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Hyaluronic acid-based gels for oral application: Comparison of *in vitro* effects on gingival cells and bacteria

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ABSTRACT

Purpose: This study aimed to evaluate the cytotoxic effects of different topical hyaluronic acid-based gels on human gingival fibroblasts and oral bacteria.
Methods: Four different hyaluronate gels - Bexident® Aftas (BA), GUM® AftaClear (AfC), Gengigel®(G), Aloclair® Plus (AlC) and a chlorhexidine gel - Bexident®Gums(BG) were selected. Human gingival fibroblasts (HGF) were seeded in 48-well plates with different gel/culture medium concentrations (v/v%) and cell viability was evaluated at 1 and 3 days of culture. Cell morphology was assessed, and alterations graded according to ISO 10993–5:2009(E). *Streptococcus oralis* CECT 907T colony was, seed on 48-well plate or spread onto the blood agar plates and exposed to the different gel's concentration. The optical density (OD) was assessed, and the diameter of the inhibition zone was measured (mm).
Results: BA and G elicited reduced HGF cytotoxicity, followed by AfC. AlC and BG were cytotoxic at concentrations up to 3% for all exposure times. PCM images of HGF showed moderate-to-severe alterations for AlC and BG and slight to mild changes, for BA, AfC and G. The highest antibacterial activity against *S.oralis* was observed on AlC and AfC, and no antibacterial activity was observed for BA and G. Inhibitory effect in sessile colonies was only observed in AlC and BG.
Conclusions: AlC demonstrated superior antibacterial activities against *S.oralis* but a higher cytotoxic potential in HGF. BA and G presented the lowest cytotoxicity with little to no antibacterial effect. AfC demonstrated bacteriostatic effects and low cytotoxicity on HGF.

1. Introduction

Oral mucosa ulcerated lesions (aphthae) are a common disease that clinicians face daily and result in significant life-quality loss for patients.[1–3] Etiology can be diverse, including physical trauma, radiation, chemical injury, and microbial infection (bacterial, viral, and fungal) but some clinical presentations are idiopathic, such as the recurrent aphthous ulcer (RAU) or recurrent aphthous stomatitis (RAS) which is the most common of the oral ulcers.[4,5]

RAU is described as the presence of single or multiple ulcers,[6] typically presented as round or ovoid with inflammatory halos.[7,8] RAU is classified into three types: minor (most prevalent form, typically found on the buccal and labial mucosa with a diameter <5–10 mm), major (less common form, larger than 10 mm) and herpetiform (appears on keratinized mucosa of dorsal tongue and palate with a diameter around 1–3 mm).[3,4,7,8] Despite the significant prevalence of patients with RAU, the etiology and pathogenesis mechanism are still unknown. However, it may be a sign of a compromised health condition due to genetic, hormonal, immunological and infectious factors.[6] It has been suggested that oral microbiota such as *Streptococcus* spp., *Helicobacter pylori*, Cytomegalovirus and a range of other microorganisms may be a potential cause of RAU, due to their ability to disturb the oral microbiota balance

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and allow pathogenic microorganism to proliferate and damage host tissues.[5]

Despite the variation in classification, all these ulcers share similar etiopathology and clinical manifestations.[3] The management of oral ulcers is challenging since they may be associated with severe pain that makes it difficult to eat, speak, and talk.[1–3] The goal should be to reduce associated pain and decrease symptoms by eliminating atrophic and ulcerative lesions using mostly topical corticosteroids, antibiotics, analgesics, and antimicrobial agents.[9] Despite their effectiveness, especially on recurrent lesions of RAU patients, the persistent use of these medications can lead to serious secondary effects as a consequence, such as drug resistance and fungal infections. Therefore, there has been an increasing interest in other treatment options to manage oral aphthae.

Chlorhexidine gluconate (CHX) has been used for many years. It may be presented as a topical gel formula or mouthwash with a notable reduction of secondary infections, increasing time intervals between events and reducing recurrence of RAU. However, its mechanism of action does not relieve associated pain immediately.[5,10] This compound is highly toxic for fibroblasts, which can impair tissue healing, associated with tissue necrosis, inflammatory reactions, and inhibition of regeneration.[11–14]

Recently, Hyaluronic Acid (HA) started being included in a variety of formulations intended for topical administration, including mouthwash and oral gels for oral lesion treatment.[3,5] It has been described as highly biocompatible, biodegradable, with low toxicity, antibacterial and healing properties.[10,15,16]

HA is a naturally occurring linear polysaccharide composed of alternating units of repeating disaccharide, D-glucuronic acid, and *N*-acetyl-D-glucosamine, which is part of the extracellular matrix (ECM) of many soft connective tissues.[17–19] HA has been used in applications such as drug delivery and tissue engineering scaffolds to enhance their biological properties, such as long-term safety, reduction of bacterial adhesion and biofilm formation. However, as a bacteriostatic agent, HA exhibits a dose-dependent effect on different microorganisms in the planktonic phase.[15,19]

In the treatment of oral lesions, HA-based gel formulations have already shown favorable outcomes for ROU treatment in clinical studies,[3,16] but it is recognized that different formulations and constitutions of gels can elicit different responses.[3] Since its mechanism of action is based on the creation of a physical barrier between the damaged epithelia and the oral cavity, multiple applications are required.[3] Therefore, it is important to know the effect of these compound on cell biology and tissue healing process. Additionally, their antibacterial efficacy, considering that HA and CHX are hypothesized to have similar levels of antibacterial efficacy against oral pathogenic bacteria, such as *Porphyromonas gingivalis* (P. gingivalis), *Treponema denticola* (T. Denticola), and *Tannerella forsythia* (T. forsythia). Although, according to a recent study by Binshabaib et al., 2020, 0.8% HA is more efficient than 0.2% CHX at lowering P. gingivalis CFUs/mL.[11,20]

However, only few *in vitro* studies have evaluated the effect of these gel formulations on the cellular behavior of human gingival cells. There is also scarce evidence on the relative efficacy of different gel formulations, regarding tissue healing and antimicrobial activity.

Hence, the aim of this study was to compare the *in vitro* biocompatibility of commercially available topical hyaluronic acid-based aphthae treatment gels on human gingival fibroblasts. A secondary objective was to evaluate their antibacterial efficacy against *S. oralis*.

2. Methods

2.1. Gel formulations

Four different HA based topical gels and a chlorhexidine gel from varied brands were selected for this study, with the respective compositions described in Table 1.

For all the tests performed, gels were diluted in cell culture medium (DMEM) (BiowhittakerTM, LonzaTM, Basel, Switzerland) for fibroblast cell culture assays or in Brain Infusion Heart (BHI) for bacteria *S. oralis* assays to reach nine different concentrations (100%, 75%, 50%, 25%, 12.5%, 6.25%, 3.13%, 0.78%, 0.195% and 0.024% $v_{(gel)}/v_{(solution)}$ %). The concentrations varied according to the nature of the assay and their relevance for the evaluated outcome.

Table 1
Composition of the topical gel formulations.

Topical Gels		List of ingredients
Bexident Aftas®	BA	Aqua, PVP, sodium Citrate, Peg-40 Hydrogenated Castor Oil, Xanthan Gum, Maltodextrin, Propylene Glycol, Gluconolactone, Sodium Benzoate, Citric Acid, Sodium Hyaluronate, Hydroxyethylcellulose, Thymus Vulgaris Extract, Mentha Piperita Leaf Extract, Aroma, Sucralose, Origanum Vulgare Leaf Extract, Calcium Gluconate, Stevia Rebaudiana Extract, Rosmarinus Officinalis Leaf Extract, Cinnamomum Zeylanicum Bark Extract, Citrus Limon Peel Extract, Hydrastis Canadensis Root Extract, Lavandula Angustifolia Flower Extract, Olea Europaea Leaf Extract, Limonene, Citral.
Bexident Gengivas®	BG	Aqua(Water), Sorbitol, Glycerin, Panthenol, PEG-60, Hydrogenated Castor Oil, Hydroxyethylcellulose, Poloxamer 188, Aroma (Flavor), Chlorhexidine Digluconate, Allantoin, Sodium Saccharin, Citric Acid, Eugenol, Limonene, BHT
Afta Clear®	AfC	Hydrogenated Starch Hydrolysate, Aqua, Propanediol, Propylene Glycol, Polycarbophil, Sodium Hydroxide, Taurine, PVP, Xylitol, Maltodextrin, Gluconolactone, Opuntia Ficus-Indica Stem Extract, Sodium Hyaluronate, Olea Europaea Leaf Extract, Sodium Benzoate, Bisabolol, Sucralose, Aroma, Stevia Rebaudiana Extract, Calcium Gluconate, CI 19140, Zingiber Officinale Root Extract, CI 42090.
Gengigel®	G	Aqua, Xylitol, Cellulose Gum, Alcohol, PEG 40 Hydrogenated Castor Oil, Polyvinyl Alcohol, Carbomer (Polycarbophil), Dichlorobenzyl Alcohol, Aroma, Sodium Hydroxide, Acid Blue 9 (CI 42090).
Aloclair	AlC	Hydrogenated Starch Hydrolysate, Aqua, Propanediol, Propylene Glycol, Polycarbophil, Sodium Hydroxide, Taurine, PVP, Xylitol, Maltodextrin, Gluconolactone, Opuntia Ficus-Indica Stem Extract, Sodium Hyaluronate, Olea Europaea Leaf Extract, Sodium Benzoate, Bisabolol, Sucralose, Aroma, Stevia Rebaudiana Extract, Calcium Gluconate, CI 19140, Zingiber Officinale Root Extract, CI 42090.

2.2. Cell culture

Human Gingival Fibroblasts HGF (HGF; Applied Biological Materials Inc., Richmond, BC, Canada) at a fourth passage were cultured at 37 °C, 5% CO_2 and 98% humidity in a culture medium composed of Dulbecco's Modified Eagle's Medium-DMEM (BioWhittaker, Lonza®, Switzerland) and supplemented with 10% fetal bovine serum (Biowest®, France) and 1% Penicillin with streptomycin (G255 Applied Biological Materials Inc., Richmond, BC, Canada) in a 75 cm^3 culture flasks (Corning).

At approximately 100% of confluence, trypsinization was (trypsin EDTA - Lonza, Veners, Belgium) was conducted according to the manufacturer protocol. After that, cells were seeded with a density of $1x10^4$ cells/ml in 48-well plates and incubated (37 °C). All experiments were conducted using a fourth cell passage.[21–23]

Eight different gel/DMEM (v/v%) concentrations of each gel (75%, 50%, 25%, 12.5%, 6.25%, 3.13%, 0.78% and 0.195%) were incubated with cells for either 15 min or 4 h (n = 8 for each gel concentration x exposure time group). Negative (medium) and positive (DMSO) controls were also used. After incubation, all culture wells were washed three times with DMEM and incubated.

2.2.1. Cell viability and proliferation

Cell viability was evaluated at 1 and 3 days after exposure (n = 8 for each gel concentration x exposure time grou), using a resazurin-based

assay (Cell Titer Blue® reagent - Promega, Madison, WI, USA), according to the manufacturer protocol and to previously validated protocols.[21–23] Conversion rate was measured as fluorescence intensity in arbitrary units (AU) and was detected at excitation/emission wavelengths of 560/590 nm using a luminescence spectrometer (PerkinElmer LS 50B, Waltham MA, USA). Results were converted to percentage of the negative control and all analyses were performed using these converted values, considering that a reduction of viability in 30% corresponded to a cytotoxic effect as described in ISO10993–5:2009(E).

2.2.2. Cell morphology and cytotoxicity analysis

Cell morphology was assessed through phase-contrast microscopy (PCM). Alterations were graded according to ISO10993–5:2009(E).

Imaging was obtained at 1 and 3 days of culture after exposure. For PCM, after cell viability, cell culture medium was changed and then images were observed in a phase-contrast inverted microscope with a magnification of 10× (n = 8).

Two calibrated researchers analyzed the images, focusing on cell morphology and spreading, and evaluated cytotoxicity accordingly.

2.3. Bacterial growth

For this study, S. oralis CECT 907T strain was cultured on an enriched blood agar plate at 37 °C for 72 h under anaerobic conditions (10% CO_2, 10% H_2 and balance N_2). A single colony was grown in 10 mL of Brain-Heart Infusion Modified Medium (BHI-2) at 37 °C under anaerobic conditions. After reaching the exponential phase, suspension growth was confirmed by measuring the optical density (OD) at 550 nm and adjusted to a final OD of 0.4 for all experiments.

2.3.1. Minimum Inhibitory concentration

Topical gels containing hyaluronic acid, or 2% chlorhexidine were evaluated at the following concentrations 50%, 25%, 12.5%, 6.25%, 3.13%, 0.78%, 0.195% and 0.024% (v/v) diluted in BHI media as previously described. The inoculum was seeded on ninety-six well plate, 100 μL for each well, and incubated in anaerobic condition for 24 h. After the incubation time, the suspension was removed and 100 μL of the different concentrations of topical gels were added to each evaluated well for 1 min. Phosphate-buffered saline (PBS) was used as positive control and ethanol 70% (v/v) was used as negative control. After exposure, each well was washed with PBS and incubated with BHI-2. The turbidity of each well was assessed after 24 h (h) of incubation by directly reading the optical density (OD) at 595 nm and the results were presented as a percentage of bacterial growth compared to the control. The presence or absence of colony growth was assessed by culturing on blood agar plates for 72 h. Three independent assays were performed with n = 3 each.

2.3.2. Inhibition halo assay

Topical gels containing hyaluronic acid and 2% chlorhexidine were evaluated at the following concentrations 100%, 75% 50% and 25% (v/v). The suspension was then diluted 1:10 and 100 μL was spread onto the blood agar plates. Four equidistant wells were made in each plate using a sterile 4.1 mm diameter circular scalpel and 50 μL of hyaluronic acid-based aphthae treatments gels were inoculated in each well, in the following concentrations 100%, 75%, 50% and 25%. Chlorhexidine 2% gel was used as a positive control. Plates with S. oralis suspension and no topical gels were used as negative controls. All plates were incubated in an anaerobic condition at 37 °C for 72 h. After the incubation time, the images were acquired using stereoscopic microscope (Leica) with camera and the diameter of the inhibition zone was measured (in mm) using a metal ruler by two calibrated observers. Three independent assays were performed with n = 3 each.

2.4. Statistical analysis

All experiments were performed in triplicate. At least three independent assays in different times were performed for all tests. Data was presented as a mean ± standard deviation (SD). For the statistical analysis, SPSS 28 statistics software for Windows (IBM) was used. The data was tested for normality with Shapiro-Wilk and Kolmogorov-Smirnov tests. Group comparisons were performed through ANOVA with Tukey *post-hoc* test and the significance were set at ($p < 0.05$).

3. Results

3.1. Cell cytotoxicity

3.1.1. Fibroblast viability

For the 15-min of exposure time at 1 day viability (Fig. 1 A), none of the gels presented cytotoxicity at concentrations of 0,781% and above. Gels BA and G presented higher viability than the other groups ($p < 0.05$) with low cytotoxic effects at high concentrations 12,5%. AfC presented intermediate results for lower concentrations and was cytotoxic for 75% dilution but still slightly recovered at 3 days of culture. BG and AlC were highly cytotoxic for concentrations up to 0,781%. Viability after 3 days of culture of the 15min group (Fig. 1 B) showed an increase for all concentrations of gels relatively to control, except for BG and AlC.

When exposed for 4h, all gels presented high cytotoxicity for the 75% dilution (viability <25%), (Fig. 1C and D). At lower concentrations (<12,5%) gels BA, G and AfC, recovered baseline viability up to 70% after 3 days of culture. AlC and BG had significant cytotoxic effects with a viability reduction of around 75% at all timepoints for concentrations >3125% for 15 min of exposure ($p < 0.05$) and for >0,781% gel concentration for 4 h of exposure.

Overall, viability results show that BA and G elicited the lowest decrease in viability, followed by AfC. AlC showed the biggest decrease in viability for gingival fibroblasts, similar to the one caused by BG.

3.1.2. Phase-contrast microscopy (PCM) and cytotoxicity evaluation

Microscopy images were obtained from cells exposed to the maximum and minimum concentrations (Fig. 2). Overall, higher signs of cytotoxicity in AlC and BG were observed comparing to other groups. AT 1 day of culture, all groups presented adhered cells, despite groups BE and AlC showing signs of cytotoxicity. After 3 days of culture, wells that presented early signs of cell alterations and cytotoxicity ended up empty as the cells present detached. It is also noteworthy that groups BA, AfC and G showed similar density in the microscopy images as the controls at 3 days of culture. Also, when comparing the 15-min inoculation to 4 h, we can observe a drastic reduction in the number of cells in all groups for the latter, as shows Table 2.

3.2. Antibacterial activity

3.2.1. Minimum Inhibitory concentration

After 1 min of exposure, there was only antibacterial effect against planktonic bacteria at 50% concentration with the exception of AfC, which did not exhibit antibacterial effect, as shown in Fig. 3. Compared with BG, at 50% concentration a reduction in bacterial growth of (21% ± 0.097) > BA (15% ± 0.115) > G (10% ± 0.099) was observed without statistically significant difference between them. For concentrations below 50%, there was no reduction in bacterial growth after 1min. S. oralis colony growth was observed at all concentrations for all topical gels after 72 h incubation. Aloclair was excluded from this analysis since due to the consistency that interfered with optical density measurements.

3.2.2. Inhibition halo

Of all hyaluronic acid gels assessed, AlC and AfC showed the highest antibacterial activity against sessile bacteria at 100% concentration (p

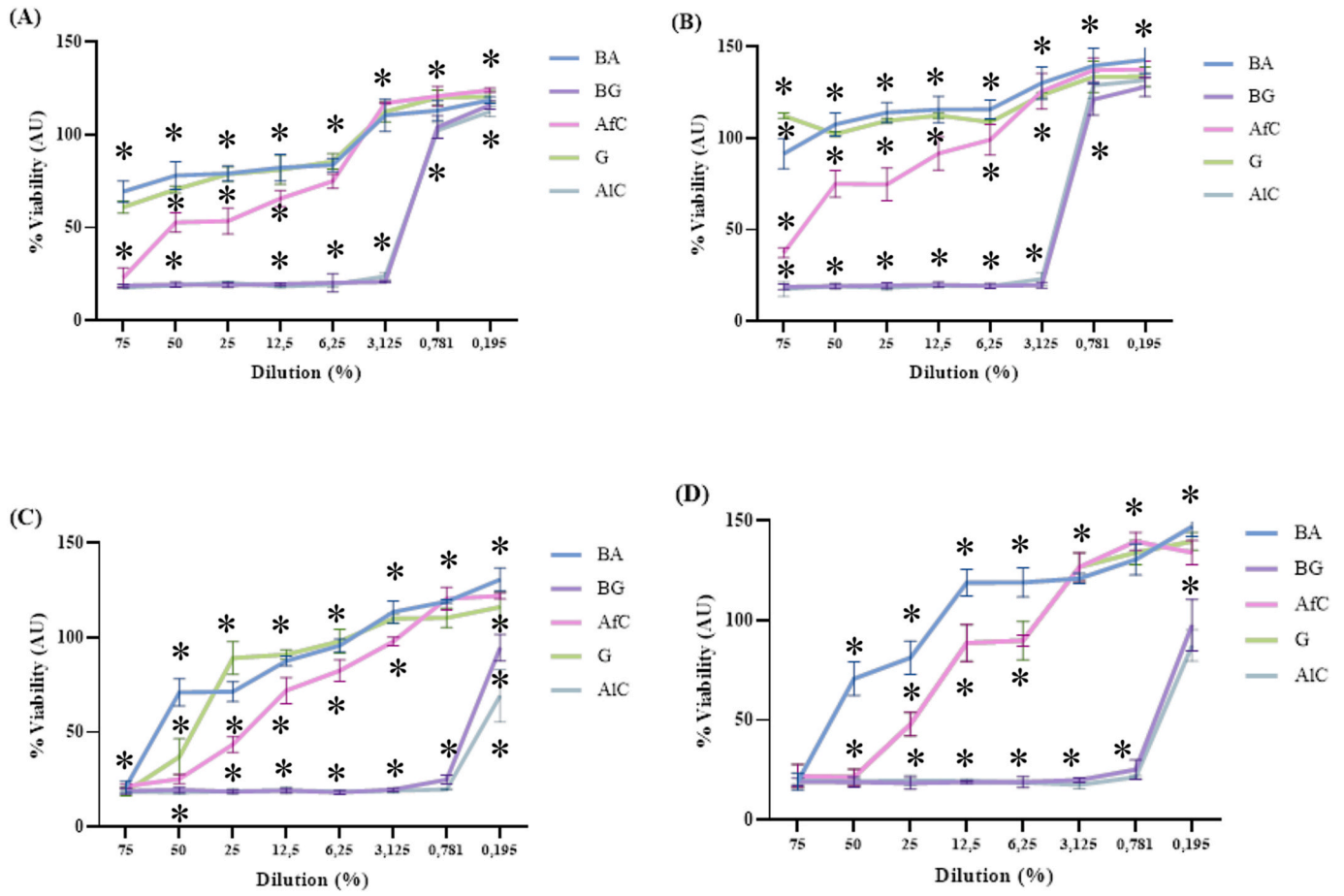

Fig. 1. Fibroblast viability results according to exposure time (15 min or 4 h) and evaluation time after exposure: (A) 15 min of exposure time, 1 day after exposure; (B) 15 min of exposure time, 3 days after exposure; (C) 4 h of exposure time, 1 day after exposure; (D) 4 h of exposure time, 3 days after exposure. Results presented as mean and standard deviation of the percentage of negative control. Statistical significance: *p < 0.05.

< 0.05), comparable to BG (positive control), as shows Fig. 4B. No statistical differences between AfC and BG antibacterial effects were observed for all concentrations (p > 0.05). BA and G showed no antibacterial activity on sessile cultures, as observed by the absence of inhibition halo (Fig. 4A). It was also observed that the antibacterial effect of BG, Aloclair and Afta Clear was dose dependent, and for Afta Clear no antibacterial effect was observed for concentrations below or equal to 50%.

4. Discussion

To date, there is no conclusive evidence on the optimal therapeutic solution for the treatment of ulcerated oral lesions, such as in RAS or RAU, which present a major toll in patients' quality of life. In order to define the most effective approach, the comparison of the direct effects of different therapeutic options on oral cells and tissues is essential, given their main function as a barrier and the need for prolonged contact and multiple applications.

Since hyaluronic acid has been proposed to be able to promote the development of a more efficient barrier, along with described healing and antimicrobial properties, this study aimed to evaluate the *in vitro* effect of three commercially available HA-based gels comparing to a reference 0.12% chlorhexidine oral gel both in human gingival fibroblasts viability and in *S. oralis* inhibition.[15,19]

The present study is the first to evaluate the cytotoxic effect of hyaluronic based gels for oral applications, along with their antibacterial activity comparing distinct formulations. The results of the biocompatibility evaluation of these HA-based gels showed that both Aloclair® and Bexident Gums® presented a high cytotoxicity (for both fibroblasts and bacteria) with a significant reduction in viability (80% reduction of viability for concentrations >0,781% for 15min exposure and >0,195% for 4h of exposure) and with considerable signs of cell cytotoxicity (general morphology, vacuolization, cell detachment, lysis and membrane integrity - as described in ISO 10993–5:2009(E)) in fibroblasts imaging analysis.

Bexident Aftas® and Gengigel® showed the least cytotoxicity, even in high concentrations, showing even some efficacy in improving fibroblast viability comparing to ontrol (>70% of viability for concentrations >50% in 15min exposure, showing an improvement in viability for concentrations <3125%; the 4h of exposure lead to similar values, <50% for Bexident Aftas® and <25% for Genegigel® with viability values > 80%).

Bexident Gums® was used in this study as a reference control. This gel contains chlorhexidine, a bisbiguanide with a well-known bacteriostatic and bactericidal mechanisms of action, depending on its concentration. Also, the base excipient does not include hyaluronic acid or another related excipient. Our results demonstrate an effective antibacterial effect of Bexident Gums®, but also a high cytotoxicity towards gingival fibroblasts even in low concentrations. Our results are in line with previous studies in periodontal cells which shows that even for very low concentrations, chlorhexidine has a very high negative effect in cell migration, mitosis, synthesis, adhesion, among other effects[12,13,24–26] Despite that, studies present a high variability in theirmethodology, product composition, exposure time and the medium used, which prevents direct comparisons.

Chlorhexidine (Bexident Gums®) was used in this study since it is

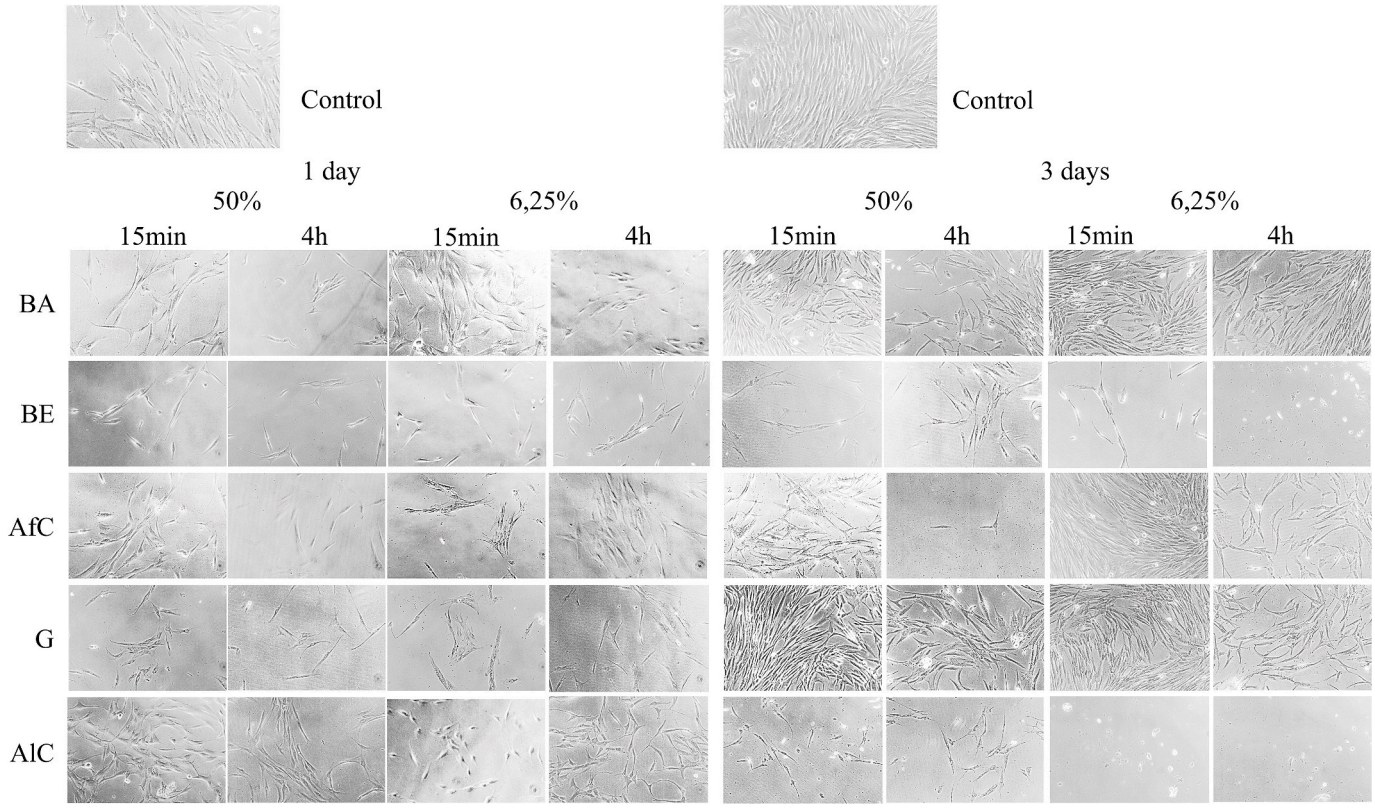

Fig. 2. Phase-contrast microscopy (PCM) of fibroblasts, subjected to 75% and 6,25% gel/medium concentrations for 15 min and 4 h of exposure time and at 1 day and 2 days after exposure.

Table 2
Grading of cytotoxic alterations according to ISO10993–5:2009(E). 0 = none, 1 = slight, 2 = mild, 3 = moderate, 4 = severe (* >2 = cytotoxic effect).

	1 day				*3 days*			
	50%		*6,25%*		*50%*		*6,25%*	
	15min	*4h*	15min	*4h*	15min	*4h*	15min	*4h*
BA	*1*	*3**	*0*	*1*	*1*	*2*	*1*	*1*
BG	*3**	*3**	*3**	*3**	*4**	*4**	*4**	*4**
AfC	*1*	*3**	*1*	*2*	*2*	*3**	*0*	*2*
G	*1*	*3**	*2*	*3**	*0*	*1*	*0*	*2*
AlC	*2*	*3**	*3**	*2*	*4**	*3**	*4**	*4**

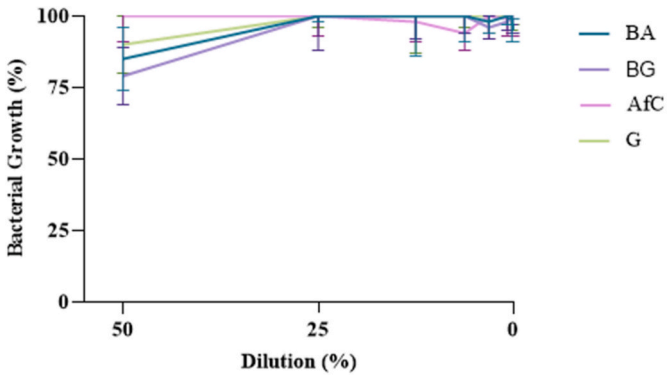

Fig. 3. Line graphs representing antibacterial effect of different concentrations of BG, BA, AfC and G against Streptococcus oralis after 1 min exposure. The results are presented as mean ± standard in percentage (%).

still considered a standard in aphthae treatment, despite the already proven cytotoxicity, association with tissue necrosis, inflammatory reactions, and inhibition of regeneration.[26] Another recent study used a chlorhexidine gel with the addition of chitosan (Bexident Post®) that showed good antibacterial effect, adequate biocompatibility, and elicited a reduction in the inflammatory cytokines on previously inflamed cells, which might suggest that the addition of other compounds to chlorhexidine products may be able to improve the cell response.[27] Despite that, this gel is recommended by the manufacturer for post-operative use and not directly as an option for aphthae treatment.

As for Aloclair®, to the best of our knowledge, there were no previous studies regarding its effect, but its composition includes several potentially cytotoxic components: polyvinylpyrrolidone (PVP), propylene glycol[3,24] and *Aloe barbadensis* extract.[28,29] Both PVP and propylene glycol are also present in AftaClear® which also showed cytotoxicity, specially at higher concentrations, suggesting that *Aloe barbadensis* extract may be the main factor that leads to the increased cytotoxicity in Aloclair®.

A previous retrospective clinical study reported a favorable effect of AftaClear® (both in gel and rinse form) in aphthae treatment, but no definitive conclusions can be taken from this study since it has a retrospective design and there wasn't a control group included.[3] In a systematic review of animal studies regarding the use of plant extracts in oral ulcers verified that only 3 articles evaluated *Aloe barbadensis* effect and that it showed no significant difference in healing.[30] This different result might be caused by the concentration of the extract not being sufficient to elicit a tissue alteration (either positive or negative) or due to the gel thickness, as Aloclair® was quite thick and difficult to remove after washing.

Regarding Bexident Aftas® and Gengigel® fibroblast viability results were quite favorable, although they did not seem to have any antibacterial effect. The proliferation results for these gels even suggest a potential ability to increase viability beyond the control. Despite the lack of

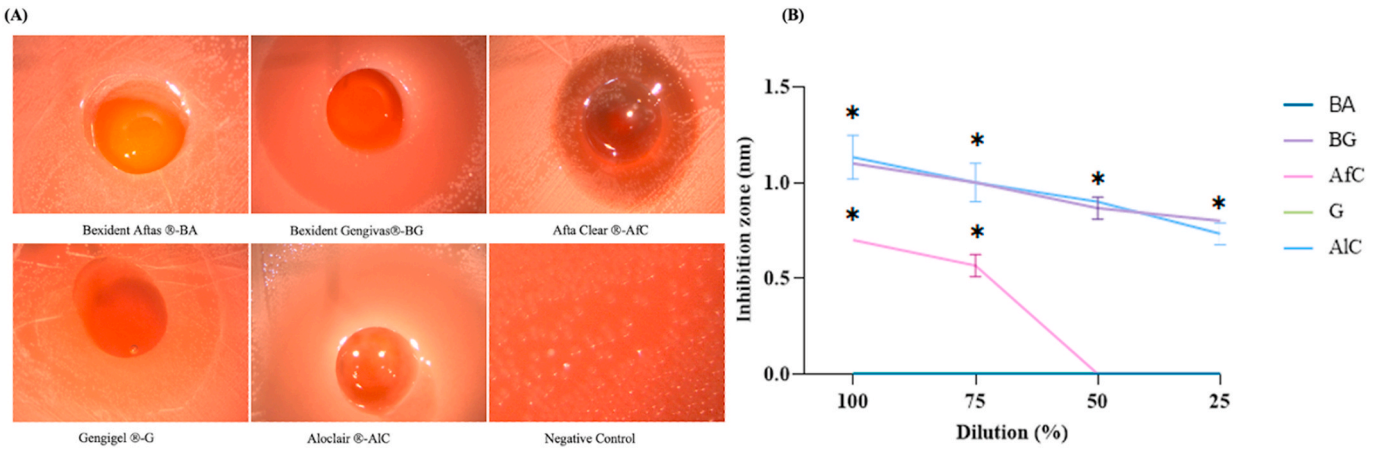

Fig. 4. (A) Images representing BA, BG, AfC, G and AlC zone of inhibition at 100% concentration taken with Lupa Leica after the incubation time. (B)Line graphs representing antibacterial effect of different concentrations of BA, BG, AfC, G and AlC against Streptococcus oralis. The line graphs for BA and G are overlapping. The zone of inhibition was measured using metal ruler. Three independent assays were performed with n = 3 each.The results are presented as mean ± standard in millimeter (mm). Statistical significance presented: p* <0.05.

studies regarding Bexident Aftas®, there are some studies using Gengigel®. In a wound closure assay, Gengigel® in spray showed a slight improvement albeit not statistically significant.[31] A clinical study used the gel as an adjunct to scale and root planning in periodontal therapy with favorable results.[32]

Despite the high heterogeneity in methodology or objectives among the literature, the little evidence that exists on hyaluronic acid-based gels suggests that they may be an appropriate approach to improve healing, despite not having a particularly good antimicrobial effect. Previous studies have shown that 0.2% of HA-based gel did not have effectiveness against microorganisms in periodontal environment and in cases of chronic periodontitis.[33,34] This is in accordance with the present results. However, different results were reported in another study in which 0.8% HA was used compared to 0.2% of CHX and the 0.8% HA was observed to be more effective.[11]

Hyaluronic acid is a viable treatment option for the improvement of cell viability, potentially leading to improved wound healing, but not for antimicrobial control. Nevertheless, product formulations must be evaluated with care and other potentially irritant substances should be avoided since they have the potential to impair the healing process, such as observed in Aloclair®. Also, there is interest in adding other therapeutic components that potentially enhance healing efficacy,[33] so in the future, embedding other substances with different therapeutic effects (like analgesic or antimicrobial properties) in hyaluronic-based gels could be an interesting strategy to improve the outcomes of treatment with these gels.

This study allowed the comparison between five different gels, evaluating both cytotoxicity and antibacterial activity which gives us a good understanding of the potential biological effect of the clinical application of this products which was never done before. Future studies should also investigate the antibacterial effect on other oral bacterial species as well as the evaluation of the treatment of multi-species biofilms, and the effect of the inclusion of other molecules with antibacterial activities in HA-based gels to enhance their antibacterial properties in order to achieve an ideal formulation.

The distinct consistencies of the gels turned the manipulation and dilution of these products challenging, which was a limitation of the methodology of this study since it turned the use of several dilutions in some tests impossible. The reduced sample size was also a limitation of this study, therefore these results should be used as reference for future studies with larger sample sizes. As an *in vitro* study, with all the limitations associated to this design, further studies should be performed to evaluate these results in a clinical context, since they are verry frequently used by patient and recommended by colleagues and there aren't many studies that evaluate their efficacy and safety.

We can conclude that Bexident® AFT and Gengigel® presented the lowest cytotoxicity of the tested hyaluronic acid-based gels for ulcerated oral lesions treatment but without significant anti-microbial effects in oral bacteria. AftaClear® had intermediate cytotoxicity and antibacterial effect and Aloclair® showed a significantly cytotoxic effect in gingival fibroblasts, while having superior antibacterial activity against *Streptococcus oralis* in sessile and planktonic forms.

Therefore, hyaluronic acid-based gels showed overall good *in vitro* results towards gingival fibroblasts, which suggests that they may enhance healing outcomes clinically, but formulations with potential cytotoxic substances like *Aloe barbadensis* might have a detrimental effect in healing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Not applicable.

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