



Article

Clinical Efficacy of a Chlorhexidine-Based Mouthrinse Containing Citrox (Citrus Aurantium Amara Extract) and Hyaluronic Acid in Early Wound Healing of Patients Undergoing Periodontal Surgery: A Triple-Blind, Parallel-Arm, Randomized Controlled Trial

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Abstract

Background: Chlorhexidine (CHX) is the reference chemical plaque control agent used after periodontal surgery, but higher concentrations are associated with adverse effects that can reduce patient compliance. This randomized controlled trial evaluated whether a mouthrinse combining a reduced CHX concentration (0.09%) with Citrox and hyaluronic acid (HA) could match the clinical performance of conventional 0.12% CHX after periodontal flap surgery, while improving tolerability. **Methods:** Thirty-two patients undergoing open flap debridement with an experimental vertical releasing incision randomly received a mouthrinse containing 0.09% CHX with Citrox and HA (TEST group) or conventional 0.12% CHX (CONTROL group) twice daily for 14 days. The primary outcome was the presence of edema; secondary outcomes were the Early Wound Healing Score (EHS) and its components, the plaque index (TQHPI), the gingival index (GI), the tooth/tongue staining index (SI), and the adverse events of oral ulcers and dysgeusia, assessed at baseline and at 3, 7, and 14 days. **Results:** Edema, EHS, and the GI did not differ significantly between the groups at any time point (edema at day 3: 37.5% in both groups; risk difference 0.0%; 95% CI −30.5% to 30.5%). The plaque index was significantly higher in the TEST group from day 7 ($p = 0.003$; median difference 0.20, 95% CI 0.10 to 0.40) to day 14 ($p = 0.005$; median difference 0.30, 95% CI 0.10 to 0.40). The distribution of staining scores differed significantly at day 14 ($p = 0.008$), with heavy staining observed only in the control group. **Conclusions:** No statistically significant differences between the reduced-concentration, multi-component mouthrinse, and 0.12% CHX were detected in the assessed wound-healing outcomes; the test formulation was associated with higher plaque scores from day 7 and with a different distribution of staining scores at day 14, without heavy staining. These preliminary findings require confirmation in larger trials.

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Keywords: biological products; chlorhexidine; hyaluronic acid; periodontal surgery; wound healing; randomized controlled trial

1. Introduction

Periodontitis remains a highly prevalent condition with a substantial global health and economic burden [1], and surgical therapy is an established part of its management when residual pockets persist after non-surgical treatment [2]. The long-term success of such surgery depends heavily on undisturbed wound healing and on effective control of the bacterial biofilm during the early post-operative period, since the main biological goal after flap elevation and suturing is to achieve a stable, passive closure of the wound margins over the instrumented root surfaces and underlying bone, allowing healing by primary intention [3,4]. In the first days after surgery, the fibrin clot that forms at the wound margin provides the structural scaffold for the subsequent phases of repair—endothelial proliferation, inflammatory cell regulation, and fibroblast migration—but this early tissue interface remains fragile and highly susceptible to mechanical disruption [5]. For this reason, mechanical plaque removal, although the mainstay of routine periodontal maintenance, must be withheld at the surgical site during the first two post-operative weeks, since premature brushing can generate shear forces capable of dislodging sutures and opening the wound margins.

Because mechanical hygiene is suspended at the surgical site, chemical plaque control becomes a necessary adjunct, rather than an optional addition during this critical window [2]. Classic clinical trials established that post-surgical rinsing with chlorhexidine (CHX) reduces plaque accumulation, limits bacterial recolonization of the wound, and improves gingival health compared with untreated controls [6,7], and subsequent work confirmed that the specific post-surgical cleansing protocol adopted materially affects early plaque control and wound healing outcomes [8]. A more recent systematic review of eleven controlled trials confirmed that CHX rinsing after periodontal or implant surgery reduces plaque and bleeding compared to a placebo, while also cataloguing its recurrent adverse effects [9].

CHX is widely regarded as the reference chemical anti-plaque agent in dentistry, owing to its broad-spectrum antimicrobial activity and pronounced substantivity to oral surfaces; as a cationic bisbiguanide, it binds to the negatively charged bacterial cell wall, disrupting membrane integrity and ultimately causing cell death [10]. Prolonged use of higher-concentration formulations, however, carries well-documented drawbacks: extrinsic brown staining of teeth, restorations, and tongue is the most frequently reported complication, and patients often describe taste alterations, a transient burning sensation, or mucosal irritation, and a detrimental impact on early wound healing has also been described [11–13]. Lowering the CHX concentration is a recognized strategy to limit these side effects: a systematic review comparing 0.12% and 0.2% CHX mouthrinses found only a small, clinically negligible difference in plaque inhibition in favor of the higher concentration and no difference in gingival inflammation [14], two commercially available rinses differing in concentration (0.12% vs. 0.2%) achieved similar plaque inhibition [15], and less concentrated formulations were indicated as the most promising option to reduce side effects while maintaining comparable clinical effects after surgery [9]. Poor tolerability is a recognized cause of early discontinuation of the prescribed rinse [13], which in the post-surgical setting exposes the unprotected wound to renewed bacterial colonization [8].

To limit these dose-dependent side effects without sacrificing antimicrobial efficacy, lower-concentration CHX formulations combined with natural adjuncts have been developed [16,17]. One such adjunct is Citrox, a complex of soluble bioflavonoids extracted

from *Citrus aurantium amara* (bitter orange) [18], with demonstrated broad-spectrum antibacterial and antifungal activity against oral pathogens and biofilms [16,19]. When combined with a reduced concentration of CHX, Citrox has been reported to preserve biofilm inhibition through a synergistic mechanism, allowing the CHX concentration to be lowered while maintaining antimicrobial coverage [17]. A recent randomized controlled trial in adults with plaque-induced gingivitis found a 0.09% CHX + Citrox mouthwash to be at least as effective as 0.12% CHX in reducing gingival bleeding over two weeks [20].

In parallel, hyaluronic acid (HA) has increasingly been incorporated into post-surgical rinses for its biological role in tissue repair. HA is a major constituent of the extracellular matrix of periodontal soft tissues, where its hygroscopic properties help maintain tissue hydration and modulate the early inflammatory response, while also acting as a scaffold that supports fibroblast migration, proliferation, and angiogenesis [21,22]. Recent systematic reviews have suggested that its adjunctive use may also improve clinical outcomes in both non-surgical [23,24] and surgical [23,25] periodontal therapy.

Despite this biological rationale, direct clinical comparisons of multi-component, reduced-dose CHX formulations against conventional 0.12% CHX in periodontal flap surgery remain limited. A short-term trial of CHX 0.12% with or without HA around submerged implants found significantly less early post-operative edema with the HA-containing rinse, but no difference in plaque, gingival, or staining indices [26]; a crossover trial of CHX with an anti-discoloration system after flap surgery found comparable gingival healing parameters between formulations, with reduced staining and better patient acceptance in the anti-discoloration arm [27]; and a triple-blind trial comparing 0.2% CHX with a CHX + HA + anti-discoloration-system rinse after flap surgery found generally comparable outcomes, with a transient gingival index difference at day 7 [28]. Most recently, a randomized trial comparing CHX + anti-discoloration system with or without HA against an untreated control after periodontal surgery reported that CHX-based rinsing improved early wound healing and that the addition of HA further enhanced soft-tissue closure [29]. None of these trials, however, tested a formulation combining a reduced CHX concentration with both Citrox and HA against the conventional 0.12% CHX reference formulation. The present triple-blind, parallel-arm, randomized controlled trial was, therefore, designed to evaluate the clinical efficacy of a 0.09% CHX mouthrinse enriched with Citrox and HA, compared with a conventional 0.12% CHX rinse, in patients undergoing periodontal flap surgery.

2. Materials and Methods

2.1. Trial Design and Ethical Aspects

This study was designed as a single-center, parallel-arm, triple-blind, randomized controlled clinical trial. The trial was conducted at the Section of Periodontics of the Department of Oral and Maxillofacial Sciences of Sapienza University of Rome, between December 2024 and September 2025. The protocol was approved by the Ethical Committee of Sapienza University of Rome (protocol code 0460/2022; Rif. 6753; date of approval: 10 June 2022), and this study was conducted in accordance with the principles of the Declaration of Helsinki (1975, as revised in 2008). Written informed consent was obtained from all patients. The trial protocol has been registered at clinicaltrials.gov (NCT06738342).

2.2. Eligibility Criteria

Patients were eligible for inclusion if they: (i) were at least 18 years old; (ii) presented an indication for surgical periodontal treatment of at least one residual pocket after non-surgical therapy [e.g., probing depth (PD) $\geq$ 5 mm with bleeding on probing (BoP) or PD $\geq$ 6 mm]; (iii)

had a full mouth plaque score (FMPS) and full mouth bleeding score (FMBS) < 20% before surgery; and (iv) had the ability and willingness to provide written informed consent.

Patients were excluded from this study if positive for one or more of the following criteria: (i) diabetes mellitus; (ii) severe blood disorders, with a documented qualitative and/or quantitative deficit of polymorphonuclears and/or platelets; (iii) the assumption of medications affecting the gingiva and/or the oral mucosa; (iv) pregnancy or breastfeeding; (v) the use of systemic or local antibiotics in the previous 4 weeks before study initiation and throughout the entire experimental phase; (vi) experimental teeth contraindicating surgery (e.g., severe mobility, root fractures, untreated endodontic lesions, or inadequate restorations); (vii) previous periodontal surgery at the same site in the previous 12 months; or (viii) heavy smokers (≥ 10 cigarettes per day).

2.3. Surgical Intervention

In accordance with current clinical practice guidelines [2], every patient underwent a two-step course of non-surgical periodontal therapy (supragingival biofilm control with professional mechanical plaque removal and individually tailored oral hygiene instructions and subgingival biofilm control). After a 6-week healing period, clinical parameters were reassessed, and only patients still meeting the inclusion/exclusion criteria were enrolled in the trial.

Surgical procedures were performed by the same expert periodontist (A.P.). Following local anesthesia, the patients were treated by open flap debridement. Each flap showed the following characteristics: (i) both the buccal and oral aspects were elevated; (ii) full-thickness flaps; and (iii) an experimental mesial vertical releasing incision (VRI) at a healthy site without clinical attachment loss. At the end of the surgery, interrupted sutures were placed to obtain passive flap closure and healing by primary intention. A 6/0 suture (PTFE, HuFriedyGroup, Chicago, IL, USA) with a 3/8 circle, cutting edge needle was used in this area. Because allocation envelopes were opened only after completion of the surgical procedure, the surgeon was unaware of group assignment throughout surgery. The VRI was deliberately placed at a healthy site without attachment loss in order to obtain a standardized wound for the assessment of early healing, independent of the pocket depth, bone defect morphology, and root instrumentation of the treated sites.

2.4. Post-Surgical Instructions and Follow-Up Visits

Immediately after surgery, each patient was randomly assigned to the TEST or CONTROL group. The TEST group used a mouthrinse containing CHX 0.09% combined with HA and Citrox (PerioPlus+ Regenerate, Curaden, Kriens, Switzerland), while the CONTROL group used a mouthrinse containing CHX 0.12% alone. Regardless of group assignment, all patients received the same instructions: use 10 mL of the assigned mouthrinse for 1 min, twice daily for 14 days, avoiding rinsing or drinking for 30 min afterwards. The patients were asked to return the mouthrinse bottles at the end of the experimental period (day 14), in order to assess the level of patient compliance.

The patients received modified oral hygiene instructions: mechanical toothbrushing was allowed in all areas of the mouth except the surgical site, where it was withheld until suture removal. No medications were prescribed, except for Paracetamol 1000 mg if needed for pain relief.

The patients were scheduled for follow-up visits at 3, 7, and 14 days. Sutures were removed at 14 days. At days 3, 7, and 14, the experimental parameters were evaluated.

2.5. Outcomes

The following clinical parameters were assessed by a calibrated examiner (G.DE.), who was blinded to group allocation throughout the follow-up period. The primary

outcome was the presence of edema, recorded as a binary event (present/absent) according to the criteria of Genovesi et al. [26], and was assessed at the site of the experimental VRI. Edema was selected as the primary outcome because its absence is an established marker of uneventful early healing, and because biofilm accumulation at an unprotected post-surgical wound contributes to local inflammation and to edema. Secondary outcomes included the Early Wound Healing Score (EHS [5], a 0–10 composite score, in which higher values indicate better healing, calculated as the sum of three components: clinical signs of re-epithelialization—CSR, clinical signs of hemostasis—CSH, and clinical signs of inflammation—CSI), also assessed at the VRI. Further secondary outcomes were: the Turesky modification of the Quigley–Hein Plaque Index [30] (TQHPI) and the gingival index, modified after Trombelli et al. [31] (GI), recorded at six tooth surfaces adjacent to the experimental site; the tooth staining index (SI), according to the Lobene index [32], as modified by Gründemann et al. [33], assessed on the buccal surfaces of the four upper incisors and the anterior two-thirds of the tongue (0 = no stains, 1 = light stains, 2 = moderate stains, and 3 = heavy stains); and two adverse events, oral ulcers and dysgeusia, each recorded dichotomously (yes/no).

Prior to the initiation of the clinical trial, the intra-examiner reliability for the experimental parameters was calculated using the intra-class correlation coefficient (ICC) and Cohen's kappa statistics (k). Calibration was accepted if the ICC and k were ≥ 0.81 .

2.6. Allocation Concealment, Randomization, and Blinding

Allocation concealment was ensured using opaque envelopes, opened only after completion of the surgical procedure, and randomization was performed using a computer-generated sequence in a 1:1 ratio. Patient blinding was achieved by dispensing the test and control mouthrinses in identical, unlabeled bottles matched for organoleptic properties (appearance, taste, and odor), so that the participants could not distinguish between the two formulations. Together with the blinding of the treating clinician and of the outcome assessor to group allocation, this triple-blind design ensured that the participants, clinicians, and outcome assessors all remained unaware of treatment allocation throughout the trial.

2.7. Statistical Analysis

The sample size was calculated based on the presence of edema at day 3, using data from a comparable study [26]. To achieve 80% power at a 5% significance level, 13 patients per group were required; to account for an expected 20% drop-out rate, 32 patients (16 per group) were enrolled. The analyzed sample was complete, with no missing data at any of the four assessment time points.

Continuous variables were expressed as the mean and standard deviation. Categorical variables were summarized as frequencies and percentages. Adherence to normal distribution was evaluated with the Shapiro–Wilk test. Continuous and ordinal scores (age, flap extent, EHS, TQHPI, and GI) were compared with the exact Wilcoxon rank-sum (Mann–Whitney U) test, and categorical variables (sex, smoking status, periodontitis stage and grade, site position, edema, EHS components, SI, oral ulcers, and dysgeusia) with Fisher's exact test. The significance level was set at $p < 0.05$. No correction for multiple comparisons was applied. Between-group differences were reported with 95% confidence intervals (CI): for edema, as the difference in the percentage of patients affected; for continuous outcomes, as the median difference, consistent with the non-parametric tests used. Outcomes were compared separately at each time point; the correlation among repeated measurements within the same patient was not modeled, given the limited sample size. Statistical analyses were performed in STATA, Release 16.0 (StataCorp LLC, College Station, TX, USA).

3. Results

3.1. Study Population

Of the patients screened and assessed for eligibility, 32 were randomized (16 per group) and completed the trial with no dropouts or protocol deviations; participant flow through screening, randomization, treatment, and analysis is summarized in the CONSORT flow diagram (Figure 1). All randomized patients were analyzed according to their allocated group (intention-to-treat). Compliance with the rinsing regimen, verified through the returned bottles at day 14, was complete in all patients in both groups.

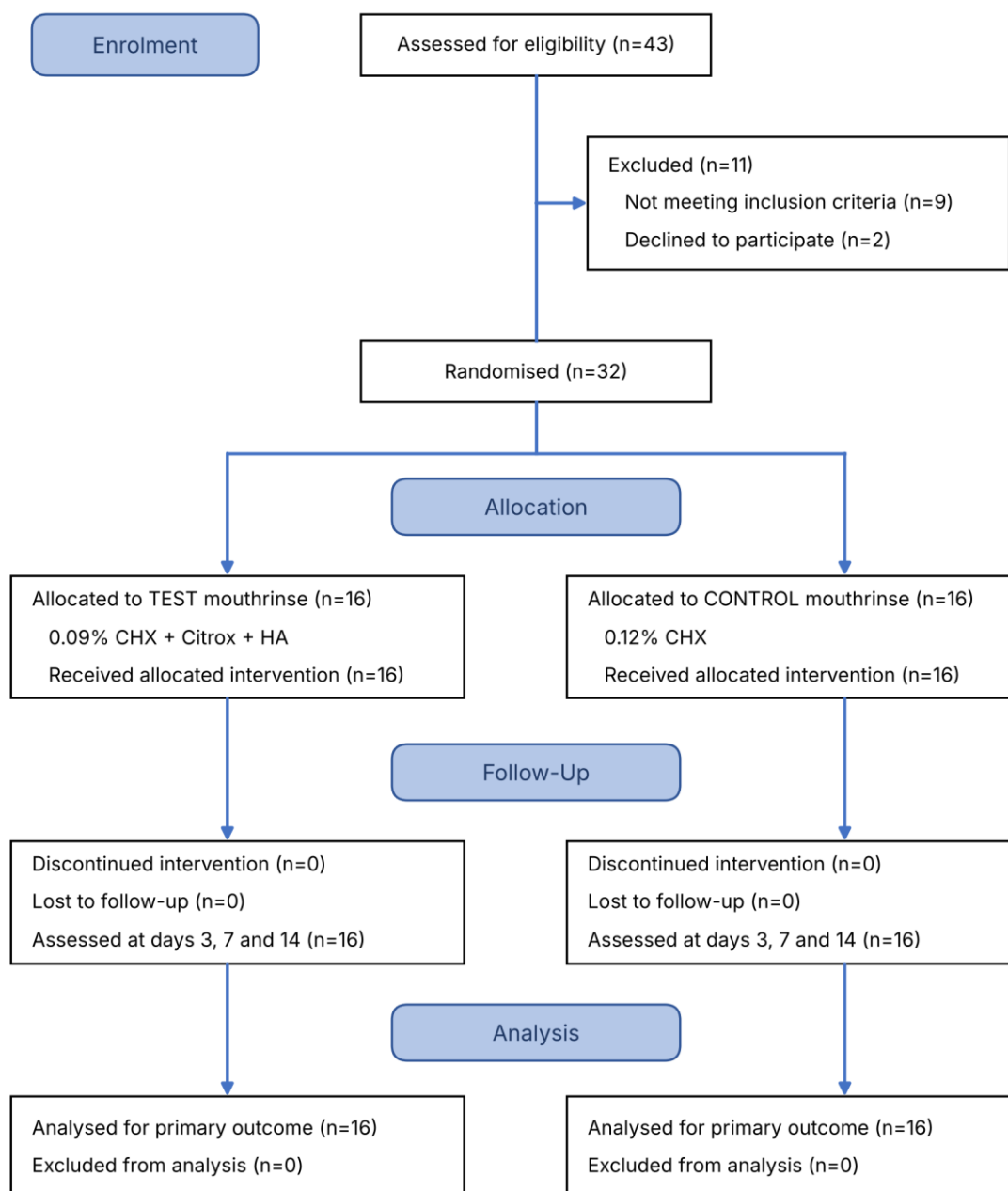


Figure 1. CONSORT flow diagram of patient enrolment, randomization, allocation, follow-up, and analysis.

All enrolled patients presented with generalized periodontitis, according to the 2018 EFP/AAP classification [34]: 29 patients (TEST 15, CONTROL 14) were diagnosed with Stage III, three patients (TEST 1, CONTROL 2) with Stage IV periodontitis, 27 patients (TEST 14, CONTROL 13) with Grade C, and five patients (TEST 2, CONTROL 3) with Grade B. The two groups were balanced at baseline for age, sex, smoking status, periodontitis stage and grade, and surgical site characteristics (Table 1). Most of the patients were non-smokers (24/32); the eight smokers (TEST 3, CONTROL 5) all smoked fewer than 10 cigarettes/day. The treated sites were located in posterior sextants in 29 patients (TEST 14, CONTROL 15), and in anterior sextants in three patients (TEST 2, CONTROL 1); flaps involved two to four teeth (mean of 2.88 ± 0.50 teeth in both groups). None of the treated sites presented intrabony defects or furcation-involved molars. The surgical duration was not recorded.

Rescue paracetamol was taken by all of the patients in both groups during the first three post-operative days, although the number of doses varied among the patients and was not quantified. No patient took paracetamol after day 3.

Table 1. Baseline patient characteristics and comparability between groups.

Variable	TEST	CONTROL	p-Value
Age (years), mean $\pm$ SD	52.47 $\pm$ 8.85	53.31 $\pm$ 9.84	$p = 0.609$
Sex (F/M)	10F/6M	8F/8M	$p = 0.722$
Smoking status, non-smokers/smokers <10 cig/day (n)	13/3	11/5	$p = 0.685$
Periodontitis extent, localized/generalized (n)	0/16	0/16	$p = 1.000$
Periodontitis stage, III/IV (n)	15/1	14/2	$p = 1.000$
Periodontitis grade, B/C (n)	2/14	3/13	$p = 1.000$
Surgical site, posterior/anterior (n)	14/2	15/1	$p = 1.000$
Flap extent (teeth), mean $\pm$ SD	2.88 $\pm$ 0.50	2.88 $\pm$ 0.50	$p = 1.000$

3.2. Primary Outcome: Edema

Edema, the primary outcome of the trial, occurred in a similar proportion of the patients in both groups at every subsequent time point (e.g., day 3: 37.5% in both groups), with no significant between-group difference at day 3, day 7, or day 14 (Fisher's exact $p = 1.000$; risk difference at day 3: 0.0%, 95% CI -30.5% to 30.5% ; Table 2). The wide confidence intervals, however, indicate that clinically relevant differences in either direction cannot be excluded.

Table 2. Edema (primary outcome) by group and time point. Risk difference with 95% CI.

Time Point	TEST n (%)	CONTROL n (%)	Risk Difference, % (95% CI)	p-Value
Baseline (T0)	0 (0.0%)	0 (0.0%)	—	—
Day 3 (T1)	6 (37.5%)	6 (37.5%)	0.0 (-30.5 to 30.5)	$p = 1.000$
Day 7 (T2)	3 (18.8%)	2 (12.5%)	6.3 (-20.2 to 32.1)	$p = 1.000$
Day 14 (T3)	1 (6.3%)	1 (6.3%)	0.0 (-22.7 to 22.7)	$p = 1.000$

3.3. Wound Healing: Early Wound Healing Score (EHS) and Its Components

The EHS increased markedly and comparably in both groups over the study period, from a mean of 5.25 (both groups) at day 3 to 9.13 (TEST) and 8.94 (CONTROL) at day 14, with no significant between-group difference at any time point (Table 3). Representative clinical photographs of the surgical site at baseline and at days 3, 7, and 14 for one patient

from each group are shown in Figure 2, illustrating the comparable course of soft-tissue healing described by the EHS. Its three components were analyzed as ordinal-categorical variables: re-epithelialization (CSR, scored 0, 3, or 6) showed no significant between-group difference at any time point, nor did hemostasis (CSH) or inflammation (CSI) (Table 4). Median EHS values were likewise similar between the groups at every time point (Table 3).

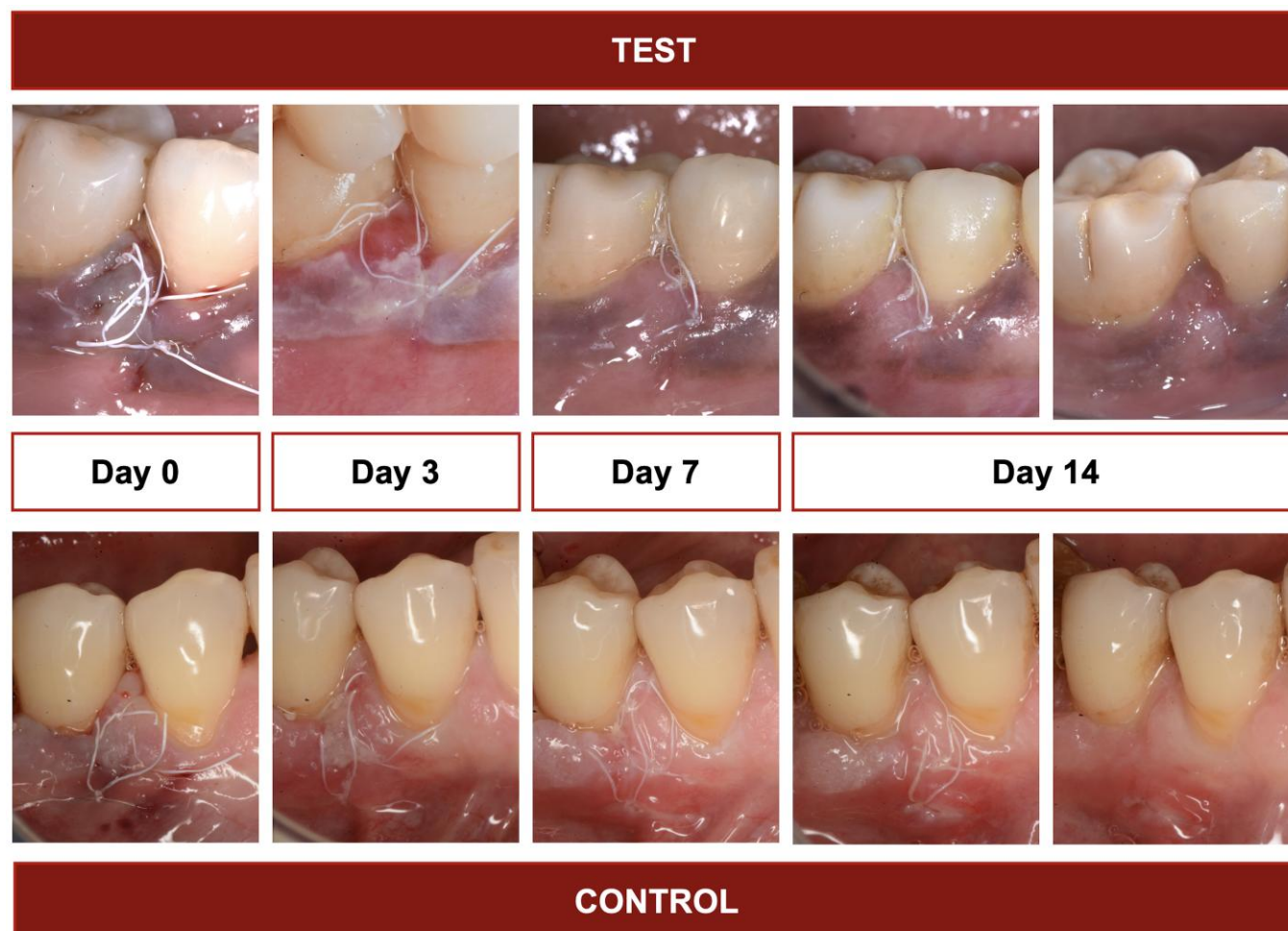


Figure 2. Representative clinical photographs of the surgical site at baseline and at 3, 7, and 14 days (before and after suture removal) for one patient from the TEST group and one from the CONTROL group.

Table 3. Early Wound Healing Score (EHS) by group and time point. Median difference with 95% CI.

Time Point	TEST Mean $\pm$ SD Median (IQR)	CONTROL Mean $\pm$ SD Median (IQR)	Median Difference (95% CI)	<i>p</i> -Value
Day 3 (T1)	5.25 $\pm$ 2.21 5.00 (4.50–6.00)	5.25 $\pm$ 2.32 5.50 (4.00–7.00)	0.00 (−2.00 to 2.00)	<i>p</i> = 0.931
Day 7 (T2)	7.31 $\pm$ 2.33 8.00 (6.00–9.00)	7.31 $\pm$ 2.60 8.50 (6.00–9.00)	0.00 (−2.00 to 1.00)	<i>p</i> = 0.769
Day 14 (T3)	9.13 $\pm$ 1.63 10.00 (9.00–10.00)	8.94 $\pm$ 1.98 10.00 (9.00–10.00)	0.00 (0.00 to 1.00)	<i>p</i> = 0.788

Table 4. EHS component (CSR, CSH, and CSI) score distribution by group and time point.

CSR:			
Time point	TEST <i>n</i> (scores 0/3/6)	CONTROL <i>n</i> (scores 0/3/6)	<i>p</i> -value
Day 3 (T1)	4/9/3 (25%/56%/19%)	3/10/3 (19%/63%/19%)	<i>p</i> = 1.000
Day 7 (T2)	1/6/9 (6%/38%/56%)	3/3/10 (19%/19%/63%)	<i>p</i> = 0.359
Day 14 (T3)	0/3/13 (0%/19%/81%)	1/2/13 (6%/13%/81%)	<i>p</i> = 1.000
CSH:			
Time point	TEST <i>n</i> (scores 0/1/2)	CONTROL <i>n</i> (scores 0/1/2)	<i>p</i> -value
Day 3 (T1)	0/12/4 (0%/75%/25%)	0/13/3 (0%/81%/19%)	<i>p</i> = 1.000
Day 7 (T2)	0/8/8 (0%/50%/50%)	0/5/11 (0%/31%/69%)	<i>p</i> = 0.473
Day 14 (T3)	0/1/15 (0%/6%/94%)	0/0/16 (0%/0%/100%)	<i>p</i> = 1.000
CSI:			
Time point	TEST <i>n</i> (scores 0/1/2)	CONTROL <i>n</i> (scores 0/1/2)	<i>p</i> -value
Day 3 (T1)	1/11/4 (6%/69%/25%)	5/5/6 (31%/31%/38%)	<i>p</i> = 0.092
Day 7 (T2)	1/9/6 (6%/56%/38%)	0/11/5 (0%/69%/31%)	<i>p</i> = 0.716
Day 14 (T3)	0/4/12 (0%/25%/75%)	0/5/11 (0%/31%/69%)	<i>p</i> = 1.000

3.4. Plaque Index (TQHPI)

The plaque index was comparable between the groups at baseline (mean 0.31 ± 0.13 TEST vs. 0.28 ± 0.14 CONTROL; *p* = 0.323). Both groups showed a progressive increase in the index over the study period, as expected, given the suspension of mechanical tooth-brushing at the surgical site. From day 7 onward, however, the TEST group accumulated significantly more plaque than the CONTROL group: at day 7, the mean values were 0.68 ± 0.23 versus 0.47 ± 0.21 (*p* = 0.003), and at day 14, they were 0.73 ± 0.25 versus 0.53 ± 0.45 (*p* = 0.005; Table 5). The median difference was 0.20 (95% CI 0.10 to 0.40) at day 7 and 0.30 (95% CI 0.10 to 0.40) at day 14.

Table 5. Plaque index (TQHPI) by group and time point. Median difference with 95% CI.

Time Point	TEST Mean $\pm$ SD	CONTROL Mean $\pm$ SD	Median Difference (95% CI)	<i>p</i> -Value
Baseline (T0)	0.31 ± 0.13	0.28 ± 0.14	0.00 (0.00 to 0.10)	<i>p</i> = 0.323
Day 3 (T1)	0.44 ± 0.08	0.48 ± 0.22	0.00 (−0.10 to 0.10)	<i>p</i> = 0.822
Day 7 (T2)	0.68 ± 0.23	0.47 ± 0.21	0.20 (0.10 to 0.40)	<i>p</i> = 0.003
Day 14 (T3)	0.73 ± 0.25	0.53 ± 0.45	0.30 (0.10 to 0.40)	<i>p</i> = 0.005

3.5. Gingival Index (GI)

The gingival index increased in both groups relative to baseline, peaking at day 3–day 7 (a mean of up to 0.94 in the CONTROL group), before decreasing by day 14. The between-group comparison never reached statistical significance at any time point (all *p* $\geq$ 0.22; Table 6), indicating a comparable time-course of the GI between the two treatments.

Table 6. Gingival index (GI) by group and time point. Median difference with 95% CI.

Time Point	TEST Mean $\pm$ SD	CONTROL Mean $\pm$ SD	Median Difference (95% CI)	<i>p</i> -Value
Baseline (T0)	0.06 ± 0.25	0.06 ± 0.25	0.00 (0.00 to 0.00)	<i>p</i> = 1.000
Day 3 (T1)	0.88 ± 0.72	0.94 ± 0.68	0.00 (−1.00 to 0.00)	<i>p</i> = 0.867
Day 7 (T2)	0.63 ± 0.62	0.88 ± 0.50	0.00 (−1.00 to 0.00)	<i>p</i> = 0.221
Day 14 (T3)	0.31 ± 0.48	0.38 ± 0.50	0.00 (0.00 to 0.00)	<i>p</i> = 1.000

3.6. Tooth/Tongue Staining Index (SI)

The staining index was comparable between the groups at baseline and increased progressively in both groups over the study period, consistent with the well-known staining potential of chlorhexidine present in both formulations (Table 7). The between-group difference reached statistical significance ($p = 0.008$) at day 14, reflecting a difference in the shape of the distribution. Heavy staining (score 3) occurred exclusively in the CONTROL group (4/16, 25%). Scores in the TEST group were concentrated at light staining (score 1: 12/16), whereas the CONTROL group showed a wider spread, with both more patients without staining (5/16 vs. 2/16) and all cases of heavy staining.

Table 7. Staining index (SI) score distribution by group and time point.

Time Point	TEST <i>n</i> (Scores 0/1/2/3)	CONTROL <i>n</i> (Scores 0/1/2/3)	<i>p</i> -Value
Baseline (T0)	15/1/0/0 (94%/6%/0%/0%)	15/1/0/0 (94%/6%/0%/0%)	$p = 1.000$
Day 3 (T1)	10/5/1/0 (63%/31%/6%/0%)	9/7/0/0 (56%/44%/0%/0%)	$p = 0.716$
Day 7 (T2)	6/7/3/0 (38%/44%/19%/0%)	7/7/2/0 (44%/44%/13%/0%)	$p = 1.000$
Day 14 (T3)	2/12/2/0 (13%/75%/13%/0%)	5/3/4/4 (31%/19%/25%/25%)	$p = 0.008$

3.7. Adverse Events: Oral Ulcers and Dysgeusia

Oral ulcers were reported more frequently in the TEST group than in the CONTROL group at every time point (e.g., day 3: 31.3% vs. 12.5%; day 7: 25.0% vs. 6.3%), but the difference did not reach statistical significance at any time point (Table 8). Dysgeusia was never reported in the TEST group throughout this study (0% at every time point), whereas an increasing proportion of CONTROL patients reported it (6.3% at day 3, 18.8% at days 7 and 14); this difference also did not reach statistical significance (Table 9).

Table 8. Oral ulcers by group and time point.

Time Point	TEST <i>n</i> (%)	CONTROL <i>n</i> (%)	<i>p</i> -Value
Baseline (T0)	0 (0.0%)	0 (0.0%)	—
Day 3 (T1)	5 (31.3%)	2 (12.5%)	$p = 0.394$
Day 7 (T2)	4 (25.0%)	1 (6.3%)	$p = 0.333$
Day 14 (T3)	2 (12.5%)	1 (6.3%)	$p = 1.000$

Table 9. Dysgeusia by group and time point.

Time Point	TEST <i>n</i> (%)	CONTROL <i>n</i> (%)	<i>p</i> -Value
Baseline (T0)	0 (0.0%)	0 (0.0%)	—
Day 3 (T1)	0 (0.0%)	1 (6.3%)	$p = 1.000$
Day 7 (T2)	0 (0.0%)	3 (18.8%)	$p = 0.226$
Day 14 (T3)	0 (0.0%)	3 (18.8%)	$p = 0.226$

4. Discussion

Mechanical plaque removal remains the safest and most effective form of daily oral hygiene, but it is deliberately withheld at the surgical site in the days following periodontal flap surgery to protect the fragile healing wound margins, making chemical plaque control a necessary adjunct rather than an optional addition during this window [2–4]. The choice of active agent must balance antimicrobial efficacy against the risk of side effects that could themselves undermine compliance and, indirectly, the quality of healing. This trial compared a reduced-concentration CHX formulation (0.09%) enriched with Citrox and hyaluronic acid against the conventional 0.12% CHX rinse that remains the reference formulation for this indication [10,15].

Edema, the primary outcome of the trial, was infrequent and similarly distributed between the groups at every time point, and the proportion of the patients free of edema increased progressively over the study period in both arms, consistent with uneventful healing [35]. Among the secondary outcomes, the EHS likewise showed similar healing trajectories in both groups: the mean values rose from just above 5 at day 3 to approximately 9 by day 14, with no significant between-group difference at any time point. Because the EHS is a composite index that separately captures re-epithelialization, hemostasis, and inflammation before combining them into a single score [5], this result suggests that none of the individual biological components of early healing was detectably compromised by the lower CHX concentration in the test rinse, although the limited sample size does not allow equivalence to be inferred. The only signal of a possible difference concerned the inflammation component (CSI) at day 3, where marked inflammation (the lowest possible score) was recorded in a larger share of the CONTROL than TEST patients; the difference approached but did not cross the significance threshold and had disappeared by later visits.

The clearest between-group difference concerned the plaque index, which was significantly higher in the TEST group from day 7 onward. A plausible, though untested, explanation lies in the composition of the formulation. CHX combines antiplaque and anti-inflammatory activity in a single molecule, and its antiplaque effect is concentration-dependent [14]; reducing its concentration from 0.12% to 0.09% would, therefore, be expected to reduce plaque inhibition unless fully compensated by the added agents. Citrox, in turn, has documented antibacterial and anti-inflammatory properties [16,17,19], but its ability to directly interfere with plaque formation and retention appears more limited [17]. This preserved anti-inflammatory profile alongside reduced plaque control would be consistent with the pattern observed across the other clinical indices. The gingival index, despite the higher plaque scores in the TEST group, was lower in the TEST group than in the CONTROL group at every post-operative time point. This represents a numerical trend that should not be over-interpreted, since the present trial did not include any mechanistic or biomarker endpoint capable of testing this hypothesis.

In absolute terms, the between-group difference in the plaque index was approximately 0.2–0.3 points on the 0–5 TQHPi scale (median difference 0.20, 95% CI 0.10 to 0.40, at day 7 and 0.30, 95% CI 0.10 to 0.40, at day 14). A difference of this size is small and of uncertain clinical relevance over a two-week period. Moreover, because mechanical toothbrushing was withheld at the surgical site in both groups, the observed plaque accumulation reflects the chemical plaque-control capacity of each rinse in the absence of any mechanical contribution, and may not extrapolate to settings in which mechanical hygiene is resumed.

Tooth and tongue staining is one of the best-known drawbacks of chlorhexidine, and it was the only secondary outcome, besides plaque, to show a clear between-group difference: at day 14 the distribution of staining scores differed significantly, with heavy staining recorded exclusively in the CONTROL group. Because staining accumulates visibly over the two weeks of rinsing, and because patients are generally well aware of this side effect, it represents a plausible trigger for reduced adherence to the post-surgical protocol, exactly during the period when biofilm and inflammation control matter most [13,27]. Since heavy staining was observed only in the control group, the lower-concentration, Citrox-containing formulation tested here may represent an alternative route, alongside dedicated anti-discoloration systems [27], to preserving patient compliance for the full duration of the critical healing window. In the present trial, however, adherence was complete in both groups, so this potential advantage could not be demonstrated directly.

Oral ulcers were numerically more frequent in the TEST group at every time point, although the difference did not reach statistical significance. Numerically, the ulcers also appeared to resolve more readily in the TEST group: the count fell from five to two

patients between day 3 and day 14 (a 60% reduction), compared with a more modest reduction from two to one patient in the CONTROL group, even though rinsing with the assigned formulation continued throughout. Very little comparable literature exists, since the two earlier trials directly comparing CHX with or without hyaluronic acid did not report mucosal ulceration as an outcome [26,28]; a plausible, though speculative, explanation is a contribution from hyaluronic acid, which is known to support soft tissue repair at both the clinical and molecular level [21,22,29,36]. However, this remains a hypothesis to be tested in future studies.

Dysgeusia was less frequent with the test formulation: no TEST patient reported taste alteration at any time point, compared with a rising proportion of the CONTROL patients (one at day 3, three at both day 7 and day 14), although this difference did not reach statistical significance in this sample. Taste alteration is a well-recognized and rather bothersome side effect of chlorhexidine [11,13], and, like staining, has the potential to reduce both post-operative comfort and adherence to the prescribed rinsing schedule.

These findings can be usefully compared with two closely related trials. Trombelli et al. [28] compared a 0.2% CHX rinse combined with hyaluronic acid and an anti-discoloration system with 0.2% CHX alone after flap surgery; gingival healing, plaque, and staining scores were generally comparable between groups, with the single exception of a significantly higher gingival index in the combination-rinse group at day 7. In the present trial, gingival inflammation (GI) did not differ between the groups, while plaque control was significantly better with the higher-concentration comparator, and the distribution of staining scores differed significantly, with heavy staining observed only with the higher-concentration comparator. More recently, Graziani et al. [29] compared a CHX + anti-discoloration system with or without HA against an untreated control after periodontal surgery, and found that CHX-based rinsing improved early wound healing, with a further benefit of HA on soft-tissue closure; our comparable EHS trajectories in both arms are consistent with a preserved healing benefit of chemical plaque control, though our design cannot isolate the specific contribution of HA.

This study has several limitations. The sample size, although calculated a priori for the primary outcome, remains modest (16 patients per arm) and is likely underpowered to detect small or moderate between-group differences in the secondary outcomes. The study used for the sample size calculation was based on data from implant-surgery procedures [26], which may not fully reflect edema incidence after periodontal flap surgery. The single-center design limits the generalizability of the findings. Follow-up was limited to 14 days; therefore, later stages of wound maturation and remodeling were not captured, and future trials should include longer follow-up. The experimental VRI was placed at a healthy site without attachment loss to provide a standardized wound [5]; while this reduces confounding from pocket depth, defect morphology and root instrumentation, the findings describe the healing of a standardized releasing incision rather than the full range of wound configurations encountered in periodontal surgery (e.g., more extensive flaps or regenerative procedures), and generalization to those settings should be made with caution. The primary outcome, edema, mainly reflects surgical trauma and the host inflammatory response. Outcomes were compared separately at each time point, without modeling the correlation between repeated measurements within patients and without correction for multiplicity. Surgical duration was not recorded, and patient-centered outcomes, such as post-operative pain, patient satisfaction, and cost, were not assessed. Future multi-center trials with larger samples should address these aspects, and all secondary findings of the present study should be regarded as preliminary.

5. Conclusions

Within the limits of this trial, no statistically significant differences were detected between the 0.09% CHX + Citrox + HA mouthrinse and the conventional 0.12% CHX reference formulation in the assessed wound-healing outcomes (edema, EHS and its components, and GI). The test formulation was associated with higher plaque scores from day 7 onward and with a different distribution of staining scores at day 14, heavy staining being observed only in the CONTROL group. These findings do not establish equivalence between the two formulations and should be confirmed in larger, adequately powered trials.

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Abbreviations

The following abbreviations are used in this manuscript:

CHX	Chlorhexidine
HA	Hyaluronic Acid
EHS	Early Wound Healing Score
CSR	Clinical signs of re-epithelialization
CSH	Clinical signs of hemostasis
CSI	Clinical signs of inflammation
TQHP	Turesky-modified Quigley–Hein Plaque Index
GI	Gingival Index
SI	Staining Index
VRI	Vertical Releasing Incision

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