Original Research
Dental Materials

Effect of alpha-humulene incorporation on the properties of experimental light-cured periodontal dressings

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Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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https://doi.org/10.1590/1807-3107bor-2022.vol36.0091

Submitted: May 10, 2021
Accepted for publication: March 7, 2022
Last revision: April 12, 2022

Abstract: The objective of this study was to formulate an experimental light-cured periodontal dressing containing alpha-humulene and to compare its physical, antimicrobial, and cytotoxicity properties with commercial gold standards (Barricaid® and Periobond®). Two periodontal dressing formulations were developed (a and b). The formulations were divided into 5 groups according to the alpha-humulene concentration as follows: Ea - control group, Ea_1 - 1%, Ea_5 - 5%, Ea_{10} - 10%, and Ea_{20} - 20%; Eb - control group, Eb_1 - 1%, Eb_5 - 5%, Eb_{10} - 10%, and Eb_{20} - 20%. Materials characterization was performed using the degree of conversion, cohesive strength, sorption, and solubility assays. Antimicrobial assay was performed using the modified direct contact test against *E. faecalis* and *S. aureus*. Cytotoxicity was assessed by the cell viability experiment using L929 fibroblasts. In general, the cohesive strength values of materials decreased as the alpha-humulene concentration increased. All the experimental dressings showed antimicrobial activity against both bacteria tested. Cell viability results for the Ea, Ea_1, Eb, and Eb_1 groups showed moderate cytotoxic effect. The formulations containing alpha-humulene showed similar behavior to the commercial references. Thus, formulations containing alpha-humulene have potential to be used as periodontal dressing.

Keywords: Anti-Bacterial Agents; Periodontal Dressings; Cell Survival.

Introduction

Periodontal dressings are materials that provide wound protection against trauma, hemorrhage, contamination, food impaction, and pain relief after periodontal surgical procedures, facilitating the healing process.[1] In non-surgical procedures, the use of periodontal dressings has been shown to be helpful in aggressive periodontitis treatment.[2]

The first dental material described for use as periodontal dressing was zinc oxide eugenol, as reported by Ward in 1923.[3] Over the years, the use of eugenol-containing materials was found to be related to allergic reactions, inflammation, delayed wound healing, and inhibition of fibroblast proliferation.[4] To overcome the problem, other periodontal dressings based on cyanoacrylate, collagen, or light-curing monomers have been introduced.[5]

Ideally, periodontal dressings should exert an antimicrobial effect against oral bacteria.[6] Thus, several agents have been added to the composition of periodontal dressings such as eugenol, tetracycline, zinc bacitracin, chlorothymol, bergamote oil, and chlorhexidine.[7] Despite the efficiency of such agents, it is important to note that chemical inactivation of the added active substances may occur during the healing process.[8] In addition, some drugs could induce staining of teeth,[9] or irritate oral mucosal tissues, inducing tissue necrosis.[4] Therefore, other alternatives should be sought.

Alternative medicine has been used as an attractive approach for the relief of post-surgery adverse effects such as inflammation, infection, and pain. Local application of phytotherapy as an adjunct to scaling and root planning has been shown to provide additional benefits in reducing pocket depth and increasing clinical attachment.[11] Alpha-humulene oil is one of the major compounds found in plants such as *Cordia verbenacea*,[11] *Santiria trimera*,[12] *Solanum macranthum*,[13] among others. Antimicrobial, analgesic, and anti-inflammatory therapeutic properties are attributed to alpha-humulene.[14] This compound has been shown to be effective against Gram-positive oral pathogens involved in the development of dental caries and persistent endodontic infection, as well as in Gram-negative bacteria involved in the development of periodontal disease.[15-17] One of the advantages of this oil is that it does not cause gastric inflammation, as most synthetic anti-inflammatories do.[18]

To the best of our knowledge, alpha-humulene has never been incorporated in a periodontal dressing. Considering the benefits that this compound may have on the healing process, it is worth testing its effect on a periodontal dressing. Hence, the aim of this study was to evaluate the effect of the incorporation of different concentrations of alpha-humulene on the physical-chemical properties, antimicrobial activity, and cell viability of experimental light-cured periodontal dressing materials. The null hypothesis to be tested is that the alpha-humulene incorporation does not alter the chemical, mechanical, cytotoxic, and antibacterial activity of the experimental light-cured periodontal dressing materials.

Methodology

Experimental design

This *in vitro* study investigated the effect of the factor 'alpha-humulene concentration' (4 levels: 1, 2, 5, and 10% wt) on the physical-chemical properties, antimicrobial activity, and cell viability of two experimental light-curing periodontal dressing materials. The control groups were the periodontal dressing materials without the alpha-humulene. Barricaid® (Lot 160624, Dentsply Caulk, York, USA) and Periobond® (Lot 132214H, Denstply, Petrópolis, Brazil) were used as reference materials. In total, 10 experimental (Ea, Ea_1, Ea_5, Ea_{10}, Ea_{20}, Eb, Eb_1, Eb_5, Eb_{10}, and Eb_{20}) and 2 commercial periodontal dressings were evaluated. The primary response-variable was CFU/mL. The sample size (n = 3 per group) was estimated based on the data of a previous study that evaluated the antibacterial activity of resin-based endodontic sealers to *Enterococcus faecalis*.[19] Secondary response variables related to the characterization of the periodontal dressing materials were the degree of C=C conversion (n = 3),[19] cohesive strength (n = 5),[20] water sorption and solubility (n = 10),[21] and cell viability (n = 4).[21]

The dressing materials were formulated according to Table 1. For the formulation of Ea material, a mixture of Exothane® 32 (Esstech Inc, Essington, USA), polypropylene glycol monomethacrylate, and dodecanodiol dimethacrylate was prepared. In the case of Eb material, only Exothane® 32 was used. For both groups, a binary photopolymerization system of camphorquinone and 4-diethylaminobenzoate in a concentration of 0.4 and 0.8% wt respectively, was used. Finally, 15% wt of silica was added using a high-speed mixer (SpeedMixer™ DAC 150.1 FV, FlackTek Inc., Landrum, USA). After mixing, all the materials were ultrasonicated for 60 minutes and maintained in a dark environment until their use.

For the reference materials, all the specimens were prepared according to the manufacturers' instructions. For Barricaid® and the experimental materials, the samples were irradiated on both sides for 20 s using the Ultra Radii® (SDI, Australia) light curing unit with an intensity of

Table 1. Distribution and composition of the experimental groups.

Group	Formulation							
	% wt							
	EXO	PPGM	DDM	CQ	DHHPT	EDAB	Silica	α-humulene
Ea								-
Ea_1								1
Ea_5	60	25	15	0.4	1	0.8	15	5
Ea								10
Ea								20
Eb								-
Eb_1								1
Eb_5	100	-	-	0.4	1	0.8	15	5
Eb_{10}								10
Eb_{20}								20

EXO: Exothane® 32; PPGM: Polypropylene glycol monomethacrylate; DDM: Dodecanodiol dimethacrylate; CQ: Camphorquinone; DHEPT: N,N-Dihydroxyethyl-p-Toluidine. Purchased from Esstech Inc, Essington, USA. EDAB: Ethyl 4-dimethyl-aminebenzoate. Purchased from Fluka, Buchs, Switzerland. Silica (7nm, Aerosil 380, Degussa, GER). α-humulene (Sigma-Aldrich, Saint Louis, USA).

900 mW/mm^2. For Periobond®, equal parts of base and accelerator were mixed until a homogeneous paste was obtained.

Degree of conversion

The degree of double bond conversion of the experimental materials and the Barricaid® (n = 3) was determined using Fourier transformed infrared (FTIR) spectroscopy (Prestige 21 spectrometer Shimadzu Corporation, Kyoto, Japan), equipped with an attenuated total reflectance attachment incorporating a horizontal diamond crystal with a 45° mirror angle (PIKE Technologies, Madison, USA). The LED curing unit was rigidly held in position with its tip placed parallel with the sample area, enabling standardization of the distance between the fiber tip and the top of the sample at 5 mm. Infrared analysis was performed at a controlled room temperature of 23°C (± 2°C) and 60% (± 5%) relative humidity. Approximately 50 mg of each sample was dispensed directly onto the diamond crystal to evaluate the degree of conversion. The spectra of uncured and cured material were obtained after 30 seconds of photoactivation. FTIR spectra were acquired between 1690 and 1.575 cm^{-1} wavenumber, averaging 12 scans using a 4 cm^{-1} resolution into absorbance mode. The degree of conversion for each material was calculated according to a formula described elsewhere.[22]

Cohesive strength

Bulb-shaped specimens (10 mm long × 10 mm wide × 1 mm thick) with a 1-mm^2 constriction were made using a metal mold (n = 5). Periobond® was not included in this analysis because the light-cured material is brittle, and it was not possible to build specimens for this type of essay. After the preparation, the specimens were immersed in distilled water for 24 hours at 37°C. The samples were measured at the constriction and fixed in a metallic device with a cyanoacrylate-based adhesive (Superbonder, Gel, Locitite, São Paulo, Brazil), positioning the constriction parallel to the traction loading axis. The specific metallic device was coupled to a universal mechanical testing machine (DL-500, Emic, São José dos Pinhais, Brazil) at a crosshead speed of 0.5 mm/min until fracture. The cohesive tensile strength value was calculated by dividing the maximal load at failure by the cross-sectional area, determining the maximum strength in MPa.

Water sorption and solubility

Cylindrical specimens (n = 10; 6 × 1 mm) were prepared using a silicon mold. Then, the specimens

were disposed in a desiccator containing freshly dried silica gel and calcium chloride and stored at 37ºC. Their weight was monitored daily on a precision scale with 0.01 mg accuracy (AUW 220D, Shimadzu; Kyoto, Japan) until a constant mass (m_1) was achieved, which was considered when the variation of two weights was less than 0.1 mg. After that, the specimens' diameter and thickness were measured to obtain the volume of each specimen (V). Then, the specimens were immersed in distilled water and stored at 37ºC. After 7 days, the specimens were removed from water, dried with a paper towel, and weighed (m_2). Then, the specimens were stored again in a desiccator at 37ºC and their weight monitored until their stabilization (m^3). The water sorption and solubility were calculated according to the formula described in ISO 4049.[24]

Antimicrobial assay

The antimicrobial assay was performed by the modified direct contact test against *Enterococcus faecalis* ATCC 4083 and S*taphylococcus aureus* ATCC 19095. *E. faecalis* and *E. aureus* were cultured overnight at 37°C in tryptic soy agar (TSA) and brain heart infusion agar (BHI) plates, respectively, in an aerobic atmosphere. The strain was inoculated in tryptic soy broth (TSB) and BHI broth, and the bacterial turbidity was adjusted to an optical density of 0.5 at 600 nm for each strain. Cylindrical specimens (n = 3; 6 × 1 mm) from each material were prepared and 20 µL of the bacterial suspension was subsequently placed above the specimens' surface. Strain suspensions (20 µL) were placed in wells without specimens and served as non-exposed controls (+Control, positive control). Materials incubated without bacteria served as negative controls. All the samples were incubated aerobically for 1 and 24 hours at 37°C in > 95% humidity; then, 180 mL of TSB or BHI broth were added and gently mixed with a pipette for 1 minute. Microbial suspensions were serially diluted using saline solution, placed onto TSA or BHI agar, and incubated in an aerobic environment for 24 hours at 37°C. The colony forming units (CFU) were counted and CFU/mL was calculated.[22]

Cell viability assay

Cell viability of mouse fibroblasts (L929) was performed according to ISO 10993:5 [25] using extracts of the test samples prepared in compliance with ISO 10993-12.[26] Disk specimens of each material (n = 4) were prepared and stored in 300 µL of Dulbecco's Modified Eagle Medium (DMEM) for 24 hours. After incubation, the conditioned DMEM was expected to contain the eluate released from the specimens. The mouse fibroblast cell line was cultured at a density of 2×10^3 cells in 96-well plates containing DMEM media supplemented with 10% L-glutamine, 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 U/mL). Cells were incubated at 37°C under 95% air and 5% CO_2 for 24 h. The culture medium was replaced with equal volumes (200 µL) of the conditioned DMEM that contained the eluate from each specimen and the plate was incubated (37°C, 5% CO_2) for 24 h. Cytotoxicity was assessed after 24 hours using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolidium bromide; Sigma-Aldrich, St. Louis, Montana, USA). The formazan content of each well was computed as a percentage of the control group (untreated cells).

Statistical analysis

The data were analyzed to verify the assumptions of normal distribution and homogeneity of variance. Eb and Ea materials were evaluated separately. A two-way ANOVA followed by Student Newman Keuls (SNK) post-hoc test was used to evaluate the effect of the independent variables (group and incubation time) on the CFU/mL. Cell viability, degree of conversion, cohesive strength, water sorption, and solubility were analyzed through a one-way ANOVA followed by a Tukey post-hoc test. All the analyses were performed using SigmaPlot 12.0® (Systat Software, Inc., Point Richmond, USA), considering $p < 0.05$ to be statistically significant.

Results

Mechanical-chemical assays

Results from the degree of conversion, cohesive strength, water sorption, and solubility are shown in Table 2. Compared to the material without

Table 2. Degree of conversion (DC), cohesive strength (CS), water sorption (WS) and solubility (SL) of the tested periodontal dressing. Data are reported as mean (standard deviation) values.

Groups	DC	CS	WS	SL
	(%)	(MPa)	(μg/mm³)	(μg/mm³)
Ea	52.0 (2.0)^{b}	2.7 (0.3)^{ab}	69.1 (18.4)^{b}	65.1 (17.8)^{b}
Ea_1	52.2 (2.2)^{b}	2.1(0.4)^{b}	65.2 (4.9)^{b}	59.3 (5.3)^{b}
Ea_5	46.2 (1.8)^{bc}	1.8 (0.2)^{c}	77.6 (12.5)^{ab}	102.3 (12.1)^{a}
Ea_{10}	40.5 (2.3)^{cd}	1.2 (0.2)^{d}	113.9 (84.5)^{a}	106.7 (14.2)^{b}
Ea_{20}	35.6 (1.5)^{d}	0.5 (0.2)^{e}	112.7 (23.1)^{ab}	153.2 (26.1)^{a}
Barricaid®	94.9 (4.2)^{a}	3.0 (0.5)^{a}	48.8 (12.5)^{b}	10.1 (2.1)^{c}
Periobond®	-	-	115.8 (14.5)^{ab}	145.3 (19.6)^{b}
Eb	93.9 (1.7)^{a}	2.6 (0.3)^{a}	47.8 (8.5)^{c}	4.8 (1.6)^{d}
Eb_1	96.3 (0.7)^{a}	2.5 (0.4)^{a}	51.3 (6.5)^{c}	6.2 (2.6)^{d}
Eb_5	94.7 (2.1)^{a}	1.7 (0.2)^{b}	66.3 (8.7)^{b}	12.2 (4.5)^{c}
Eb_{10}	96.4 (1.9)^{a}	1.5 (0.3)^{b}	64.2 (6.2)^{b}	24.1 (2.4)^{b}
Eb_{20}	96.5 (3.9)^{a}	0.6 (0.1)^{c}	46.9 (8.1)^{c}	18.4 (2.6)^{b}
Barricaid®	94.9 (4.2)^{a}	3.0 (0.5)^{a}	51.4 (14.4)^{c}	10.1 (2.1)^{c}
Periobond®	-	-	110.3 (22.0)^{a}	145.3 (19.6)^{a}

Different lowercase letters (comparisons in the same column) indicate statistically significant differences between means ($p < 0.05$).

alpha-humulene, the incorporation of 5, 10, and 20% wt of alpha-humulene significantly decreased the degree of conversion of the Ea periodontal dressing material ($p < 0.05$). On the other hand, the incorporation of alpha-humulene into the Eb formulation did not affect its degree of conversion ($p > 0.05$). There were no significant differences among Eb groups and Barricaid® ($p > 0.05$).

The incorporation of alpha-humulene at 5, 10, or 20% wt significantly reduced the cohesive strength of Ea and Eb formulations ($p < 0.05$). Compared to Barricaid®, the values of the cohesive strength of Ea and Eb periodontal dressings, without the incorporation of alpha-humulene, were statistically similar ($p > 0.05$).

The incorporation of 10 and 20% wt. of alpha-humulene significantly increased both the water sorption and solubility of Ea and Eb periodontal dressings ($p < 0.05$). Among the commercial references, Periobond® achieved significantly higher values ($p < 0.05$).

Antimicrobial assay

Figure 1a-b shows the CFU/mL count of *S. aureus* after direct contact with the specimens. Compared to the positive control, after 1 and 24 hours of incubation, all the experimental materials significantly reduced the CFU/mL ($p < 0.001$). For both Ea and Eb materials, the incorporation of alpha-humulene at 10 and 20% wt promoted the highest antibacterial activity ($p < 0.05$). Of the commercial materials, only Barricaid® significantly reduced the CFU/mL after 1 and 24 hours of incubation ($p < 0.05$). The differences for the CFU/mL between 1 hour and 24 hours were statistically significant for all the groups.

Figure 2a-b shows the antimicrobial activity of the experimental and commercial periodontal dressings against *E. faecalis*. Compared with the control, after 1 and 24 hour incubation, all the experimental and commercial materials significantly reduced the CFU/mL ($p < 0.001$). The commercial materials did not present antimicrobial activity after 24 hours of incubation ($p > 0.05$). The differences in CFU/mL between 1 hour and 24 hours were statistically significant for all groups ($p < 0.05$).

Cell viability assay

Cell viability of L929 cells cultured in the conditioned medium from the different materials is summarized in Figure 3a-b. The incorporation

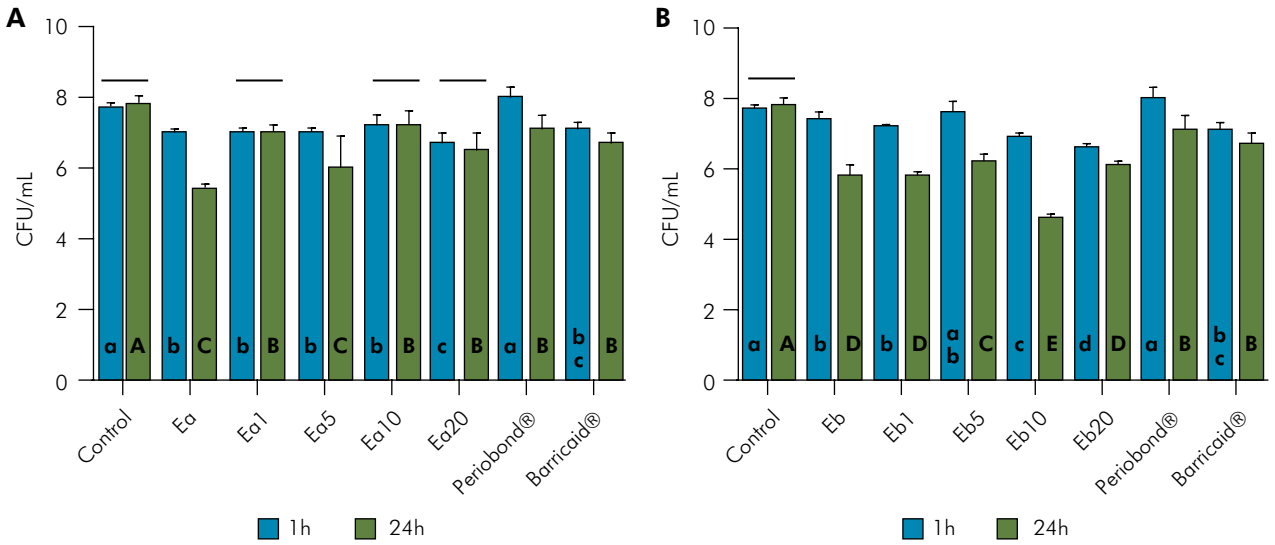

Figure 1. Modified direct contact test against *S. aureus* at 1 and 24 hours. For each material (Ea or Eb), different lowercase letters indicate significant differences for CFU/mL at 1 h incubation ($p < 0.05$); different uppercase letters indicate significant differences for CFU/mL at 24 h incubation ($p < 0.05$); columns under the same horizontal line indicate no differences within the incubation time for each group.

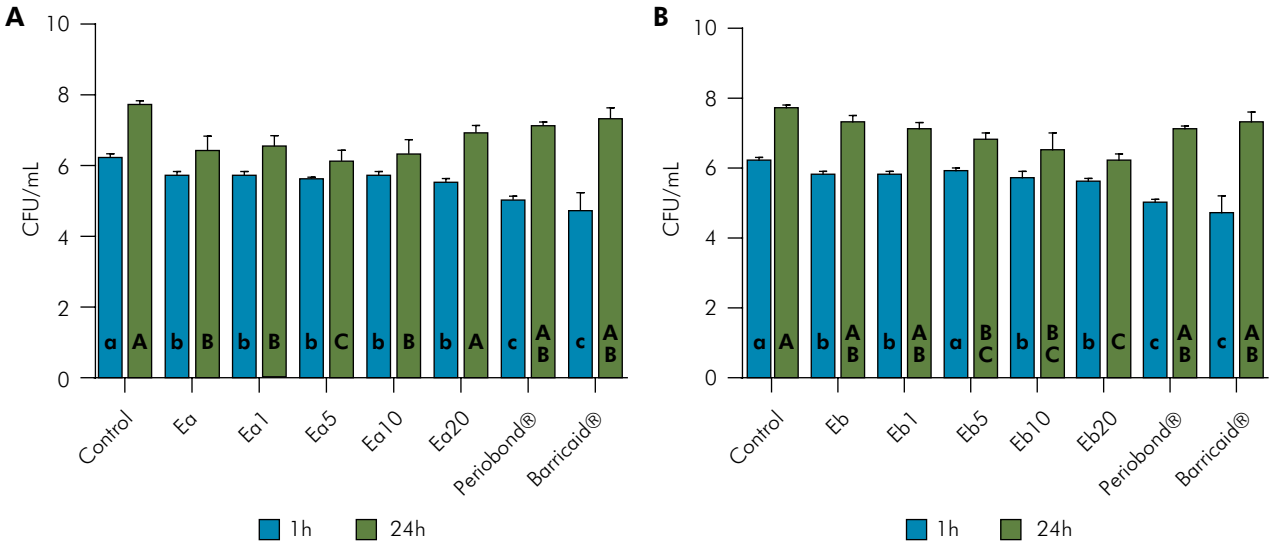

Figure 2. Modified direct contact test against *E. faecalis* at 1 and 24 hours. For each material (Ea or Eb), different lowercase letters indicate significant differences for CFU/mL at 1 h incubation ($p < 0.05$); different uppercase letters indicate significant differences for CFU/mL at 24 h incubation ($p < 0.05$); columns under the same horizontal line indicate no differences within the incubation time for each group.

of alpha-humulene at 5, 10, or 20% wt promoted a significant cytotoxic effect for both Ea an Eb formulations ($p < 0.05$). When analyzing the commercial reference materials, Barricaid® was the most cytotoxic material.

Discussion

In this study, different experimental light-cured periodontal dressings were formulated and their antibacterial, biological, and chemico-

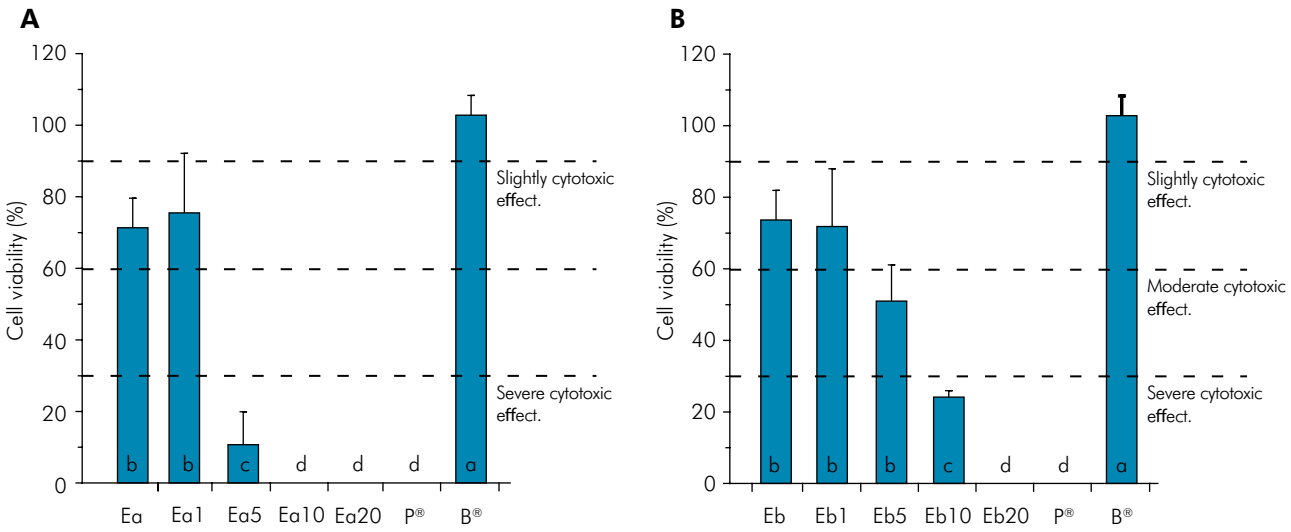

Figure 3. Cell viability of L929 fibroblasts after exposure of eluates to different periodontal dressings. Different lowercase letters indicate statistically significant differences among groups ($p < 0.001$). Dotted lines indicate the percentages of cell viability considering a material to exert a slight (<90%), moderate (<60%) or severe (<30%) cytotoxic effect.

mechanical properties were evaluated after the incorporation of four different concentrations of alpha-humulene. The results obtained suggested that the incorporation of different concentrations of alpha-humulene significantly affected the properties of the experimental materials evaluated, therefore, the null hypothesis tested was rejected.

Only a limited number of studies has evaluated the physical and mechanical properties of periodontal dressings. Actually, there are no current standardized reproducible techniques to evaluate their properties.[27] Considering the most recent findings, it would be very interesting to establish quality standards (properties) that an ideal periodontal dressing must have. Adequate working time, low surface roughness that does not cause mechanical trauma and avoids food retention, adequate elastic modulus to resist distortion and displacement, satisfactory adhesiveness, and adequate dimensional stability to prevent contamination and plaque accumulation are some of the ideal properties of periodontal dressings.[28]

The reaction of the carbon-carbon double bonds (C = C) in the functional monomer methacrylate groups is important for the analyses of mechanical and physical properties of resin-based materials used in dentistry.[29] Among the experimental materials, the incorporation of alpha-humulene significantly reduced the degree of conversion only for the Ea materials ($p < 0.05$), while this effect was not observed for the Eb materials ($p > 0.05$). Ea materials were formulated using a mixture of Exothane® 32, polypropylene glycol monomethacrylate, and dodecanodiol dimethacrylate, while for the Eb material, only the Exothane® 32 monomer was used. Considering that alpha-humulene acts a plasticizer, and therefore, a reduction in the degree of conversion was expected,[30] it seems that its effect is less pronounced when an homopolymer is used. Despite this, it seems that the reduction in the degree of conversion observed in the Ea formulation does not have a significant effect in cell viability, and therefore, its use would not have any disadvantage from a clinical point of view.

The maximum cohesive strength under tensile force was evaluated.[32] This mechanical property is important since adequate mechanical properties are highly desirable in a periodontal dressing to protect the wound.[32] The experimental dressing groups Ea and Eb base materials showed similar results to Barricaid®, which in turn, indicates that the experimental materials would have a similar clinical performance. Despite this, the incorporation of alpha-humulene significantly impaired the mechanical properties of both Ea and Eb formulations ($p < 0.05$). This result could be due to the fact that as alpha-

humulene does not copolymerize with the base material, it would work as an impurity that reduces the material mechanical properties,[33] especially at concentrations as high as 10 and 20% wt. Periobond® was not used in this assay because its polymerization process is based on chemical reactions and therefore the specimens cannot be obtained.

The water sorption and solubility phenomena precede a variety of chemical and physical processes that lead to deleterious effects on the polymer structure and may compromise its clinical performance.[34] The sorption of water dispersed in the polymer matrix acts as a plasticizer, causing polymer expansion. The amount of water absorbed thus depends on the equilibrium between free spaces, the physicochemical affinity of polymer groups to water, and the resistance of polymer chains to the deformation force.[35] These factors may also influence strength values.

The sorption and solubility values of Barricaid were taken as the acceptable standard because it is a light-cured material and a commercial surgical dressing. This study was also based on the ideal sorption and solubility values established for restorative materials from ISO 4049 (2009) - lower than or equal to 40 μg/mm^3 and 7.5 μg/mm^3, respectively. The water sorption of Ea_1 and Ea_5 was statistically similar to Barricaid®, and for solubility, all the materials with Ea formulation were statistically different to Barricaid®. However, all the Eb formulations were statistically similar to Barricaid® for water sorption and solubility and similar to Periobond® only for solubility ($p < 0.05$). According to these results, only the Eb materials, with or without alpha-humulene, are within the limits established by the international standard.

Antimicrobial ability was tested using the modified direct contact model against *E. faecalis* and *S. aureus* in 1 and 24 hours as previously described.[24] A reduction in the *S. aureus* growth was found for all the experimental materials evaluated, being the materials containing 10 or 20% wt those with the highest antibacterial effect. Alpha-humulene is a sesquiterpene compound that has been shown to have anti-inflammatory, analgesic, and antimicrobial activity against several bacterial and fungi species.[36] The proposed antimicrobial mechanism of action of this essential oil is by membrane disruption, which can be explained by the presence of carbon double bond arrangements within the chemical structure of alpha-humulene that creates high electronegativity.[37] The antimicrobial effect observed in this study is consistent with the findings presented by Carvalho Junior et al.[11] who observed an antimicrobial activity against Gram-positive bacteria (*Staphylococcus* ATCC 5051) and fungi (eight *Candida* species, including *C. albicans*), but only against one Gram-negative genus (*Protium mirabilis* and *P. vulgaris*).

Concerning *E. faecalis*, the incorporation alpha-humulene promoted a significant antibacterial effect for the Ea and Eb formulation (Table 3), and this effect was maintained after 24 hours of incubation. The commercial reference materials presented antibacterial activity after 1 hour of incubation, but lost their antimicrobial effectiveness after 24 hours. The effect of alpha-humulene on *E. faecalis* has been previously described.[38,39] In this case, the antibacterial activity is due to monoterpenes within the chemical structure of the material, whose mechanism of action is through the loss of integrity or function of the bacterial cell membrane.[40] This result is similar to that reported for a *Santiria trimera* oil (containing 34.6% α-humulene), which was evaluated by microdilution and disk diffusion methods on agar, presenting high

Table 3. Materials used as commercial reference and their components.

Material	Manufacturer	Components
Barricaid®	Caulk/Dentsply, Milford, USA	Polyether urethane dimethacrylate resin, silanized silica, VLC photoinitiator and accelerator, stabilizer, colorant.
Periobond®	Dentsply, Petropolis, Brazil	Base: rosin, cellulose, natural gums and waxes, liquid coconut fatty acid, chlorothymol, zinc acetate, denatured alcohol, methanol, petrolatum, lorothidol. Accelerator: zinc oxide, vegetable oil, mineral oil, chlorothymol, silica, magnesium oxide, synthetic resin, coumarin.

effectiveness against *Bacillus cereus* and *E. faecalis*, although it was less sensitive to Gram-negative bacterial strains.[12]

Another example could be observed in a study on the inhibitory potential of *Cordia verbenacea* extracts against Gram-positive bacteria,[41] where alpha-humulene was the molecule with the greatest antimicrobial effect. Other studies have tested essential oils from plants containing alpha-humulene and most concluded that volatile oils were more active against Gram-positive bacteria and some fungal species.[42]

The antibacterial effect of commercial periodontal dressings was previously reported only for those based on zinc oxide eugenol, which have antifungal properties. In other words, the current available evidence indicate that periodontal dressings act simply as a physical barrier.[32,43]

A slight cytotoxic effect was found for the base material. Experimental dressing materials were formulated using camphorquinone as polymerization initiator, and the presence of this compound could be related to the cytotoxic effect observed. The mechanism by which camphorquinone is toxic is not well known, but reports have indicated that it is dose-dependent.[44] Also, the incorporation of 5, 10, and 20% wt of alpha-humulene drastically reduced cell viability of L929 mouse fibroblast cells. Prior studies that assessed the cytotoxic effect of alpha-humulene suggested that it induced a decrease cellular glutathione and an increase in the production of reactive oxygen species,[45] and this cytotoxic effect is dose-dependent, as occurred in this study. An interesting finding was that Periobond® promoted a severe cytotoxic effect. In previous studies, periodontal dressings containing eugenol delayed healing patterns,[46] increased allergenic reactions, and inhibited fibroblast proliferation.[45] Also, the release of zinc, rosin, or resin acids had a toxic effect on gingival fibroblasts.[48,49] On the other hand, the light-cured periodontal dressing Barricaid® demonstrated high cell viability (Figure 3). These findings corroborate previous studies that found no cytotoxic changes in fibroblasts and HeLa cells using completely polymerized Barricaid®.[50]

The Eb formulation presented better laboratory performance. Among the experimental dressings containing alpha-humulene, Eb_1 and Eb_5 were the most favorable for clinical use, due to their higher cell viability and cohesive strength. These experimental dressings are easy to use because of the differential in viscosity, working time, and setting time. Further tests should be performed to evaluate physical-mechanical properties, such as dimensional alteration, adhesion to dental structures, adhesion to soft tissues, and alpha-humulene release from the resinous material, and anti-inflammatory properties by *in vitro* or *in vivo* assays.

Conclusion

Within study limitations, the results indicate that formulations containing alpha-humulene showed similar behavior to the commercial references. Thus, alpha-humulene has potential to be used as an additive in periodontal dressings.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) Finance Code 001.

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