolated. [14] In the second study, the microbiological analysis of both periodontal and peri-implant areas showed a complex bacterial community of gram-positive and gram-negative anaerobe facultative microorganism, having 14 species being isolated and evaluated (*A actinomycetemcomitans, A israelli, Actimomyces spp, B forsythus, C rectus, Capnocytphaga spp, E corrodens, Fusobacterium spp, P gingivalis, P intermedia/nigrescens, P micros, P sputigena, Streptococci spp, Veionella spp).*[16]

DISCUSSION

The purpose of this review was to clarify the conditions under which t immediate implants in contaminated sockets are indicated, rate of success/ survival found as well as decontamination protocol employed.

The greatest advantages of immediate implant placement are decreased alveolar bone resorption after extraction, reduction of treatment time and less psychological stress for the patient, by avoiding a second surgical procedure, not to mention preservation of soft peri-implant tissue. [17,18,19,20]

Minimally traumatic extraction, debridement of the socket and minimally invasive surgical technique, associated with primary implant stability, are some of the criteria which contribute to the success of immediate implants. [21,22,23]

Some authors establish the presence of periapical lesions as an absolute contraindication to immediate implant placement[24,25], something the articles studied in this review refute. In a study involving 30 implants immediately installed in infected socket after debridement, only one was not osseointegrated after a period of 12 to 72 months. [6]

This technique is reported in several studies, in which it is aimed maximum cleanliness of the socket before preparing it for implant placement, thus avoiding its contamination. [10,26,27] Similarly, in an experiment carried out in 655 patients and involving 922 implants, with 285 being installed in sockets with chronic periapical lesions, the reported success rate was 97, 5%, with no significant difference concerning control group. [28]

In a study in which 159 implants were installed immediately after tooth extractions in sites where the inclusion criteria were no previous periodontal treatment and presence of acute infection, the success rate was 91.8% after 10 years. This result was due to several factors such as monitoring of severe oral hygiene and good biomechanical of installed prosthesis. [29] Other review involving the installation of 50 implants, out of which 26 were immediate implants and 24 were early ones, showed no significant difference between groups, referring both techniques as safe and with high survival rate. [30]

The use of antibiotic therapy before and after immediate implant placement is a well-established procedure for cases with periapical and/or periodontal lesions[1,6,13,14,15,16,31] ; in addition to that, such practice has also been

referred to sites with no injuries. [29] Except for the debridement of the socket, no alternative therapy to the use of systemic antibiotics in order to reduce local bacterial contamination was found. The protocols include use of 600mg of clindamycin or even 1.5 g of amoxicillin, starting 1 hour up to 4 days before procedure and keeping medication for no longer than 10 days. [6] Antimicrobial postoperative local control was conducted with 0.2% chlorhexidine mouthwash for periods ranging from 5 to 15 days. [1,13,15]

Knowing that primary endodontic infections are mixed infections dominated by strict anaerobic bacteria, these ones are restricted to the root canal. The extraction of the involved tooth and efficient debridement of the socket tissues may partially remove this contamination. The presence of periapical lesion does not necessarily contraindicate immediate implant placement, provided that appropriate clinical procedures are performed, such as: administration of antibiotics combined with meticulous cleaning and alveolar debridement before preparation for the implant. [11,12]

The study of periodontal microbiota on endangered sites before and after installation of implants shows that this environment is changed after the removal of the involved tooth and the mechanisms of host defense. Twenty periodontally endangered sites and 20 peri-implant sites were analyzed showing a complex bacterial flora consisting of facultative anaerobic gram-positive and gram-negative, reduction and modification in samples after one year. All isolated microorganisms are considered severely pathogenic to peri-implant sites in different concentrations, though. In these sites, increase in beneficial microflora was also observed (*Actinomyces spp, Streptococci spp* and *Veillonella spp*) as well as the reduction of periodontopathogenic elements(*Fusobacterium nucleatum, spp, P gengivalis and P intermedia/nigrescens*).[16]

Randomized controlled trials with longer periods of follow-up are needed to ensure the use of immediate implants in contaminated sockets. The establishment of an alternative protocol to antibiotic therapy is of clinical interest due to the difficulty in carrying out the procedure correctly, as it depends on the participation and cooperation of the patient. Studies for detailed definition of the involved microbiota would also be important to help in the search for effective antimicrobial therapy.

FINAL CONSIDERATION

The use of immediate implants is well accepted in the literature with high success rates. Their use in contaminated sites has been indicated when criteria that favor the success of this technique are respected, such as the local bacterial decontamination by means of alveolar tissue debridement, favoring the initial stability as well as prophylactic systemic antibiotic therapy, showing no significant difference when compared with non-contaminated sites.

No alternative therapy was found for bacterial decontamination that could be associated with the existing protocol or even replace it.

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3.2 CAPÍTULO 2

"EFFECTS OF ANTIMICROBIAL PHOTODYNAMIC THERAPY IN THE DECONTAMINATION OF SOCKETS WITH PERIAPICAL LESION – A CONTROLLED CLINICAL STUDY"

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ABSTRACT

The present study evaluated the effect of photodynamic therapy (PDT) mediated by methylene blue 0.005 % (MB) associated with a 40mW laser and 660 nm in the decontamination of dental sockets with periapical lesion after tooth extractions. There was a selection of 16 patients with indication for extraction of two single-rooted elements. Blood samples were collected after extraction. One of the sockets was filled with MB solution, and after 5 minutes, irradiated (P+L+). The second socket was filled with only MB solution for 5 minutes (P+L-). Subsequent blood collection was carried out. After incubation, the number of colony forming units (CFU) was obtained and submitted to the Student's t test ($\alpha = 0.05$). The results showed, in groups P+L+, reduction in number of CFU / mL for aerobic bacteria (2.5 log) and anaerobic ones (1.5 log), without significant difference compared to P+L- groups ($p > 0.05$). PDT showed a tendency to reduce contamination in sockets with periapical lesion, as well as MB has shown to have an antimicrobial potential regardless irradiation.

DESCRIPTORS: Methylene Blue, Photochemotherapy, Dental Socket, Decontamination, Dental Extraction

INTRODUCTION

Since their emergence in the last century, laser lights have expanded their therapeutic use in all fields of knowledge. Such lights can be classified into two types: surgical or high power lasers and non-surgical or low power lasers, or even therapeutic lasers.[1] The first ones have an effect in reducing the pathogenic microflora associated with well-established oral infections, having thermal action.[2] Low-power lasers act in the reestablishment of the biological balance by promoting conditions for the vitality of tissue, besides their analgesic, biomodulatory, and anti-inflammatory actions. These benefits are due to photochemical and photoelectric effects, not generating antimicrobial effect when used isolatedly. [1,2]

The combination of a low power laser light or LED (light emitting diode) and a photosensitizing agent or chromophore is called photodynamic therapy (PDT). [3,4,5,6] Dyes such as methylene blue (MB) and toluidine blue O (TBO), which are classified as phenothiazines, are commonly used for marking the bacteria to be photosensitized. [3,7,8] MB has been used clinically in the treatment of bladder cancer , against inoperable esophageal tumors, skin virulence , psoriasis and adenocarcinomas, in addition to viral disinfection in the blood. [9] The cytotoxicity of these substances on human fibroblasts culture was proved to be insignificant, suggesting safe applicability on connective tissue. [10] PDT action mechanism involves the activation of the chromophore by the light, which absorbs photons, converting them into an excited state. This energy transferred to neighboring molecules results in the formation of active molecules (singlet oxygen, superoxide ions, hydroxyl and other free radicals) that kill or damage the bacterial cell, besides neutralizing its virulence factors after death. [11] The advantages of using PDT are based on two principles: preferential accumulation of the photosensitizer in the target cell and precise light irradiation, resulting in a selective and localized action of therapy. [1]

The use of antimicrobial PDT has been used and studied with emphasis on Periodontics, Endodontics and Implantology, in search of an additional alternative to the existing treatment protocols for bacterial decontamination aiming at the elimination and/or reduction of bacteria resistant to systemic antibiotics. [2,12,13,14] In Periodontics, the use of PDT seeks to eliminate the contamination of periodontal pockets in areas where access is difficult, as in a study conducted by Campos *et al* in 2013, using diode laser with a 660 nm wavelength,129 J/cm² energy density

associated with methylene blue chromophore at a concentration of 10 mg/ml. [15] In Implantology, antimicrobial PDT acts by reducing or eliminating the formation of potentially pathogenic microflora in the surgical site immediately after implant placement. Its use is related with laser diode with low intensity for 2 min and associated with methylene blue dye 0.005% on the microbiota of peri-implant crevice. [11] In Endodontics, PDT application relates to the reduction of microorganisms in root canals with a 660nm laser diode, 40mW power, and having chloro-polietilenamine as a photosensitizer, associated with endodontic treatment for patients previously submitted to endodontic therapy and antibiotics. Authors confirmed the use of PDT as an effective means for removing bacteria resistant to chemo-mechanical preparation of root canals. [16]

Bacterial colonization of the oral cavity presents a vast microbiota and corresponds to almost half of those present throughout the human body, with a predominance of aerobic bacteria. Some areas, however, are colonized predominantly by anaerobic organisms, as it occurs with periapical lesions. Microbiological studies of these lesions stated that facultative anaerobic bacteria and strict anaerobic bacteria were isolated, and the predominance of strict anaerobes on the other. *Fusobacterium nucleatum*, *Prevotella intermedia* and *Porphyromonas gengivalis* are some of the predominant species in the pulp and periapical lesions. [17]

Thus, the use of PDT after extraction of elements with periapical lesions may have great clinical utility when considering the possibility of immediate implant placement. Its action can serve as manner of contamination reduction of the surgical site, reducing the need for using systemic antimicrobials, promoting greater efficiency in local decontamination of these sockets, therefore enabling osseointegration. [11,12,18,19,20] The purpose of this controlled clinical trial study was to test the null hypothesis that PDT mediated by both methylene blue and red wavelength laser light source would not show any effect on the decontamination of fresh socket with periapical lesion.

MATERIALS AND METHODS

This research project was registered in *Plataforma Brasil* in January 2013 and was approved by the Ethics Committee in Research of the Federal University of Ceara.

Subjects and eligibility criteria

A number of 16 patients took part in this study. The subjects met the following inclusion criteria, regardless gender or age: low plaque index (less than 10% - O'Leary index) [21], indicated for extraction of at least two single-rooted elements, presence of apical or periodontal lesion.

Patients who used systemic antibiotics in the last three months, had history of allergy to dyes, were smokers or immunocompromised could not be included in the study. Cases that would have to be removed from the survey include the ones in which the procedure of tooth extraction needed retail, osteotomy or odontossection, or those there was fracture of the tooth into the socket or alveolar bone plate during its dislocation.

All participants were screened and treated at the Faculty of Pharmacy, Dentistry and Nursing of the Federal University of Ceara, as well as they read and signed an Informed Content Form (Appendix).

Sample size

Based on the results obtained from an initial sample in a pilot test, the calculation of the statistical power was carried out by means of the BioEstat 5.0 software (Institute of Sustainable Development of Mamirauá, Manaus, AM, Brazil). It was verified that the number of required patients for a minimum of 80% power with significance level of $\alpha = 0.05$ and $\beta = 0.80$ would be n equal to 16.

Photosensitizer and light source

The photosensitizer selected for the study was the methylene blue at a concentration of 0.005% (Vetec Chemical LTD., Duque de Caxias, Brazil) sterilized by filtration from 0.22 μm (Millex-GS, Merck Millipore, Billerica, MA, USA) and the light source used was a laser of 660 nm and 40 mW (Fig 1), applied inside the socket through an optical fiber of 300 μm diameter at the tip (Twim Flex Evolution MM Optics, San Carlos, Brazil).[11]

Fig 1: Twim Flex Evolution (MM Optics).

Experimental Design

Each patient had indication for extraction of at least two dental elements. By draw, one of them was assigned to be the control of the photosensitizer (P + L-) and the other was the experimental (P + L +) (n = 16). After extraction, the P + L- alveoli were filled with methylene blue, while the P+ L+ ones were filled with methylene blue and irradiated with the laser.

Microbiological analysis of the blood of each socket was carried out after extraction, before (P-L-) and immediately after (P+L-) and (P+L+) treatment. The amount of viable aerobic and anaerobic bacteria was analyzed by counting the colony forming units (CFU).

Clinical intervention

The extraction surgeries were performed in the clinic the Faculty of Pharmacy, Dentistry and Nursing of the Federal University of Ceara. All procedures followed a biosafety and technical protocol recommended by the course, being carried out by pre-calibrated surgeon dentists. The intervention, object of the research, which is the application of photodynamic therapy after extraction as well as the collection of alveolar blood, was performed by a single trained researcher (researcher 1).

The extraction technique employed was the conventional and minimally traumatic one, starting by syndesmotomy, followed by dislocation of the tooth with the aid of elevators and forceps. The anesthetic selected for infiltration of the surgical area was based on Mepivacaine Hydrochloride 2% with epinephrine 1:100,000 (DFL, Taquara, RJ, Brazil).

After extraction, the socket was irrigated with 1 ml of sterile saline solution, with a relative isolation of the area with gauze and insertion of three sterile absorbent paper cones for 20 seconds for both alveolar (P+L-) and for alveolar submitted to photodynamic therapy (P+L+). After removal, the cones were immediately transferred to separate Eppendorf tubes containing 2 ml of RTF (Reduced Transport Fluid) transport media.

In the (P+L+) socket, there was the removal of blood and serum excess, with the aid of oral evacuator and gauze, and then it was filled with 1 ml methylene blue 0.005% solution (Fig. 2). After a five-minutes wait for dye impregnation, the excess was sucked for the application of low intensity laser of 660 nm wavelength (Double Flex Evolution MMOptics) by means of optical fiber (MMOptics) inserted in the socket, for 90 seconds of irradiation time (Fig 3).

The procedures described above were carried out in the (P+L-) socket; however, after dye impregnation and removal of excess laser irradiation was not applied, it was only waited 90 seconds for its removal.

At the end of that process, there was another irrigation of socket with 1 mL of sterile saline solution to remove the dye and also new blood collection using three sterile absorbent paper cones, following the same previously mentioned procedures. The patient was then released to the completion of the surgical procedure, which corresponded to suture of the socket.

The tubes containing the cones collected before and after treatment were labeled with codes that did not allow the identification of the patient or if that collection corresponded to alveoli (P+L-) or (P+L+) or even if the procedure was conducted before (P-L-) or after (P+L-) and (P+L+). Thus, the second researcher, who was responsible for laboratory study stages, worked blindly.

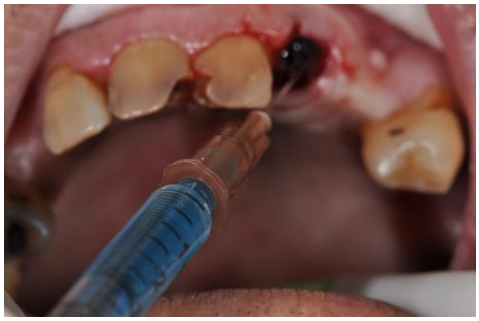


Fig 2: Filling the socket with 1 mL of MB solution 0.005%.

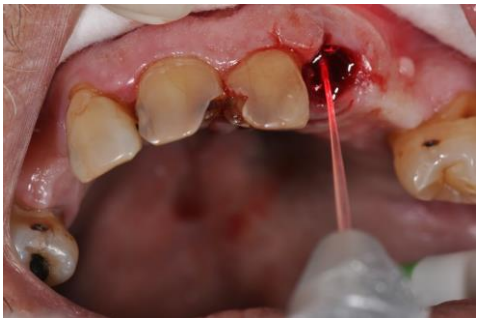
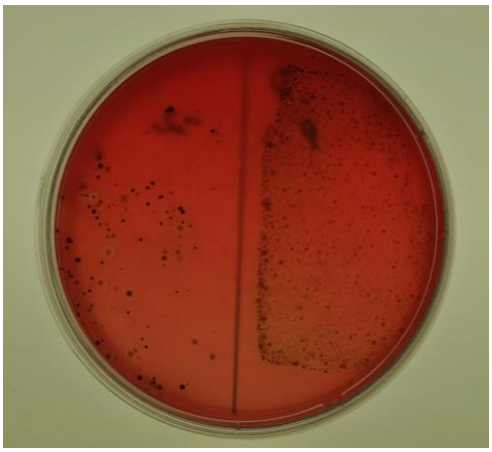


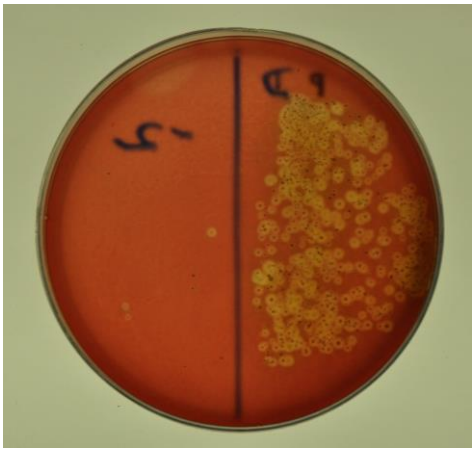
Fig 3: Application of 660 nm low intensity laser with aid of optic fiber.

Laboratory procedures

In the laboratory of Microbiology of the Graduate Program in Dentistry of the Federal University of Ceara, in laminar flow hood, the tubes containing cones soaked with alveolar blood were placed in shaker (Vertex QL-901, São Paulo, SP, Brazil) and 100 ul aliquots of the suspension of each sample underwent serial dilutions 1:100, 1:1000, 1:10000, 1:100000 and 1:1000000 in sterile saline solution. After that, 25 ul of each dilution were plated in triplicate by means of drops in Petri dishes containing blood agar culture medium (Eximlab LTDA., São Paulo, SP, Brazil) for cultivation of aerobic bacteria and Brain Heart Infusion (BHI) agar supplemented with defibrillated blood sheep/hemin/menadione/cysteine for cultivation of anaerobic bacteria (Eximlab LTD.).

Subsequently, the plates were coded and incubated in a bacteriological incubator for 48 hours at 37°C (aerobic) or under anaerobic conditions in jars with ANAEROBAC (Probac, São Paulo, Brazil) establishing low level of oxygen and higher carbon dioxide levels for 7 days at 37°C. Then, there was a total count of CFU on each plate. (Figs. 4 and 5)





Figs 4 e 5: Aerobic bacterial growth on blood agar plate and anaerobic bacterial growth on BHI plate enriched with blood, Hemin, Cysteine and Menadione.

Outcome variable and analysis methods

After counting CFU on the plates, data were converted to CFU/mL and transformed into logarithms to the base 10. The number of CFU/mL of aerobic and anaerobic bacteria was compared before (P-L-) and after treatment (P+L-) and (P+L+).

Data analysis was carried out using the paired Student's t statistical test at a confidence interval of 95% ($\alpha = 0.05$). Graph Pad Prism 5.0 software (GraphPad Software Inc., La Jolla, CA, USA) was used for analysis.

Counting and statistical procedure were conducted by a third researcher (researcher 3), who was unaware of the origin of the samples.

RESULTS

A total of 25 patients living in Fortaleza, Ceara, Brazil came to the clinic interested in extraction procedures. Concerning to gender, 62.5% were female and 37.5% male and subjects´ mean age was 49.5 years; however, seven patients were excluded because they did not meet the inclusion criteria. The main reasons for exclusion were: indication for posterior teeth extraction, use of antibiotics in the last three months and smokers. Of the 18 patients who had inclusion criteria, two were excluded due to dropout, having only 16 volunteers at the end. (Fig. 6)

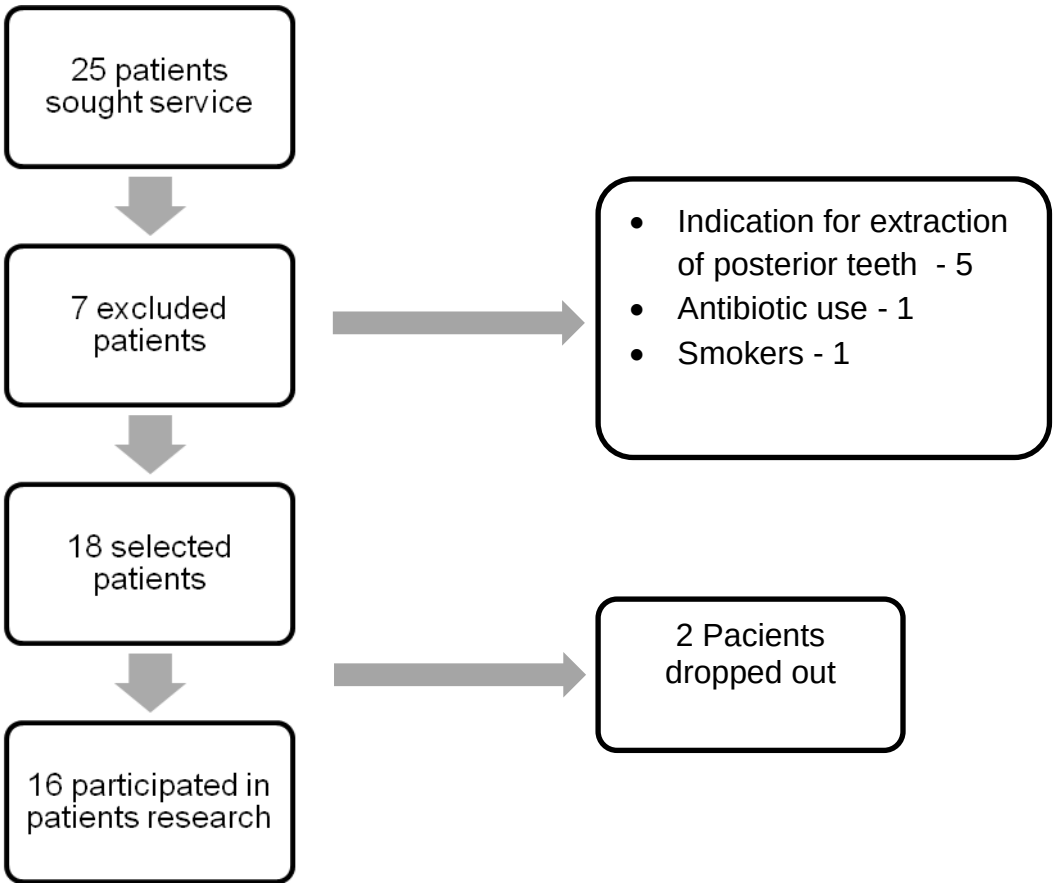


Fig 6: Sample fluxogram

Figure 7 shows the result of the counting of CFU/mL viable aerobic bacteria found in the alveolar blood before and after treatment with methylene blue 0.005% (P+L-). There was a 1.5 log reduction, that is, average CFU/mL before treatment was 21566 and then decreased to 1292 after treatment. However, such reduction was not statistically significant (p=0.07). Figure 8 shows the result of the counting of CFU/mL found in the alveolar blood before and after treatment with photodynamic therapy (P+L+). A log reduction of 2.5 was observed, in which the average CFU/mL, which was 226326 before treatment, changed to 827.5 after treatment. However, this reduction was not statistically significant either (p= 0.29).

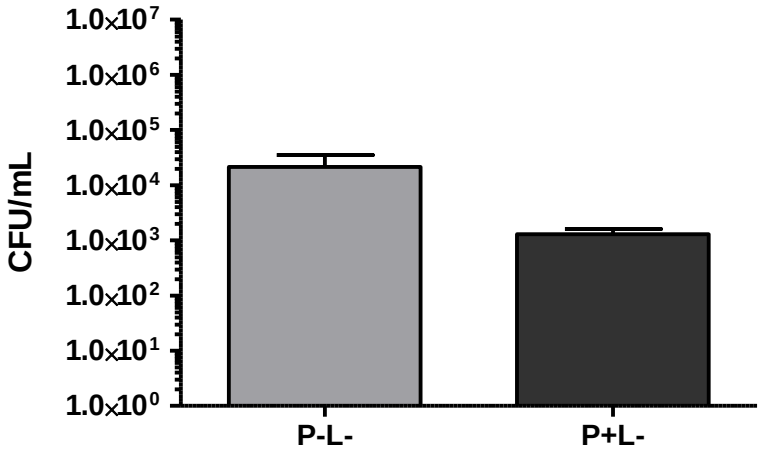


Fig 7: Counting CFU/mL of aerobic bacteria in the alveolar blood before and after treatment with methylene blue 0.005% (p=0.07).

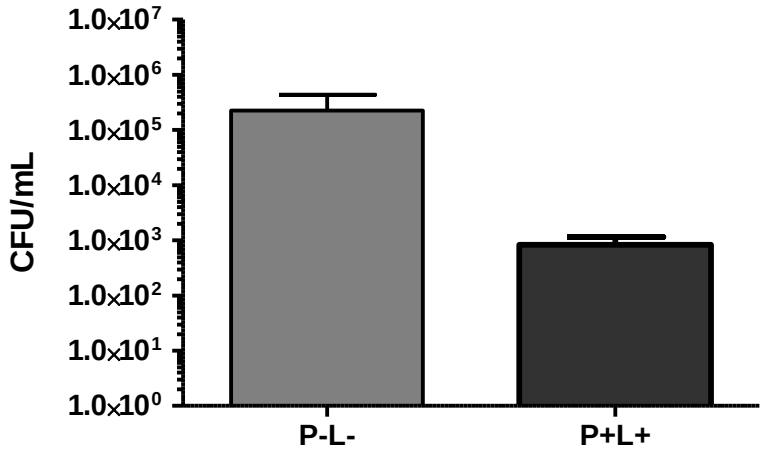


Fig 8: Counting CFU/mL of aerobic bacteria in the alveolar blood before and after treatment with photodynamic therapy (p=0.29).

Figure 9 shows the result of the count of CFU / mL of viable anaerobic bacteria found in the alveolar blood before and after treatment with methylene blue 0.005% (P+L-). There was a 2 log reduction, or the average CFU / mL before treatment was increased to 3326 and 331484 after treatment. This reduction was not statistically significant (p = 0.16). The figure 10 shows the result of the count of CFU / mL alveolar found in the blood before and after treatment with photodynamic therapy (P+L+) a 1.5 log reduction was observed in the average CFU / ml before treatment was 382827 and passed 14320 after treatment. This reduction was not statistically significant (p = 0.16)

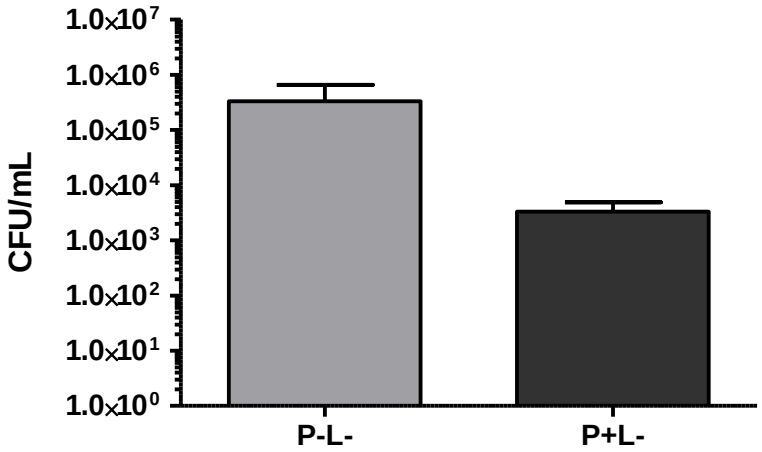


Fig 9: Counting CFU/mL of anaerobic bacteria in the alveolar blood before and after treatment with methylene blue 0.005% (p=0.16).

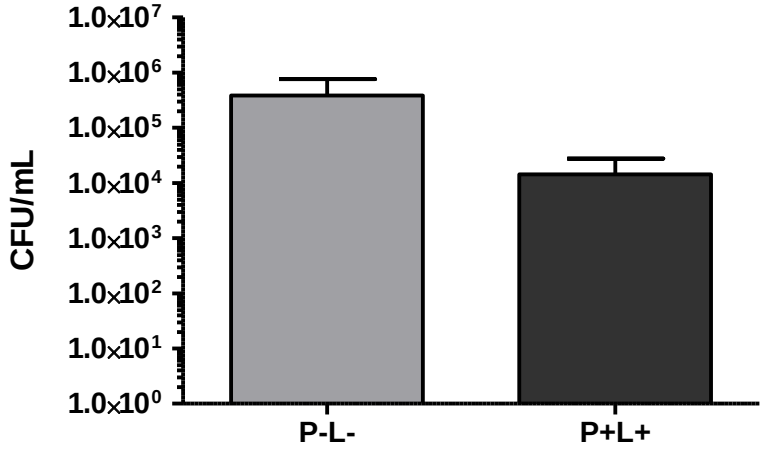


Fig 10: Counting CFU/mL of anaerobic bacteria in the alveolar blood before and after treatment with photodynamic therapy (p=0.16).

DISCUSSION

The results of this study show that the null hypothesis was confirmed. But, clinically thinking, it cannot be said that photodynamic therapy (PDT) was totally ineffective in this study, as the results showed that there was a reduction in bacterial count after PDT in aerobic and anaerobic cultures. This trend showed that there is antimicrobial activity which can be improved by adjusting the methodological protocol. The analysis carried out for the P+L- showed statistically similar results to those obtained by P+L+, suggesting an antimicrobial activity of methylene blue

photosensitizer, regardless laser irradiation. Still, for aerobic cultures, it was observed a higher antimicrobial activity to the sockets which were also irradiated.

The results of this study showed log reduction between 1.5 and 2.5, similar to a study by Komerik et al (2003), who used the toluidine blue at concentration 0.1 mg/ml and 0.01 mg/mL associated with a 100 mW and 660 nm laser, achieving log reduction between 1 and 2 for *P. gengivalis* in periodontitis induced in rats. [5] This means that better results would be achieved if there were some change in the protocol by increasing the power density of the laser, besides rising irradiation time or the laser power. However, an increase of irradiation time would be critical for the time of clot formation within the socket and having no delay in a possible immediate implant placement. It is also important to highlight that high power or high intensity lasers are not recommended because they present photothermal effects and heating the socket would not be desirable for future osseointegration of implants. In this case, it would be necessary a low-intensity laser device with a little more power to the red laser. Caution is also required when it comes to increasing the concentration of photosensitizer dye due to its possible cytotoxicity.

The methodology of this work was inspired in the study by FRANCO et al (2010), who sought a way to reduce the microbiota of peri-implant area by means of TFA, in which the peri-implant sulcus was irrigated with 1 mL of the PM 0.005 % for 5 minutes with occasional applications with low power laser wavelength of 660 nm by continuous irradiation of 120 J/cm². Such density power was divided into four points of 30 J/cm² per implant, for 30 seconds per point, in a power of 40 mW, obtaining a statistically significant reduction in microbial $p = 0.003$.[11] Similarly, NIKOLAOS et al (2006) evaluated the effects of TFA on pathogenic microorganisms in 60 freshly extracted human teeth, obtaining the elimination of all bacteria by applying a solution of MB (25 mg/mL), leaving it for 5 minutes and then irradiating laser diode with a wavelength of 665 nm, 30 J/cm2, through the introduction of optical fiber within the root canal, which may lead to different results than those found in sockets. [22]

In a randomized clinical trial conducted by CAMPOS et al (2013), it was evaluated the effects of TFA as an adjuvant treatment for residual periodontal pockets in single-rooted teeth. They used diode laser with wavelength 660 nm, power 60 mW and energy density of 129 J/cm², associated with the MB dye as photosensitizer at a concentration of 10 mg/mL or 1%. The results showed significant benefits in the use

of TFA. [15] In a study with animals ALMEIDA et al (2007) used four groups divided into: untreated, which is the control group; the group topically treated with methylene blue 100 mg / mL; Laser Therapy (685 nm, 120 seconds, 4.5 J/cm²) and TFA histologically and radiographically evaluating its effect on the progression of induced periodontal disease, concluding that TFA reduced the destruction of periodontal tissue. [14] Analyzing the referred studies, it is observed that there is great variability in literature on the concentration of photosensitizer and energy fluency employed by the light source. These variations may affect the results, as well as the tissue being irradiated may have an important influence.

In part, the results obtained in this work can be related to its uniqueness. The literature showed a deficit of articles that use TFA in strict anaerobic cultures, making the design of the methodology of this study difficult. There is no established protocol for laser application in the studied circumstances, requiring further studies employing variations in its power as well as its irradiation time, in addition to testing other sources of light at different wavelengths. Variations in the use of the dye especially involve its concentration, ranging from 0.005%, as used in this methodology, to 1% for MB, as employed by CAMPOS et al, 2013. [15]

It is suggested conducting microbiological studies *in vitro* by the making of artificial anaerobic biofilms in bovine bone arrays, and *in vivo*, microbiological and histological, with animal models in order to enable the exploration of different photosensitizers and light sources at different concentrations and energy fluency, respectively. Controlled randomized clinical studies with longitudinal follow-up will also be needed in the future to check for survival and success rates of implants immediately placed in contaminated sockets treated with TFA.

CONCLUSION

Given the limitations of this study and the obtained data, it was concluded that, although the differences observed have not been statistically significant, there was a promising trend in susceptibility of aerobic and anaerobic bacteria in periapical lesions with the use of PDT, using methylene blue 0.005% and a 660 nm laser.

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CONSIDERAÇÕES GERAIS

Os objetivos desta dissertação consistiram em, através de uma revisão sistemática da literatura, esclarecer as condições para a indicação dos implantes em alvéolos contaminados, a taxa de sucesso/sobrevivência encontradas e o protocolo de descontaminação empregado e,por meio de um estudo clínico controlado, observar os efeitos da terapia fotodinâmica antimicrobiana (TFA) na descontaminação de alvéolos com lesão periapical imediatamente após exodontias, o que confirmou a hipótese nula.

Alguns autores estabelecem a presença de lesão periapical como uma contraindicação absoluta à instalação de implantes imediatos (QUIRYNEN et al, 2003; ROSENQUIST et al , 1996), o que os artigos estudados nesta revisão refutam (SIEGENTHALER et al, 2007; CASAP et al,2007; CORNELINI et al, 2005; LINDEBOOM et al, 2006; CRESPI et al, 2010; TRIPODAKIS & NAKOU, 2011).

As maiores vantagens atribuídas a instalação imediata dos implantes são diminuição da reabsorção óssea alveolar após a extração, redução do tempo de tratamento e menor stress psicológico para o paciente (DEL FABRO et al, 2009; BHOLA et al, 2008; MISSIKA, 1994; WERBITT et al, 1992).

Em um estudo envolvendo 30 implantes imediatamente instalados em alvéolos infectados após o debridamento, exceto 1 implante não mostrava-se osseointegrado após um período de 12 até 72 meses (CASAP et al, 2007). Esta técnica é relatada em diversos estudos, em que se busca a máxima limpeza do alvéolo antes do preparo do mesmo para a instalação do implante, assim evitando sua contaminação (JOFRE et al, 2012; PEÑARROCHA-DIAGO et al, 2012; BEAGLE et al, 2006). Similarmente, um experimento realizado em 655 pacientes e envolvendo 922 implantes, 285 instalados em alvéolos com lesão periapical crônica, referiu uma taxa de sucesso de 97,5%, não apresentando diferença significativa em relação ao grupo controle (BELL et al, 2011).

A presença de lesão periapical não necessariamente contraindica a instalação de implantes imediatos, desde que procedimentos clínicos adequados como a administração de antibióticos combinado com meticulosa limpeza e debridamento alveolar antes do preparo para o implante sejam realizados (NOVAES et al, 1998; SUNDVIST, 1992)

O uso de antibioticoterapia antes e após a instalação de implantes imediatos é uma prática bem estabelecida para casos com presença de lesão periapical e/ou periodontal (CORNELINI et al, 2005; CRESPI et al, 2010; LINDEBOOM et al, 2006; SIEGENTHALER et al, 2007; TRIPODAKIS & NAKOU, 2011; MARCONINI et al, 2013) e também tem sido referida para sítios sem lesões (COVANI et al, 2012). Excetuando-se o debridamento do alvéolo, nenhuma outra terapia alternativa ao uso dos antibióticos sistêmicos como forma de diminuir a contaminação bacteriana local foi encontrada. Os protocolos incluem uso de 600 mg de clindamicina ou até 1,5 g de amoxicilina, iniciados de 1h até 4 dias antes do procedimento e mantendo-se por até 10 dias (CASAP et al, 2007). O controle antimicrobiano pós-operatório local foi realizado com bochechos de clorexidina a 0,2% por períodos variando de 5 a 15 dias (CORNELINI et al, 2005; CRESPI et al, 2010; SIEGENTHALER et al, 2007).

Os resultados apontados pelo estudo clínico controlado mostram que a hipótese nula foi confirmada. Entretando, tratando-se da aplicação clínica, não se pode afirmar que a TFA foi totalmente ineficaz, uma vez que os resultados obtidos mostram uma redução na contagem bacteriana após a TFA em culturas aeróbia e anaeróbia, o que permite afirmar que há atividade antimicrobiana e que poderá ser melhorada através do ajuste do protocolo metodológico. A análise realizada para os alvéolos apenas irrigados com azul de metileno (P+L-) demonstrou resultados estatisticamente semelhantes aos obtidos pelos alvéolos irrigados com azul de metileno (AM) e irradiados com laser vermelho (P+L+), sugerindo uma atividade antimicrobiana do fotossensibilizador, independente da irradiação com laser. Ainda assim, para as culturas aeróbias, observou-se maior atividade antimicrobiana para os alvéolos também irradiados.

A metodologia deste trabalho foi inspirada no estudo de FRANCO et al (2010), que buscou uma forma de redução da microbiota da área peri-implantar com a TFA, no qual o sulco peri-implantar foi irrigado com 1 mL de solução de AM a 0,005% por 5 minutos com aplicações pontuais com laser de baixa potência de comprimento de onda de 660 nm, por meio da irradiação contínua de 120 J/cm² de densidade de energia, dividida em 4 pontos de 30 J/cm² por implante, 30 segundos por ponto, numa potência de 40 mW, sendo obtida uma redução microbiana estatisticamente significante. Estudos similares realizados por NIKOLAOS et al (2006), CAMPOS et al (2013), ALMEIDA et al (2007) apontaram uma grande

variabilidade na literatura sobre a fluência de energia empregada pela fonte de luz e a concentração do fotossensibilizador, que podem interferir nos resultados, assim como o tecido irradiado também pode ter influência.

O déficit de artigos que empregam a TFA em culturas anaeróbias estritas dificultou o delineamento metodológico do estudo clínico. Não existe um protocolo estabelecido para a aplicação do laser nas circunstâncias estudadas, necessitando de mais estudos que empreguem variações em sua potência e seu tempo de irradiação, além do teste de outras fontes de luz, em diferentes comprimentos de onda. As variações no uso do corante envolvem especialmente sua concentração, podendo variar de 0,005%, como empregado nesta metodologia, até 1% para o AM, como empregado por CAMPOS et al, 2013.

Sugere-se a realização de estudos microbiológicos *in vitro* por meio da confecção de biofilmes anaeróbios artificiais em matrizes de osso bovino *e in vivo,* microbiológicos e histológicos*,* com modelos animais, para possibilitar a exploração de diferentes fotossensibilizadores e fontes de luz, em diferentes concentrações e fluências de energia, respectivamente. Estudos clínicos controlados e randomizados, com acompanhamento longitudinal, também serão necessários subsequentemente para verificação de índices de sobrevivência e sucesso de implantes imediatos colocados em alvéolos contaminados tratados com a TFA.

CONCLUSÕES GERAIS

Baseado no exposto nos capítulos 1 e 2 desta dissertação, conclui-se:

- As indicações para implantes imediatos incluem a reposição de dentes decíduos retidos, fratura radicular vertical ou horizontal, dentes com impossibilidade de restauração, falha endodôntica e estética deficiente.
- A presença de sítios contaminados constitui uma contraindicação relativa a instalação de implantes imediatos.
- Os implantes imediatos apresentam elevadas taxa de sucesso e sobrevivência, não apresentando diferença em relação aos implantes tardios.O protocolo existente hoje para instalação dos implantes imediatos inclui o debridamento mecânico e a antibioticoterapia.
- A aplicação da TFA em alvéolos contaminados mostrou redução na contagem microbiana, mas estatisticamente insignificante, confirmando a hipótese nula. No entanto, sob o ponto de vista clínico, reduções microbianas de 1 a 2 $\log_{10}$ não podem ser consideradas ineficazes.
- Existe uma atividade antimicrobiana do fotossensibilizador azul de metileno a 0,005%, independente da irradiação com laser.

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APÊNDICE I– TESTE T DE STUDENT PARA AERÓBIOS NO GRUPO CONTROLE

	Before	After
Minimum	0.0	10.00
25% Percentile	403.8	246.7
Median	1500	1100
75% Percentile	10873	2183
Maximum	165083	3380
Mean	21566	1292
Std. Deviation	48609	1115
Std. Error	13482	309.4
Lower 95% CI	-7808	618.3
Upper 95% CI	50940	1966

Table Analyzed	Data 1
Column A	Before
vs	vs
Column B	After
Paired t test	
P value	0.0775
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	One-tailed
t, df	t=1.518 df=12
Number of pairs	13
How big is the difference?	
Mean of differences	20274
95% confidence interval	-8837 to 49385
R square	0.1610
How effective was the pairing?	
Correlation coefficient (r)	0.4043
P Value (one tailed)	0.0853
P value summary	ns
Was the pairing significantly effective?	No

APÊNDICE II– TESTE T DE STUDENT PARA ANAERÓBIOS NO GRUPO CONTROLE

	Before	After
Minimum	40.00	42.20
25% Percentile	194.1	393.5
Median	2138	1110
75% Percentile	8769	2904
Maximum	4.583e+006	20660
Mean	331484	3326
Std. Deviation	1.224e+006	5742
Std. Error	327006	1535
Lower 95% CI	-374969	10.21
Upper 95% CI	1.038e+006	6641

Table Analyzed	Data 1
Column A	Before
vs	vs
Column B	After
Paired t test	
P value	0.1671
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	One-tailed
t, df	t=1.003 df=13
Number of pairs	14
How big is the difference?	
Mean of differences	328158
95% confidence interval	-378501 to 1.035e+006
R square	0.07183
How effective was the pairing?	
Correlation coefficient (r)	-0.09634
P Value (one tailed)	0.3716
P value summary	ns
Was the pairing significantly effective?	No

APÊNDICE III – TESTE T DE STUDENT PARA AERÓBIOS NO GRUPO TERAPIA

	Before	After
Minimum	12.00	0.0
25% Percentile	118.8	102.6
Median	1007	358.5
75% Percentile	37750	966.3
Maximum	2.934e+006	3950
10.00% Percentile	49.10	35.75
90.00% Percentile	1.524e+006	3475
Mean	226326	827.5
Std. Deviation	780126	1193
Std. Error	208497	318.8
Lower 95% CI of mean	-224106	138.7
Upper 95% CI of mean	676757	1516

Table Analyzed	Data 1
Column A	Before
vs	vs
Column B	After
Paired t test	
P value	0.2987
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.082 df=13
Number of pairs	14
How big is the difference?	
Mean of differences	225498
95% confidence interval	-224481 to 675478
R square	0.08268
How effective was the pairing?	
Correlation coefficient (r)	0.5447
P Value (one tailed)	0.0220
P value summary	*
Was the pairing significantly effective?	Yes

APÊNDICE IV – TESTE T DE STUDENT PARA ANAERÓBIOS NO GRUPO TERAPIA

	Before	After
Minimum	84.00	96.00
25% Percentile	474.5	302.3
Median	1730	576.6
75% Percentile	4325	1590
Maximum	5.303e+006	190000
Mean	382827	14320
Std. Deviation	1.416e+006	50569
Std. Error	378482	13515
Lower 95% CI	-434834	-14877
Upper 95% CI	1.200e+006	43518

Table Analyzed	Data 1
Column A	Before
Vs	vs
Column B	After
Paired t test	
P value	0.1655
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	One-tailed
t, df	t=1.010 df=13
Number of pairs	14
How big is the difference?	
Mean of differences	368507
95% confidence interval	-419825 to 1.157e+006
R square	0.07272
How effective was the pairing?	
Correlation coefficient (r)	0.9999
P Value (one tailed)	< 0.0001
P value summary	***
Was the pairing significantly effective?	Yes

ANEXO I

FICHA N°_____

ACEITO ( )
NÃO ACEITO ( )

UNIVERSIDADE FEDERAL DO CEARÁ

FACULDADE DE FARMACIA, ODONTOLOGIA E ENFERMAGEM

EFEITOS DA TERAPIA FOTODINÂMICA ANTIMICROBIANA NA DESCONTAMINAÇÃO DE ALVÉOLOS DENTÁRIOS COM LESÃO PERIAPICAL APÓS EXODONTIA: ANÁLISE CLÍNICA.

DADOS PESSOAIS

Nome :_____

Endereço :_____

Bairro:_____ Cidade:_____

Estado:_____ Telefones :_____

HISTÓRIA MÉDICA

( ) Está em acompanhamento médico? Qual? _____

( ) Apresenta alguma alteração de saúde, como Hipertensão, Problema Cardíaco, Diabetes, Imunossupressão?

( ) Toma algum medicamento regularmente? Qual? _____

_____

( ) Apresenta algum tipo de Alergia (Medicamento, Alimento, Corante, etc)? Qual?

_____

( ) Tomou antibiótico há pelo menos 3 meses?

( ) Já teve alguma hemorragia?

( ) Já teve câncer em alguma parte do corpo? Qual? Há quanto tempo?

_____

( ) Já fez Quimioterapia? Há quanto tempo? _____

( ) Já fez Radioterapia? Há quanto tempo? Onde?_____

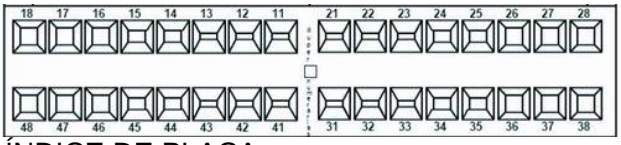
( ) É Fumante?

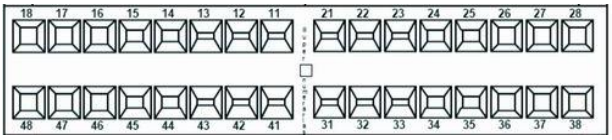
HISTÓRIA ODONTOLÓGICA

Quando foi a última vez que foi ao Dentista? _____

Que tipo de tratamento foi realizado? _____

( ) Apresenta sangramento na gengiva ou mobilidade dental?





ÍNDICE DE PLACA

Data: ___/___/___ _____% Data: ___/___/___ _____%

Observações:

_____

_____

DADOS CIRURGIA

Data: ___/___/___ Elementos Dentais: (C)_____ (T)_____

Pressão:_____

Cirurgia realizada por: _____

Coleta realizada por: _____

Observações:

_____

_____

FASE LABORATORIAL

Data da cultura: ___/___/___ Procedimento realizado por: _____

Data prevista para a contagem: ___/___/___

Contagem das unidades formadoras de colônia:

Contagem de UFC - AEROBIOS - TERAPIA							
	Antes				Depois		
	Triplicata 1	Triplicata 2	Triplicata 3		Triplicata 1	Triplicata 2	Triplicata 3
Puro							
-1							
-2							
-3							

Contagem de UFC - AEROBIOS - CONTROLE							
	Antes				Depois		
	Triplicata 1	Triplicata 2	Triplicata 3		Triplicata 1	Triplicata 2	Triplicata 3
Puro							
-1							
-2							
-3							

Contagem de UFC - ANAEROBIOS - TERAPIA							
	Antes				Depois		
	Triplicata 1	Triplicata 2	Triplicata 3		Triplicata 1	Triplicata 2	Triplicata 3
Puro							
-1							
-2							
-3							

Contagem de UFC - ANAEROBIOS - CONTROLE							
	Antes				Depois		
	Triplicata 1	Triplicata 2	Triplicata 3		Triplicata 1	Triplicata 2	Triplicata 3
Puro							
-1							
-2							
-3							

Observações complementares:

_____

_____

_____

_____

_____

ANEXO II

Termo de Consentimento Livre e Esclarecido (T.C.L.E.)

Prezado paciente,

Você está sendo convidado a participar da pesquisa " EFEITOS DA TERAPIA FOTODINÂMICA NA DESCONTAMINAÇÃO DE ALVÉOLOS PÓS-EXODONTIA ". Você não deve participar contra a sua vontade. Leia atentamente as informações abaixo e faça qualquer pergunta que desejar, para que todos os procedimentos desta pesquisa sejam esclarecidos.

Você deverá estar ciente que:

1. O dente que precisa ser extraído, será removido normalmente, com anestesia local e todos os procedimentos triviais.
2. Após a extração do dente, será realizada a coleta de uma pequena amostra de sangue com três pequenos palitos de papel esterilizado, colocados e retirados rapidamente no local onde o dente estava.
3. Em seguida, será colocado neste mesmo local, um líquido corante azul por 5 minutos para a aplicação de uma luz laser dentro do espaço onde o dente estava presente. A luz ficará acesa por 1 minuto e meio.
4. Quando a luz apagar, o corante será removido com soro fisiológico e uma nova coleta de amostra de sangue semelhante à anterior será realizada. A pesquisa termina aqui, com duração máxima de 8 minutos. A finalização da cirurgia, com a sutura, será realizada normalmente.
5. Não existe nenhum desconforto ou dor relacionado à coleta de sangue ou à aplicação do corante associado à luz laser.
6. Também não haverá prejuízo na cicatrização da cirurgia.
7. Trata-se de estudo experimental testando a hipótese de que a aplicação da luz associada a um corante poderá reduzir ou eliminar totalmente as bactérias que podem causar infecções após a extração.
8. Fotografias poderão ser feitas durante os procedimentos, com finalidade didática e científica, não permitindo a sua identificação quando de sua divulgação.
9. Você tem a liberdade de retirar seu consentimento a qualquer momento e deixar de participar do estudo, sem prejuízo para seu tratamento na Instituição.
10. As informações obtidas serão analisadas em conjunto com outros pacientes, não sendo divulgada a identificação de nenhum paciente.

11. Você será mantido atualizado sobre os resultados parciais da pesquisa, se desejar.
12. Não há despesas pessoais para o participante em qualquer fase do estudo, incluindo exames e consultas. Também não há compensação financeira relacionada à sua participação.
13. Os dados coletados serão utilizados somente para esta pesquisa.

Responsável: Profa. Dra. Karina Matthes de Freitas Pontes
Rua Monsenhor Furtado S/N – Rodolfo Teófilo – Fortaleza – CE - Fone: 33668403

ATENÇÃO: Para informar qualquer questionamento durante a sua participação no estudo, dirija-se ao Comitê de Ética em Pesquisa da Universidade Federal do Ceará, na rua Cel.Nunes de Melo, 1127 – Rodolfo Teófilo – Fortaleza – CE. Fone: 33668338.

O abaixo assinado, _____, _____anos, RG: _____, declara que é de livre e espontânea vontade que está participando como voluntário da pesquisa. Eu declaro que li cuidadosamente este T.C.L.E. e que, após sua leitura tive a oportunidade de fazer perguntas sobre o conteúdo do mesmo, como também sobre a pesquisa e recebi explicações que responderam por completo minhas dúvidas. E declaro ainda estar recebendo uma cópia assinada deste Termo.

_____	_____
Assinatura do voluntário ou responsável legal Data___/__/__	Karina Matthes de Freitas Pontes Data___/__/__ Pesquisadora responsável
_____	_____
Profissional que aplicou o T.C.L.E Data___/__/__	Assinatura da testemunha Data___/__/___

(Para casos de pacientes menores de 18 anos, analfabetos, semi-analfabetos ou portadores de deficiência auditiva ou visual).