Archives of Oral Biology 81 (2017) 1–6

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Stimulation of human gingival fibroblasts viability and growth by roots treated with high intensity lasers, photodynamic therapy and citric acid

Paula Stephania Brandão Hage Karam[a,*], Rafael Ferreira[a], Rodrigo Cardoso Oliveira[b], Sebastião Luiz Aguiar Greghi[a], Maria Lúcia Rubo de Rezende[a], Adriana Campos Passanezi Sant'Ana[a], Mariana Schutzer Ragghianti Zangrando[a], Carla Andreotti Damante[a]

[a] Discipline of Periodontics, Bauru School of Dentistry, University of São Paulo, Brazil
[b] Discipline of Biochemistry, Bauru School of Dentistry, University of São Paulo, Brazil

ARTICLE INFO

Keywords:
Lasers
Photochemotherapy
Cell culture techniques
Demineralization

ABSTRACT

Objective: The aim of this study was to compare the effect of root biomodification by lasers, citric acid and antimicrobial photodynamic therapy (aPDT) on viability and proliferation of human gingival fibroblasts (FGH).
Design: Groups were divided in control (CC – only cells), and root fragments treated by: scaling and root planing (positice control – SC), Er:YAG (ER–60 mJ,10pps,10 Hz,10s,2940 nm), Nd:YAG (ND–0.5W,15 Hz,10s,1640 nm), antimicrobial photodynamic therapy (PDT–InGaAIP,30 mW,45J/cm^2,30s,660 nm,toluidine blue O), citric acid plus tetracycline (CA). Fibroblasts (6th passage, 2×10^3) were cultivated in a 24-h conditioned medium by the treated root fragments. Cell viability was measured by MTT test at 24, 48, 72 and 96 h. In a second experiment, FGH cells (10^4) were cultivated on root fragments which received the same treatments. After 24, 48, 72 h the number of cells was counted in SEM pictures. In addition, chemical elements were analyzed by energy dispersive spectroscopy (EDS). Data was analyzed by two-way ANOVA (first experiment), repeated measures ANOVA (second experiment) and ANOVA (EDS experiment) tests complemented by Tukey's test ($p < 0.05$).
Results: ND, PDT and CA promoted higher cell viability ($p < 0.05$). ND and ER groups presented higher number of cells on root surfaces ($p < 0.05$). ER group presented higher calcium and CA group a higher carbon percentages ($p < 0.05$).
Conclusions: All treatments but scaling and root planing stimulated fibroblast viability while Er:YAG and Nd:YAG treated root surfaces presented higher number of cells.

1. Introduction

One of the main challenges in periodontal treatment is how to eliminate the subgingival bacteria and to convert the root surface in a biocompatible environment. Bacterial invasion in radicular cementum and dentinal tubules reach 300 µm and elimination by mechanical treatment is challenging. Therefore adjuvant methods for root biomodification are proposed (Adriaens, Edwards, DeBoever, & Loesche, 1988). Among the options are chemical treatment (Register & Burdick, 1975; Sant'Ana, Marques, Barroso, Passanezi, & de Rezende, 2007), high-energy lasers (Feist et al., 2003; Gaspirc & Skaleric, 2007; Hamaoka, Moura-Netto, Marques, & Moura, 2009; Qadri, Javed, Poddani, Tunér, & Gustafsson, 2011) or recently, antimicrobial photodynamic therapy (Andrade et al., 2013; Kö et al., 2013; Qin, Luan, Bi, Sheng, Zhou, & Zhang, 2008; Salmeron, et al., 2013; Sgolastra et al., 2013). All of these treatments are adjunctive to scaling and root planing and show distinct advantages. These therapies also promote root surface modification and demonstrated antimicrobial effects (Andrade et al., 2013).

Hard surface demineralization has been used in periodontology since 1973, when Register showed an accelerated reattachment of fibers and cementogenesis in surgically exposed and demineralized roots *in situ* (Register, 1973). Various acid products were tested aiming hard tissue demineralization without side effects. The best results were obtained with citric acid, at pH 1, for 3 min (Register & Burdik, 1975). Citric acid demineralization removes smear layer, lipopolysaccharides and exposes collagen fibrils, which improves blood clot formation with higher retention of fibrin (Leite et al., 2010). The

* Corresponding author at: Bauru School of Dentistry, Alameda Octávio Pinheiro Brisolla 9-75, 17012-901, Bauru, SP, Brazil.
E-mail addresses: paulinhakaram@yahoo.com.br (P.S.B.H. Karam), rafael2.ferreira@usp.br (R. Ferreira), rodrigocardoso@usp.br (R.C. Oliveira), slagregh@fob.usp.br (S.L.A. Greghi), maluzrezende@usp.br (M.L.R. de Rezende), acpsantana@usp.br (A.C.P. Sant'Ana), mariana@fob.usp.br (M.S.R. Zangrando), cdamante@usp.br (C.A. Damante).

http://dx.doi.org/10.1016/j.archoralbio.2017.04.012
Received 8 June 2016; Received in revised form 20 March 2017; Accepted 17 April 2017
0003-9969/ © 2017 Published by Elsevier Ltd.

association of tetracycline with citric acid optimizes these effects (Sant'Ana et al., 2007).

High intensity lasers (Er:YAG) promote root biomodification with efficient calculus removal, resulting in a rough surface (Feist et al., 2003; Passanezi, Damante, de Rezende, & Greghi, 2015; Aoki et al., 2000). Root surfaces treated by Er:YAG are superior for fibrin clot formation and blood cell attachment (Cekici, Maden, Yildiz, San, & Isik, 2013) and are attractive to gingival fibroblasts (Feist et al., 2003). Clinically, Er:YAG as an adjunctive treatment for periodontal disease results in reduced probing depths and gingival index (Gaspirc & Skaleric, 2007). Nd:YAG lasers promote significant black pigmented bacteria reduction in class two furcation lesions (Andrade et al., 2008). The improved root biocompatibility is due to surface modification and bactericidal effect (Hamaoka et al., 2009). Also, additional benefits were demonstrated in smokers in relation to conventional therapy for treatment of periodontal disease (Eltas & Orbak, 2012). A recent *meta*-analysis suggested that the adjunctive treatment with Nd:YAG to scaling and root planing is responsible for probing depth and gingival crevicular fluid reduction (Sgolastra et al., 2013).

Antimicrobial photodynamic therapy (aPDT) is indicated to lethal photosensitization of bacteria. The basis of aPDT is the activation of a photosensitizing drug as toluidine blue O (TBO) by a red laser. The chemical reaction releases oxygen reactive species capable of destroying target microorganisms. aPDT exerts an additional effect on periodontopathogenic bacteria and reduces virulence factors. This therapy is successfully associated to nonsurgical and surgical periodontal therapy modulating the extracellular matrix and bone remodeling (Andrade, Garlet, Silva, Fernandes, & Milanezi, 2013). A *meta*-analysis demonstrated that aPDT exerts additional benefits on periodontal treatment outcomes as probing depth reduction and clinical attachment level gain in a short-term period (Sgolastra et al., 2013). Recent researches of our group have proven that Toluidine Blue O [100 μg/ml] and pH [4.31] commonly used in aPDT causes demineralization and loss of Knoop microhardness on dentin surfaces without damage to gingival fibroblasts (Damante et al., 2016). This result could be useful for root surface decontamination and demineralization in periodontal treatments.

There is no data in literature addressing the biocompatibility of roots demineralized by aPDT with toluidine blue O. Thus, the first step is to test these effects in cell culture tests. Cell culture presents advantage of selecting a specific cell type and enable the study of cell behavior without the influence of nervous and endocrine systems. Besides that, the effects can be compared to common treatments as high intensity lasers and citric acid in a controlled environment.

The aim of this study was to compare the effects of root biomodification by lasers (Er:YAG, Nd:YAG) citric acid and antimicrobial photodynamic therapy on the viability and proliferation of human gingival fibroblasts.

2. Materials and methods

This research was approved by Ethical Committee on Human Research of Bauru School of Dentistry – University of São Paulo (#086/2011).

2.1. Preparation of root fragments

Sixty teeth extracted for severe periodontal disease were selected and stored in saline solution at 4 °C. Dental roots were cut with a disk at the following dimensions: 4 mm length, 2 mm width, 2 mm height. One hundred and twenty fragments were obtained and sterilized in autoclave. All fragments were scaled with Gracey curettes (Hu Friedy, Chicago, USA) (20 strokes/fragment) and washed with saline for 30 s.

2.2. Experimental groups

The experimental groups were divided as follows:
CC – cell control (first experiment only)
SC – scaling and root planing control
ER – laser Er:YAG
ND – laser Nd:YAG
PDT – antimicrobial photodynamic therapy – Toluidine Blue O (pH 4.31) + Laser InGaAlP
CA – citric acid + Tetracycline − pH1

The CC group had only cells cultured in ideal conditions of growth and it was used only on the first experiment. The SC group consisted in fragments treated with 20 strokes of curette. The ER group was treated with an Er:YAG laser (Fotona, Twinlight, Slovenia) – 2940 nm, scanning mode, 60 mJ, 10pps, 10 Hz, 10s, focal distance 12 mm, diameter 0.466 mm^2, water spray (0.34 ml/s). The ND group was treated with a Nd:YAG laser (Fotona, Twinlight, Slovenia) – 1640 nm, contact mode, 5J, 0.5W, 15 Hz, 10s. The PDT group was treated with Toluidine Blue O in deionized water(TBO – 100 μg/ml) for 60 s and irradiated with an InGaAlP red laser (Thera Lase – D.M.C. Equipamentos Ltda, São Carlos, Brazil) – 660 nm, 30 mW, 45J/cm^2, 30s, sweeping mode, 0.028 cm^2 spot area, 360J, 1.07W/cm^2. The CA group was treated with a gel containing 50% citric acid plus 10% tetracycline (pH1). After all treatments, fragments were washed with saline solution for 30s.

2.3. First experiment – conditioning of the medium with treated root fragments

Cells used in this study were human gingival fibroblasts (FGH lineage). This lineage was obtained by primary culture and was stored in liquid nitrogen. Cells were cultured in a conventional Dulbecco Modified Eagle's medium (DMEM) supplemented by 10% fetal bovine serum (FBS) and 1% antibiotic–antimycotic solution (Penicillin 10.000UI, streptomycin 0,050 g/L, Amphotericin B) in a humidified air–5% carbon dioxide (CO_2) atmosphere. Cells from the 6th passage were used for the experiment in a concentration of 2×10^3 cells/well in 96-well plates. The experimental groups were in sextuplicate.

A conditioned medium was prepared by submerging the treated root fragments (10/group) in conventional DMEM (supplemented by 10% FBS and 1% antibiotic–antimycotic solution (Penicillin 10.000UI, streptomycin 0,050 g/L, Amphotericin B). After preparation, the conditioned medium was maintained in incubator for 24 h following the ISO standard 10993-12 (International Organization for Standartization, 1996). After 24 h, root fragments were removed and the conditioned medium was used to substitute the conventional medium in the wells with the exception of CC group.

The cell viability was measured after 24, 48, 72 and 96 h by MTT assay. The MTT assay measures cell mitochondrial activity involving the conversion of the water-soluble 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to an insoluble formazan. The formazan is then, solubilized, and the concentration determined by optical density (OD) at ≅ 570 nm.

Results were statistically analyzed by a software Statistica 11.0 (www.statsoft.com/company). The Two-way ANOVA complemented by Tukey's test was applied ($p < 0.05$).

2. Second experiment – SEM analysis of cell growth on treated root surfaces

Human gingival fibroblasts were cultured as described above. Cells from the 6th passage were used for the experiment in a concentration of 10^4 cells/well in 24-well plates. The experimental groups were in triplicate. After treatment, the root fragments were sterilized and placed in 24-well plates. FGH cells were plated over the fragments and cultured in DMEM (supplemented by 10% FBS and 1% antibiotic–antimycotic solution) for 24, 48 and 72 h.

Samples were prepared for SEM analysis by fixation in Karnovsky

Download English Version:

https://daneshyari.com/en/article/5637973

Download Persian Version:

https://daneshyari.com/article/5637973

Daneshyari.com