Journal of Photochemistry & Photobiology, B: Biology 160 (2016) 172–177

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

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

In vitro inactivation of *Enterococcus faecalis* with a led device

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ARTICLE INFO

Article history:
Received 4 August 2015
Received in revised form 29 March 2016
Accepted 6 April 2016
Available online 16 April 2016

Keywords:
LED
Enterococcus faecalis
Endodontics
Sodium hypochlorite
Light therapy

ABSTRACT

Non-coherent light-emitting diodes (LEDs) are effective in a large variety of clinical indications; however, the bactericidal activity of LEDs is unclear, although the effectiveness of such lights is well known. Currently, no studies have examined the effects of NIR-LED on bacteria. The aims of this study were to verify the antibacterial activity of 880-nm LED irradiation on a bacterial suspension of *Enterococcus faecalis* and to compare it with the actions of sodium hypochlorite (NaOCl) and the concurrent use of both treatments. Before we proceeded with the main experiment, we first performed preliminary tests to evaluate the influence of such parameters as the distance of irradiation, the energy density, the irradiation time and the presence of photosensitizers on the antimicrobial effects of LEDs.

After treatment, the colony forming units per milliliter (CFU/mL) was recorded and the data were submitted to ANOVA and Bonferroni post hoc tests at a level of significance of 5%.

The results showed that LED irradiation, at the parameters used, is able to significantly decrease *E. faecalis* viability *in vitro*. The total inhibition of *E. faecalis* was obtained throughout concurrent treatment of LED and NaOCl (1%) for 5 min.

The same antimicrobial activity was confirmed in all of the experiments ($p < 0.05$), but no statistically significant differences were found by varying such parameters as the distance of irradiation (from 0.5 mm to 10 mm), energy density (from 2.37 to 8.15 mJ/s), irradiation time (from 5 min to 20 min) or by adding toluidine blue O (TBO).

1. Introduction

Enterococcus faecalis forms a part of the normal micro-flora of the human body, but relatively low counts are found in the mouth. It is a Gram-positive, non-motile, facultative anaerobic microbe [1]. *E. faecalis* can survive in extreme environmental conditions, such as acid or alkaline pH, high concentrations of salts and heavy metals as well as low nutrient concentrations. It can grow in the range of 10 to 45 °C and is resistant to a temperature of 60 °C for 30 min [2].

E. faecalis is known to invade dentinal tubules and to bind collagen. It has the ability to survive in root canals as a single body without the support of other bacteria and can resist a wide range of antibiotics and intra-canal drugs. It is highly virulent, producing lytic enzymes, cytolysin, aggregation substances, pheromones, and lipoteichoic acid and is able to alter the host responses and to suppress the actions of lymphocytes [3].

E. faecalis can be found in primary endodontic lesions and is involved in the pathogenesis of secondary endodontic lesions, despite the combined use of root canal irrigants, intra-canal medicaments and mechanical instrumentation during the root canal treatment [4,5].

Among irrigants, sodium hypochlorite is the most commonly used; it is effective against the endodontic flora with some tissue-dissolving properties. However, it is highly toxic, and its efficacy is susceptible to temperature, concentration, and exposure time [6].

These issues and the increasing problem of microbial antibiotic resistance have ignited the interest of researchers into the use of additives and/or alternative antimicrobial treatments [7].

Novel approaches for disinfecting root canals include the use of high-power lasers because the photosensitization of bacterial cells is independent of their antibiotic resistance spectrum [8,9]. However, lasers function by dose-dependent heat generation and, if incorrectly used, they have the potential to cause damage, such as dentin charring, ankylosis, cementum melting, root resorption, and periradicular necrosis [10].

On the contrary, non-coherent light-emitting diodes (LEDs) are safe, non-thermal, nontoxic and noninvasive, and to date, no side effects have been reported from their use [11].They are complex semiconductors that convert electrical currents into incoherent narrow spectrum light at wavelengths ranging from the ultraviolet (UV) to the visible to the near infrared (NIR). The bactericidal activity of LEDs is unclear,

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http://dx.doi.org/10.1016/j.jphotobiol.2016.04.015
1011-1344/

although the effectiveness of such lights is well known [12]. A possible mode of action has been explained in the literature through photon absorption mediated by endogenous (intracellular) sensitizers, such as porphyrins and flavins, or exogenous (extracellular) sensitizers, such as humic compounds [13]. Excited sensitizers transfer energy or electrons to other parts of the cell, causing damage, or to molecular oxygen, producing reactive oxygen species (ROS) that cause photooxidative damage [14]. Depending on whether energy or electrons are transferred to molecular oxygen, ROS, such as singlet oxygen, superoxide, hydrogen peroxide, and hydroxyl radicals, are formed and can damage membrane lipids, proteins, enzymes, or nucleic acids.

The effects of LED irradiation are dependent upon the wavelength, power density, quantity (or number) of bacteria, and microbial species [15]. For example, the wavelength influences the depth of penetration. Lights that operate at the near infrared spectrum (NIR) are characterized by a greater depth with respect to those characterized by an inferior wavelength [16]. When LED and LASER are used at very low intensities (low-level laser therapy, LLLT), the energy is enough to activate the target cells, but without a rapid or significant increase in tissue temperature [17]. LED-LLLT is effective in a large variety of clinical indications, such as pain attenuation, wound healing, skin rejuvenation, some viral diseases, allergic rhinitis, and other allergy-related conditions [18].

Currently, no studies have been published about the effects of NIR-LEDs used at low intensities (LLLT) on bacteria *in vitro*.

The aim of this study is to assess the antibacterial activity of LED irradiation and sodium hypochlorite (NaOCl) alone and then to evaluate the effects of the concurrent use of both treatments on *E. faecalis*. Before proceeding with the main experiment, we first performed preliminary tests to verify the influence of the parameters, such as the distance of irradiation (d), the energy density, the irradiation time and the presence of photosensitizers on LED activity.

2. Materials and Methods

2.1. Light Source and Irradiation Parameters

A NIR-LED device characterized by an 880 nm-wavelength was used as the light source (PhaseTech, Bergamo, Italy).

The hand-piece was constituted by 6 LEDs (12-mm diameter) disposed in two lines, Fig 1A. To simplify the comprehension of the methods used, we will refer throughout the text to the energy output (mJ/s) emitted by a single led. In all of the experiments, the LED hand-piece was mounted perpendicularly to the wells through the use of a particular polystyrene box to maintain a constant distance of irradiation (d), as shown in Fig 1B. Irradiation was performed under a laminar flow hood in the dark under aseptic conditions in all of the experiments.

2.2. Bacteria and Culture Conditions

E. faecalis ATCC 29212 was cultured at 37 °C for 24 h in Brain Heart Infusion (BHI) broth. The bacterial suspension was evaluated using a spectrophotometer (Agilent Technologies 8453 UV, Santa Clara, USA) to assure an optical density of 0.5 McFarland corresponding to 10^8 Colony Forming Units (CFU)/mL. *E. faecalis* solution was prepared for 25-well (dimension: 20 * 20 mm) flat-bottom plates with lids separately for several experiments (test).

Aliquots of 1 mL were dispensed in triplicate into micro-titer plates for each treatment group, and all of the tests included a positive control (C+) and a negative one (C−).

2.3. Test 1

Two groups, characterized by a different distance of irradiation (d), were tested: LED 5 (d = 10 mm) and LED 5C (d = 0.5 mm). Both groups were irradiated for 5 min at an energy output of 2.37 mJ/s, Fig 2.

2.4. Test 2

Two groups, irradiated by a different energy output (e), were tested: PROG A (e = 2.37 mJ/s) and PROG B (e = 8.15 mJ/s). Both groups were irradiated for 5 min at a constant distance d = 10 mm, Fig 3.

2.5. Test 3

Three groups were tested and characterized by a different irradiation time (t) IR 5 (t = 5 min), IR 10 (t = 10 min) and IR 20 (t = 20 min). Among these groups, another subcategory was characterized by the addition of toluidine blue before the LED irradiation (IR + TBO). Toluidine blue O (TBO) powder (Diapath S.p.A.—Italy) was prepared in deionized water and subsequently dissolved at concentration 25 μM. All irradiations were performed for 5 min at d = 10 mm at a measured energy output of 2.37 mJ/s, Fig 4.

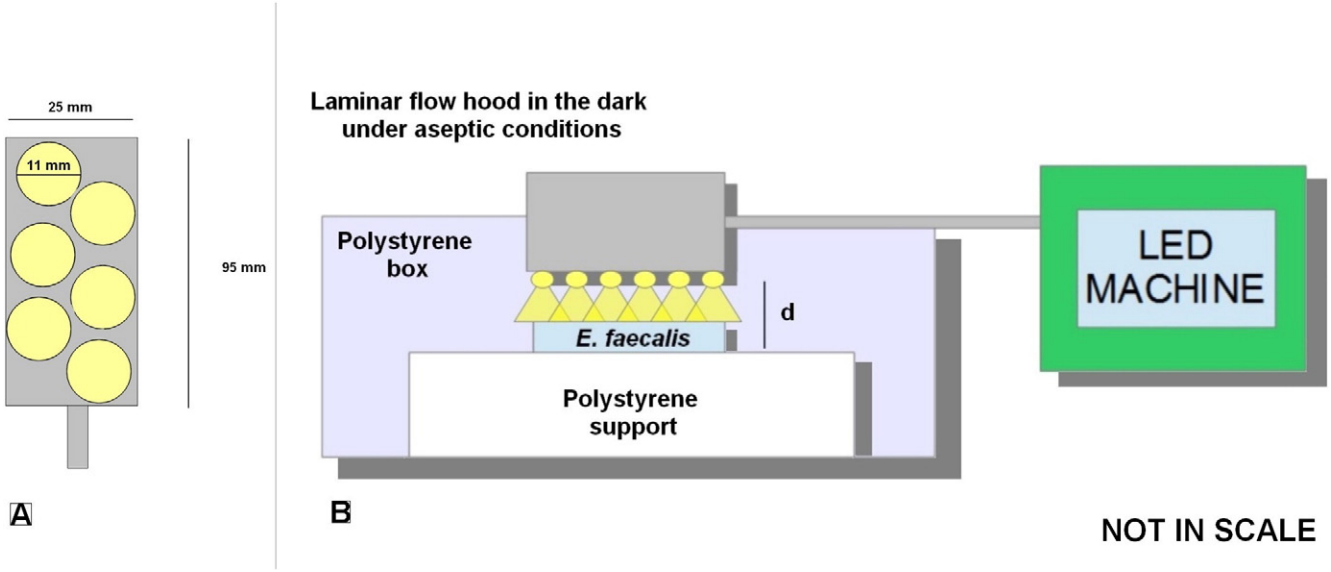

Fig. 1. Schematic illustration of the apparatus utilized to obtain uniform LED irradiation of the samples, maintaining a constant distance (d) from the LED bulbs to the samples.