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ORIGINAL ARTICLE

Comparative antifungal efficacy of light-activated disinfection and octenidine hydrochloride with contemporary endodontic irrigants

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Abstract The aim of this study was to evaluate the antifungal effects of light-activated disinfection (LAD) in comparison with contemporary root canal irrigation solutions: sodium hypochlorite and 2 % chlorhexidine gluconate and a new wound antiseptic, octenidine hydrochloride. Seventy extracted teeth having single root canals were contaminated with *Candida albicans* for 14 days. The samples were divided into five experimental (n=10) and two control (positive and negative) groups (n=10): (1) LAD with toluidine blue O, (2) octenidine hydrochloride (OCT), (3) 2.5 % sodium hypochlorite (2.5 % NaOCl), (4) 5.25 % sodium hypochlorite (5.25 % NaOCl) and (5) 2 % chlorhexidine. Five millilitres of each test solution was applied for 3 min, and irradiation time used for LAD was 30 s. After treatment, the dentin chips were collected from inner canal walls into vials containing phosphate buffered saline, vortexed, serially diluted, seeded on Tryptic Soy Agar plates and incubated (37 °C, 48 h). The number of colony-forming units was then counted. Differences between LAD group and positive control group were statistically significant (P<0.05). All *Candida* cells were totally eliminated in root canals irrigated with OCT, 2.5 % NaOCl, 5.25 % NaOCl and 2 % chlorhexidine groups (CFU=0). Within the limitations of this ex vivo study, LAD had minimal antimicrobial effect on *C. albicans* when used 30 s, and further modifications in LAD protocol are required to improve its antifungal capability. A new wound antiseptic, octenidine hydrochloride, demonstrated better potential than LAD in elimination of *Candida albicans* cells and may be a promising alternative to NaOCl and chlorhexidine solutions in future.

Keywords Irrigation solutions · *Candida albicans* · Octenidine · Light-activated disinfection · Photodynamic therapy · Toluidine blue

Introduction

The elimination of microbial infection from the root canal system is one of the crucial factors in the success of endodontic treatment [1]. Persistent microbial flora that cannot be removed by using contemporary antimicrobial agents frequently result in post-treatment disease [2, 3]. *Candida albicans* is one of the common members of the oral microbiota that have been isolated from obturated root canals in which treatment has failed [4]. The presence of *C. albicans* in persistent apical periodontitis may depend on its ability to penetrate deep into dentinal tubules and on its resistance to antimicrobial agents [5, 6].

Sodium hypochlorite (NaOCl) has been widely used as an endodontic irrigant due to its antimicrobial efficacy [7–9] and its organic tissue dissolution properties [10]. However, an alternative solution is still being researched because of NaOCl's high toxicity on host tissues [11], its bad smell and taste and its potential allergic effects [12]. Chlorhexidine gluconate has been suggested as an alternative to NaOCl due to its antibacterial activity, substantivity and relatively less toxicity [7]. On the other hand, the disadvantage of chlorhexidine is that it has no tissue dissolution capacity [13].

Octenisept is a new antiseptic having 0.1 % octenidine hydrochloride as an active component with potential usage for traumatic, acute, chronic and surgical or burn wounds, for mucous membrane disinfection and as a mouth rinse. Octenidine hydrochloride [*N*,*N*′-(1,10 decanediyldi-1[4H]-pyridinyl-4-ylidene)bis(1-octanamine)dihydrochloride] possesses broad

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spectrum antimicrobial effects against both Gram-positive and Gram-negative bacteria and fungi and some virus species [14, 15]. It has previously been tested as a root canal irrigant or medicament on *Enterococcus faecalis* and has been compared to some other antiseptics [16–19], but few studies have focused on the antifungal potential of this disinfectant [15, 17–19].

Light-activated disinfection (LAD), also called photodynamic therapy, functions with the action of light and a non-toxic photosensitiser. It was developed as a therapy for cancer and for localised microbial infections. The light-sensitive photosensitiser reacts with molecular oxygen to generate highly reactive oxygen species that are cytotoxic to cells of the target tissue [20]. Several studies have reported not only the antimicrobial effectiveness of LAD treatment ex vivo [21–25] and in vitro [26–28] but also its relatively lower toxicity to periodontal ligament fibroblasts than chlorhexidine [29].

There is no study in the literature comparing the antifungal effectiveness of LAD with the new promising antimicrobial octenisept solution. Therefore, the aim of this ex vivo study was to evaluate the effectiveness of LAD in comparison with OCT and other common irrigants such as NaOCl and chlorhexidine on *C. albicans* by using a modified dentin block model.

Materials and methods

Specimen preparation and infection

A total of 70 freshly extracted single rooted human teeth were stored in 0.5 % NaOCl for 2–4 weeks. Root surfaces were cleaned of any calculus or soft tissues. Specimens were decoronated to a standard 8-mm root length with a diamond bur (MANI Inc., Tochigi, Japan) under continuous water cooling. The root canal preparation was performed with ProTaper series (Dentsply Maillefer, Swiss made, Ballaigues) NiTi rotary files in a crown-down manner. The ProTaper files were used as Sx-F3 according to the manufacturer's instructions. The canals were irrigated with 1 mL of 1 % NaOCl solution (Caglayan Kimya, Konya, Turkey) between the instruments. After canal preparation, the smear layer was removed in an ultrasonic bath (Bandelin Sonorex, Berlin, Germany) with sequential use of 17 % ethylene diamine tetraacetic acid (EDTA) (pH 7.3) (Merck KGaA, Darmstadt, Germany) and 5.25 % NaOCl (Caglayan Kimya, Konya, Turkey) for 5 min each [30]. Subsequently, the root specimens were rinsed with distilled water and placed into vials containing phosphate-buffered saline (PBS; Sigma-Aldrich, St. Louis, MO, USA) solution and were autoclaved (HMC Hirayama, Saitama, Japan) at 121 °C for 15 min. Finally, the root canals were dried using sterile paper points (Gapadent CO, Hamburg, Germany).

A *C. albicans* strain was obtained (ATCC 90028, Refik Saydam Institute, Ankara, Turkey) and was plated on Brain Heart Agar (BHA) (bioMerieux, France) for 24 h at 37 °C. A loopful of this yeast was then transferred to 10 mL of Brain Heart Infusion (BHI) and was incubated at 37 °C for 48 h, and 0.5 aliquots of this suspension were added to 100 mL of BHI. After 12-h incubation, the suspension was spectrophotometrically adjusted to an optical density of 500 at 600 nm to obtain a standard fungal solution.

The root specimens were randomly divided into five experimental (n=10) and two control groups (n=10) and placed into separate tubes. All specimens except negative controls were infected with *C. albicans* suspension for 14 days at 37 °C, and the media was changed every second day with a new suspension having same optical density, to achieve sufficient *Candidal* growth.

Testing procedures

The antimicrobial agents used in each group and their manufacturers are given in Table 1. The canals were irrigated with 5 mL of each test solution in a standard way, using a 27-gauge needle reaching three quarters of the working length for 3 min in all irrigant groups. The canals were then dried with sterile paper points.

In the LAD group, the canals were treated according to the manufacturer's recommendations. In clinical practice, FotoSan™ is used in combination with a powerful red light with wavelength of 620–640 nm with a peak of 630 nm and a photosensitiser (FotoSan Agent). This agent contains 'toluidine blue O' at a concentration of 0.1 mg/ml dissolved in a 1 % xanthan gel as an active ingredient for catalysing the photochemical process (see Fig. 1a). The photosensitiser is available in low, medium and high viscosities, with low viscosity being recommended for endodontic treatments and used in the present study. A fibre optic tip (Endo tip) which is conic and which ends with a diameter of 0.5 mm was used in this study.

The root canals were filled with the photosensitiser agent, using a lentulospiral (MANI Inc., Tochigi, Japan) of size 30, to the level of the access cavity; they were inoculated for a fixed period of time (60 s) to allow the agent to come into contact with all of the surfaces. Subsequently, the endodontic fibre tip was placed as close as possible to working length with spiral movements from apical to cervical third of the root samples in order to permit adequate distribution of the light throughout the root canal. The irradiation was carried out for 30 s in each root canal according to the manufacturer's suggestion (Fig. 1b). Afterwards, the root canals were irrigated with 10 mL of sterile saline solution to remove the agent [22] and were dried with sterile paper points. All samples were stored in a freezer for 1 h at −27 °C before

Table 1 Experimental antimicrobial agents and their manufacturers according to the groups

Group fino	Antimicrobial agents	Company
Group 1	Light-activated disinfection (LAD) with toluidine blue O solution	FotoSan™, CMS Dental ApS, Copenhagen, Denmark Fotosan Agent®, CMS Dental ApS, Copenhagen, Denmark
Group 2	Octenidine hydrochloride (OCT)	Octenisept®, Schülke & Mayr, Germany
Group 3	2.5 % Sodium hypochlorite (2.5 % NaOCl)	Caglayan Kimya, Konya, Turkey
Group 4	5.25 % Sodium hypochlorite (5.25 % NaOCl)	Caglayan Kimya, Konya, Turkey
Group 5	2 % Chlorhexidine	Klorhex®, Drogsan, Turkey

obtaining the dentin powder, in order to avoid killing *Candida* cells due to the excessive heat created by the friction from using Gates Glidden burs on the dentin.

Sampling procedures

After 1 h of freezing at −27 °C, a 3-mm apical portion of the root samples was resected to eliminate differences arising from the apical delta and apical lateral canals [31]. The dentin powder was obtained from inner root canal lumen with the sequential use of sterile Gates Glidden burs (nos. 3, 4 and 5). Dentin samples were obtained directly over separated test vials containing 2 mL of PBS and glass beads. Vials were then vigorously shaken on a Vortex mixer (VWR, Bedfordshire, UK) for 30 s. PBS with resuspended *Candida* cells was serially diluted (1:10, 1:100, 1:1,000, 1:10,000), and two droplets of 25 μL from each of the four parallel dilutions were inoculated on BHA plates and incubated at 37 °C for 48 h [32]. All procedures were performed inside a laminar flow chamber using sterile instruments in order to achieve strict asepsis. A classical bacterial counting technique was used for recovery of viable *C. albicans*, and the number of visible colony-forming units (CFU) was determined for each sample.

Scanning electron microscopy

Seven more samples were incubated with *Candida* cells as in other root samples and were divided into seven groups and treated with one of the treatment groups mentioned above. Scanning electron microscopy (EVO LS10, Zeiss, Oberkochen, Germany) was used to visualise the establishment of fungal colonisation on dentinal walls after the various disinfection methods. Scanning electron microscopy (SEM) microphotographs were obtained at different magnifications (×1,500 to×5,000) in representative areas of the samples.

Statistical analysis

The CFU values were transformed to log10 values. Mean log10 CFU values with standard deviations were calculated. Levene's test was done to analyse the homogeneity of the variances. Statistical analysis was performed by SPSS 17.0

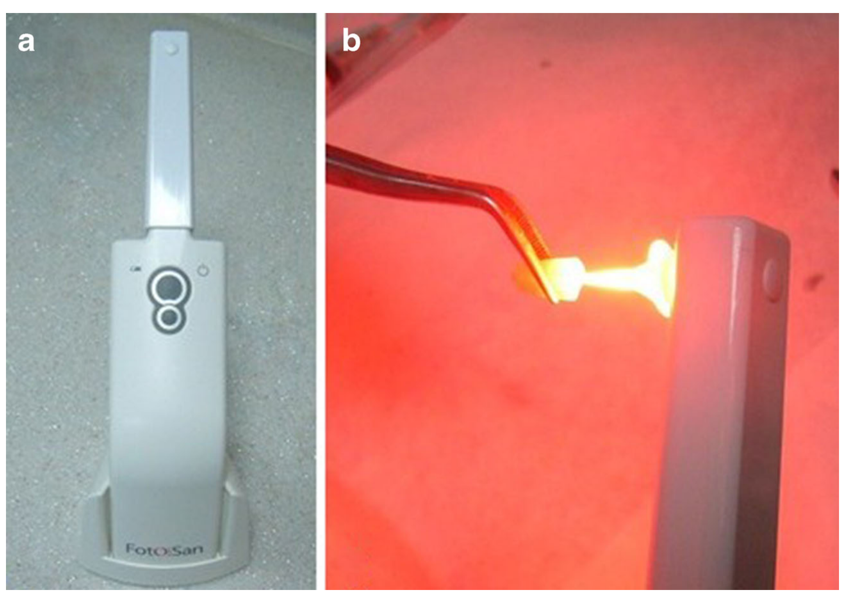

Fig. 1 **a** LAD was performed using a LED lamp emitting in the red spectrum with a peak frequency at 630 nm (FotoSan®). **b** The endodontic fibre tip was placed as close as possible to the working length of the root samples for adequate distribution of the light throughout the root canal

(SPSS Inc., Chicago, IL, USA), using an independent two-sample *t* test with assumed equal variances. The level of statistical significance was set at .05.

Results

The negative control group showed no growth after all procedures (CFU=0), whereas the positive control group yielded vigorous growth, confirming the yeast infection (log10 CFU=4.04±0.52). All specimens in the positive control group yielded positive cultures. The number of colony-forming units in the LAD group (log10 CFU=3.63±0.03) was lower when compared with the positive control group, and the differences between the two groups were statistically significant (P=0.009). The tested irrigants OCT, 2.5 % NaOCl, 5.25 % NaOCl and chlorhexidine groups totally eradicated all *Candida* cells (CFU=0; Fig. 2).

Scanning electron microscopy

SEM images of various test groups are shown in Fig. 3. SEM pictures of the positive control group showed sufficient *Candidal* growth on root canal walls after a 14-day infection period (Fig. 3a). Damaged fungal cells were seen covering the dentinal walls after LAD treatment (Fig. 3c). Total eradication of *C. albicans* was observed after irrigation with OCT, 2.5 % NaOCl, 5.25 % NaOCl and chlorhexidine (Fig. 3d–g). No fungal cells were visible in the negative control group (Fig. 3b).

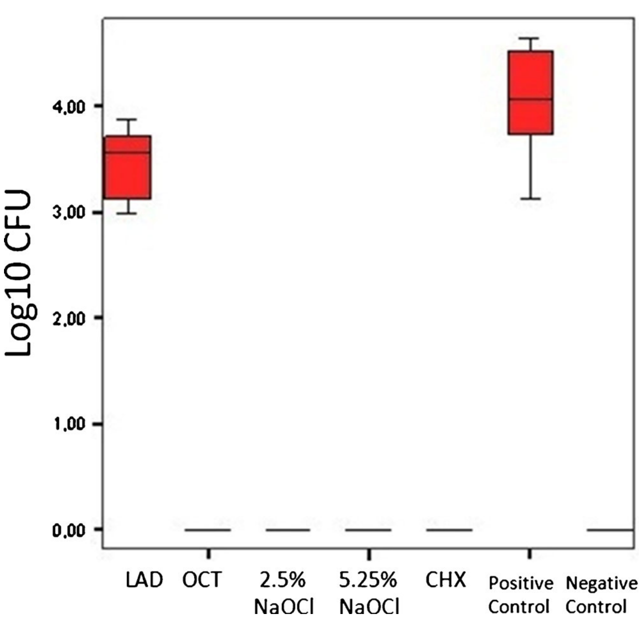

Fig. 2 Antifungal activity of LAD, OCT, 2.5 % NaOCl, 5.25 % NaOCl and Chlorhexidine groups on *C. albicans*. Recovered mean CFU counts from various experimental groups

Discussion

The absorbent paper cones method [22, 24, 25], one of the conventional culturing methods, has limitations because the paper points can only detect planktonic microorganisms. The paper points also cannot access irregularities and other regions of the root canal system. As a result, this method might fail to harvest viable bacteria in biofilms or bacteria that reside within the dentinal tubules [33]. Thus, the dentin-powder model developed by Haapasalo and Ørstavik [30], with some modifications, was preferred because of the reported good correlation between histology and the culturing of dentin dust in their study. That study was also later confirmed by Peters et al. [1], who showed that the grinding and culturing of dentin gave better quantitative information about the extent of the infection. Consequently, we used Gates Glidden burs at low speed to remove dentin from the root canal walls and dentinal tubules, allowing more predictable sampling.

C. albicans was chosen as a test microorganism in the present ex vivo study because of its pathogenic characteristics such as binding to dentin collagen and invasion to deeper dentinal tubules, biofilm formation, and its activation of host defences as well as for its resistance to antimicrobial agents used in endodontics [4–6]. It was also proved that the fungal cells could be found in the resorption lacunae of periapical root surfaces and also in periapical granuloma [4].

No deactivating agent was used to reduce the carry-over effect of the disinfectant solutions, not only because of a lack of any "universal" neutraliser agent appropriate for all the disinfectants which were tested but also due to the limited carriage possibility of the disinfectants, while sampling with Gates Glidden burs, because of their evaporation. Some of the previously suggested neutralising agents, such as 0.6 % sodium thiosulfate for NaOCl; 3 % Tween 80 (a detergent) and 0.3 % Lecithin for chlorhexidine; and 3 % Tween 80, 0.3 % Lecithin and 0.1 % Cystein for OCT [18], have various antimicrobial effects which might also deteriorate the results [34], and therefore, all groups were tested under same conditions in the present study.

LAD has been suggested for the endodontic treatment protocols owing to its root canal disinfection ability [22–25, 35] and the fact that its cytotoxic effects on host tissues are lower than those of NaOCl and chlorhexidine solutions [29]. However, it is difficult to compare the results of this study with previous studies that tested the antimicrobial activity of LAD because of the differences of type and concentration of the photosensitiser, the agitation of the photosensitiser, the photosensitiser incubation period before the light irradiation, the exposure period and the density of laser energy. In this study, LAD treatment was applied to the infected root canals according to the manufacturer's instructions suggested for endodontic treatment. Nevertheless, the LAD group was the only group that

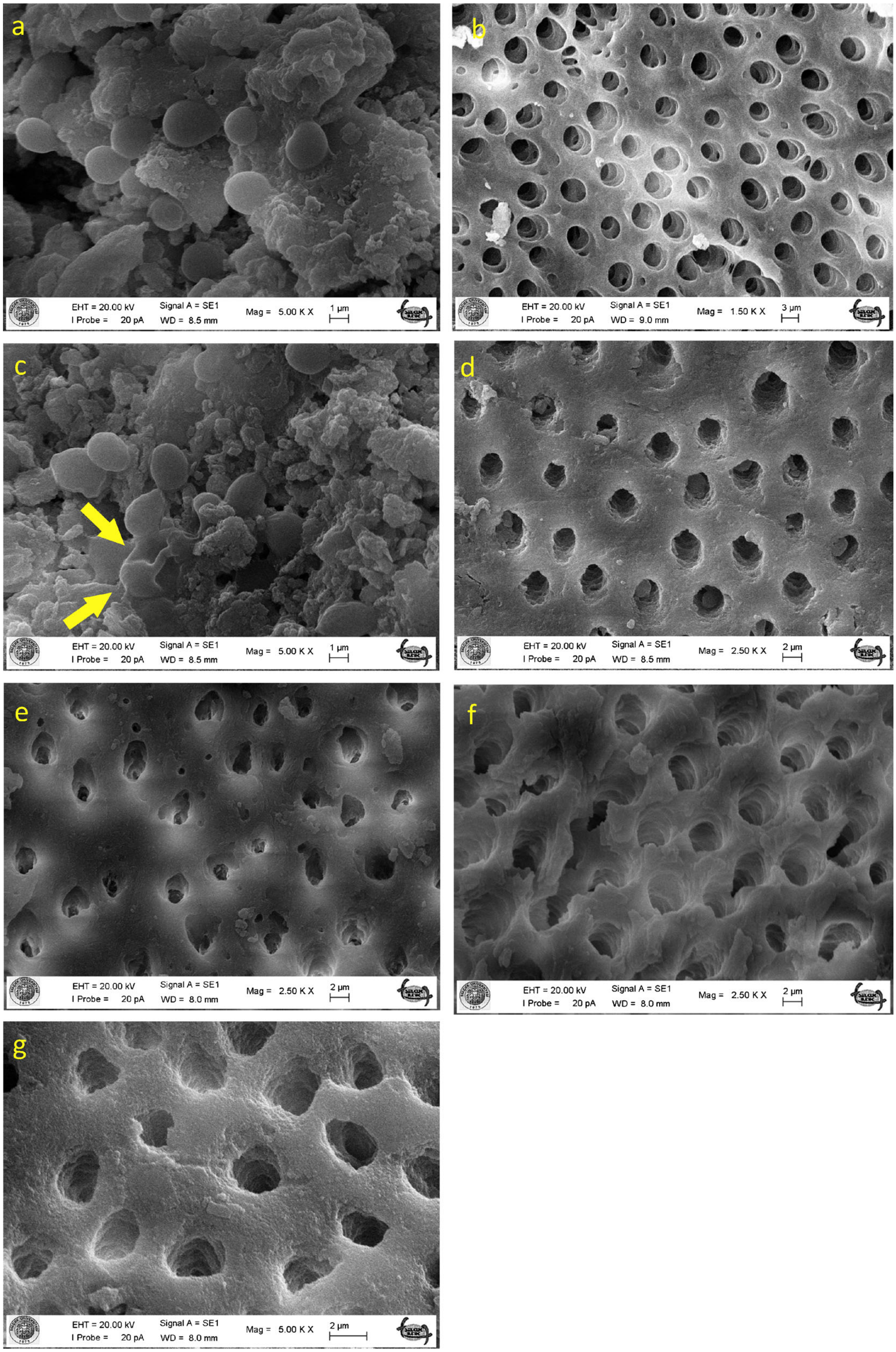

Fig. 3 Scanning electron microscopic micrographs of root canal dentin. **a** Positive control group; note the dense fungal colonisation on dentinal walls (original magnification ×5,000); **b** negative control group; presence of no fungal cell (original magnification ×1,500); **c** dentin section treated with LAD; *arrows* denote the damaged fungal cells (original magnification ×5,000); **d** OCT group; **e** 2.5 % NaOCl group; **f** 5.25 % NaOCl group; no fungal cells are visible (original magnification ×2,500); **g** chlorhexidine group; no apparent fungal cells (original magnification ×5,000)

did not succeed in fungal elimination in agreement with the reports of some previous researchers [25, 28]. The limited irradiation time of 30 s (as per the manufacturer's instructions) could be effective on bacteria such as *Streptococcus intermedius*, *Escherichia coli*, *E. faecalis* and *Fusobacterium nucleatum*, but it seems that the irradiation time may not be sufficient for the total elimination *of Candida* cells as Schlafer and colleagues [24] reported. These researchers showed in their additional experiments that a higher antifungal effect could be achieved when the irradiation time was prolonged from 30 to 120 s.

Additionally, unsatisfactory results found in our study might have occurred due to a greater resistance of *C. albicans* to the LAD treatment than that of bacteria [24, 28, 36]. The presence of a nuclear membrane in the structure of fungi and also the greater cell size and reduced concentration of singlet oxygen may result in a stronger resistance of *C. albicans* to photoinactivation [28]. Also, the photosensitiser agent might not have diffused well into the dentinal tubules and may not have achieved direct contact with the fungi on the root canal walls, especially in tubules and irregularities, when compared with the test irrigants. These findings do not necessarily mean that the LAD system cannot be beneficial in fungal elimination from infected root canals, but they emphasise the fact that further research is necessary to establish the appropriate laser parameters and application time for endodontic treatment protocols.

Octenisept is composed of 0.1 g octenidine dihydrochloride and 2.0 g 2-phenoxyethanol, a derivate of ethanol. Although the concentration of phenoxyethanol is higher than octenidine in solution, it has been proposed that octenidine itself is the active agent and provides the antimicrobial property of OCT [16]. Furthermore, the resistance of OCT to blood, albumin and mucin [17] and its constant efficacy in the presence of organic matter [37] are known from medical literature. It has previously been shown that not only does the presence of such organic materials in the root canal system have an inhibitory effect on antimicrobial agents but also that the dentin, composed of organic and inorganic materials, may cause a reduction in antimicrobial efficacy [38]. With the experimental procedure described above, it may be assumed that OCT could retain its high antimicrobial properties due to its resistance against the organic substances present in the dentin structure, and this warrants further investigation. The manufacturer of octenisept does not specify any usage for endodontic disinfection and only recommends a 2-min wash of the mouth when OCT is used for rinsing purposes. We preferred to use a 3-min irrigation time with this solution in the root canals due to the difficulty in reaching the inner part of the dentinal tubules.

To our knowledge, there is no study in the literature about allergic reactions of the relatively new disinfection solution, octenisept, on gingiva and oral tissues. The allergenic potential is classified as low, based on the data obtained from a previous animal study [15]. Some eczematous reactions were reported related to octenidine treatment from 2 to 8 weeks in 11 of 251 patients (4.4 %) who had chronic wounds [39]. Previously, some clinical cases were published about allergic reactions to sodium hypochlorite [40–43], but there is no data about the percentage of these reactions in the literature.

Within the limitations of this ex vivo study, the LAD protocol suggested for endodontic treatment exhibited minimal antifungal activity. Therefore, further modifications in the LAD protocol may be required to enhance the capacity of fungal elimination. In contrast, OCT showed the potential for use as an effective fungicidal agent for patients in whom fungal infection is suspected.

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