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ORIGINAL ARTICLE

A randomized clinical trial on the clinical and microbiological efficacy of a xanthan gel with chlorhexidine for subgingival use

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Abstract
Background The main indication of the adjunctive use of local antimicrobials lies around situations in which the outcome of non-surgical mechanical treatment results in a limited number of residual pockets. The purpose of this investigation was to evaluate the clinical and microbiological effects of the subgingival application of a xanthan-based 1.5% chlorhexidine (CHX) gel (Xan–CHX), adjunctive to scaling and root planing (SRP) in localized periodontitis.
Methods Periodontitis patients with four to ten residual (after conventional SRP) or relapsing (during supportive periodontal treatment) pockets were recruited and randomized to receive SRP plus the subgingival application of (Xan–CHX) or SRP plus a placebo gel. Supragingival plaque, bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment level were evaluated with a computerized probe at baseline, and after 1, 3, and 6 months. Subgingival samples were also collected for the microbiological analysis. Statistical analysis used ANOVA and chi-square tests.
Results Overall, the clinical results were better in the test group, with significant changes in BOP (between baseline and 3 months) and with a significant increase in the proportion of shallow pockets (1–3 mm) at 6 months. These results did not result in significant intergroup differences. The microbiological impact was limited in both treatment groups.
Conclusion The adjunctive use of Xan–CHX may improve, although to a limited extent, the clinical outcomes (BOP and PPD), in chronic periodontitis patients with "residual" or "relapsing" pockets, but no significant differences were detected between groups. No side effects, neither clinical nor microbiological, were detected after the use of the test product.
Clinical relevance Adjunctive use of slow-released chlorhexidine might be considered in the management of periodontal disease and gingival inflammation to reduce the need for periodontal surgery.

Keywords Chlorhexidine · Local antimicrobials · Xanthan gum · Periodontitis · Therapy

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Introduction

Periodontal diseases are plaque-induced chronic inflammatory conditions affecting the periodontium. In periodontitis, the disease process involves destruction of the tooth-supporting tissues that if left untreated, can lead to mobility and subsequent tooth loss [1]. Periodontitis is caused by microorganisms residing in the subgingival biofilm that require a susceptible host to elicit the chronic inflammatory reaction responsible of the tissue destruction. Although more than 500 different microorganisms can be found in the subgingival microbiota, only a limited number of bacterial species, the so-called periodontal pathogens, such as *Aggregatibacter actinomycetemcomitans*, *Porphyromonas*

gingivalis, and *Tannerella forsythia*, have been strongly associated to periodontitis [2].

Even though there is still controversy on the specific role of these pathogens in the pathogenesis of periodontitis, the main focus of the therapeutic interventions to treat and to prevent this disease is still based on the control of these pathogenic bacteria [3]. The gold standard in the treatment of periodontitis is the mechanical debridement of the pockets by scaling and root planing (SRP), thus aiming for the elimination or disruption of the subgingival biofilm, which together with the adjunctive supragingival plaque control have widely proven their efficacy in the prevention and treatment of most periodontal diseases [4, 5]. Mechanical debridement, however, is a highly demanding therapeutic procedure and presents a number of shortcomings and limitations, mainly related with the inability to access to deep, tortuous pockets, and furcations, as well as to control certain pathogens. Moreover, there are well-documented secondary effects of this therapy (gingival recession, loss of tooth substance, dentin hypersensitivity, etc.) [4, 6].

To overcome these limitations, different adjunctive therapies have been proposed, mostly associated to the adjunctive use of antimicrobial agents, either systemically or locally [5, 7, 8]. The local application of antimicrobials may be cumbersome and time consuming when the periodontitis is generalized, but has clear advantages in presence of localized pockets or in the treatment of non-responding and recurrent sites [8–10]. In these cases, locally applied antimicrobials lack of the adverse effects associated with systemic medications and do not depend on the patient's compliance.

The main indication of the adjunctive use of local antimicrobials lies around situations in which the outcome of non-surgical mechanical treatment results in a limited number of residual pockets and this mode of therapy might be an alternative to surgical debridement [8, 11, 12]. Other indications are the frequent situations during the course of supportive periodontal treatment (SPT), where there is a local relapse in the periodontitis, characterized by localized deep probing pockets depths in combination with bleeding on probing [13–16]. In all these situations, patients may be unwilling to undergo surgical therapy [17–19] and furthermore, the cost–benefit ratio of such surgical interventions might be questioned [20], which highlights the need for alternative treatment options, such as the effective use of locally applied antimicrobials. For a locally applied antimicrobial to be effective in the adjunctive treatment of deep pockets, its antimicrobial activity must reach high concentrations inside the pocket and maintain these high levels during a period of at least 7 days [21, 22].

Among the broad range of available antimicrobial agents, a limited number has shown efficacy when applied locally in the treatment of periodontitis. They are mainly antibiotics, such as minocycline, doxycycline, metronidazole, and tetracycline, although the antiseptic agent chlorhexidine (CHX) has also demonstrated adjunctive clinical efficacy [8, 11, 13, 23, 24]. In fact, the main requirement for a local agent to be effective, more than its nature, is its permanence and bio-availability in the subgingival environment [25]. Using antiseptics versus antibiotics, however, has the clear advantage of reducing the chances of developing multi-bacterial resistances [7]. CHX when used as an irrigant or vehicled in gels has the important limitation of its high clearance from the pocket due to the cleansing action of the crevicular fluid [7, 26–28]. Any CHX formulation aiming to provide a sustained effect in the subgingival environment must, therefore, include a vehicle with intrinsic capacity to maintain antimicrobial levels beyond concentration breakpoints during sufficient time. A xanthan gel chemically linked to the CHX molecule has demonstrated in vitro its capacity to maintain adequate CHX concentrations and a highly stable pharmacokinetic profile inside the periodontal pocket [29]. Using this formulation, a CHX-based local antimicrobial has been recently marketed as a xanthan-based syringable gel system (ChloSite®, Casalecchio di Reno, Bologna, Italy). This gel is a combination of two CHX formulations: 0.5% CHX digluconate and 1.0% CHX dihydrochloride. CHX digluconate is liberated in the first day and achieves a concentration >100 μg/ml, which is maintained for an average of 6–9 days. CHX dihydrochloride is released in the following days and maintains the bacteriostatic and bactericidal concentrations for at least 2 weeks (>0.10 μg/ml) [24].

The efficacy of this local antimicrobial formulation has been recently tested in a multicenter clinical trial [30] demonstrating that the adjunctive use of a xanthan gel with CHX (Xan–CHX) promoted greater probing pocket depth (PPD) reductions and clinical attachment level (CAL) gains than the standard therapy consisting on SRP alone. This study, however, selected patients with generalized periodontitis and used a split-mouth design site-based analysis that may limit the evaluation of the real efficacy of the adjunctive use of (Xan–CHX). With the purpose of evaluating the clinical and microbiological effects of the subgingival application of a xanthan-based 1.5% CHX gel adjunctive to SRP in localized periodontitis, we have designed this parallel clinical trial comparing the adjunctive subgingival application of versus the application of a placebo. Our hypothesis was that the (Xan–CHX) subgingival application had a microbiological added impact on the subgingival biofilm, when used as an adjunct to mechanical debridement in the treatment of localized periodontitis.

Patients and methods

Study population

Consecutive patients from the Graduate Clinic of Periodontology at the Complutense University, Madrid, were enrolled

for this study. All patients signed an informed consent after receiving detailed information about the purpose, the benefits, and the possible risks associated with the trial. The Clinical Research Committee of the San Carlos University Hospital had previously approved the protocol, the patient's information, and informed consent forms.

Individuals satisfying the following entry criteria were recruited:

- Adult patients, older than 30 years old with at least 16 remaining teeth and at least 3 teeth in each quadrant.
- History of periodontal disease as demonstrated by generalized radiographic bone loss (greater than one third of the root length).
- Prior periodontal treatment (non-surgical) in the previous 6 months or patients in a supportive periodontal therapy for at least 1 year.
- Presence of a limited number (between four and ten) of deep pockets (PPD>4 mm) that bled on probing at the post-treatment evaluation ("residual" pockets) or at a programmed supportive visit ("relapsing" pockets).
- No systemic antimicrobial treatment in the previous 4 weeks.
- No acute periodontal conditions, such as necrotizing periodontal diseases or periodontal abscesses.
- No known allergies to CHX or any of the components in the tested products.

Study design and interventions

The study was as a randomized, placebo-controlled, parallel-designed 6-month clinical trial.

Treatment phase I: instruction of oral hygiene procedures After having entered the study, all patients received the following procedures:

- Individualized oral hygiene instructions together with the provision of a new toothbrush, dental floss or interdental brushes, and a fluoridated dentifrice.
- Full-mouth periodontal examination and retrieval of microbiological plaque samples from selected sites.
- Full-mouth supragingival professional prophylaxis using ultrasonic/hand-instruments.

Treatment phase II: re-instrumentation of selected sites plus application of the assigned local antimicrobial therapy The treatment was performed in one session. Under local anesthesia, an experienced operator, blinded to the treatment assignment, scaled the selected sites by means of an ultrasonic device and Gracey curettes. The instrumentation was carried out until the operator felt a planed and well-debrided root surface.

Once the roots were debrided, the experimental or placebo gel formulations were applied. The test product contained two CHX formulations in a xanthan vehicle. The placebo gel contained the same vehicle without any active ingredients. After isolating and drying the selected sites, the assigned gel formulation was subgingivally applied using a needle with a blunt tip and a lateral opening for avoiding any trauma to the tissues. Once the selected pockets were filled with the gel, a periodontal dressing (Peripac®, Dentsply, Germany) was applied and left in place for at least 3 days to protect the site and avoid any spill over of the gel.

In each subject, an external agent through a computer-generated list randomly assigned test or placebo treatments by coding identical syringes with either test or placebo gels with consecutive numbers. These treatment assignment codes were kept in the appropriate registration forms by the central registrar (D.H) who was blind to the therapist and the clinical examiner. Allocation concealment was performed by opaque sealed envelopes, sequentially numbered, which were opened immediately after completing the root debridement.

Follow-up visits were scheduled after 1, 3, and 6 months. At these visits, microbiological samples were taken from the same selected sites followed by a clinical examination assessing the study clinical outcome measurements. Oral hygiene was reinforced at each visit, but no further treatment was provided. The occurrence of any adverse events was recorded, including staining of both teeth and oral mucosa. After the last visit, patients were provided with a full-mouth professional prophylaxis.

Clinical measurements

The following clinical outcome variables were recorded at baseline, 1, 3, and 6 months at the selected teeth, at six sites per tooth, by means of a computerized, electronic periodontal probe (Florida® probe, Florida Probe Corporation, Gainsville, FL, USA) by two calibrated blinded examiners:

- Plaque index (PlI) using a dichotomous scale (present/absent).
- Bleeding on probing (BOP), through visual inspection 20 s after probing, using a dichotomous scale (present/absent) [31].
- Probing pocket depth (PPD) in millimeters, measured from the gingival margin to the deepest stop of the periodontal pocket at the standardized force.
- Recession (REC) in millimeters, measured from the gingival margin to the cemento–enamel junction or to the margin of a cervical restoration.
- Clinical attachment level (CAL), calculated by adding PPD and REC at each site.

- Degree of furcation involvement (0–3), based on the amount of horizontal periodontal tissue destruction that had occurred in the inter-radicular area [32].
- Tooth mobility (0–3).

Microbiological study

Prior to the clinical examination, a pooled sample was obtained from the selected sites. Microbiological samples were taken by inserting two paper points per site that were kept in place for 10 s, consecutively, and then pooled in the same 1.5 mL RTF (reduced transport fluid) vial [33] with the other plaque samples. They were transported to the laboratory within 2 h, where they were dispersed (30 s of Vortex), serially diluted and inoculated on two different media:

(a) Blood agar medium (no. 2 of Oxoid; Ltd. de Oxoid, Basingstoke, UK), with horse serum at 5%, and with haemin (5 mg/l) and menadion (1 mg/l).
(b) Dentaid-1 medium [34].

The blood agar plates were checked after 7 and 14 days of anaerobic incubation in an 80% N_2; 10% H_2; 10% CO_2 at 37°C atmosphere; the plates in Dentaid-1 medium were studied after 3–5 days at 37°C in a micro-aerophilic atmosphere (5% CO_2).

Total microbial counts were calculated from the blood agar plates. On these plates, *P. gingivalis, Prevotella intermedia*, *T. forsythia, Parvimonas micra*, *Campylobacter rectus*, and *Fusobacterium nucleatum* were identified through their colony morphology and the different chemical tests used to confirm the preliminary identification. For every specific bacterial species, counts and percentages relative to the total flora were calculated. *A. actinomycetemcomitans* was identified on the Dentaid-1 medium plates based on the colony morphology and the positive reaction to catalase.

Adverse effects

At every visit, the occurrence of any undesirable side effects or adverse circumstances that could be related to the treatment was recorded.

Statistical analyses

Sample size calculation The sample size was calculated using α=0.05 and the power (1−β)=80%. For the variability (σ = SD), the value of 0.5 mm was used, considering PPD change from baseline to 6 months as the main outcome variable, with a desired difference of 1.03 mm. On the basis of these data, the number of enrolled patients to conduct this study was calculated as ten patients per arm. However, considering the possibility of having a certain amount of drop out patients (20%), the total number of requested patients was 12 per treatment group.

Statistical methods Only the data from the selected sites were processed, but the patient was considered as the statistical unit. The obtained clinical outcome variables were calculated by patient, and then by treatment group.

For the evaluation of the intragroup changes between baseline and 1, 3, and 6 months the ANOVA test was used (once the normality of the distribution was proven), being the visit the factor and smoking the covariate.

For the evaluation of the intergroup comparisons, assessing the differences at each follow-up visit with baseline the ANOVA test was used, being the factor, the treatment group, and the baseline values and smoking, the covariates.

Microbiological outcome variables were considered as secondary variables. For comparing the results from the microbiological quantitative outcome variables (log-transformed total anaerobic counts), similar statistical methods were utilized. For the comparisons from the qualitative microbiological variables, we generated frequency distributions of the different pathogens detected in each treatment group and visits, and a chi-square test in 2×2 contingency tables was utilized as statistical test.

Demographic and qualitative variables, such as smoking and gender were also compared with the chi-square test in 2×2 contingency tables.

Results

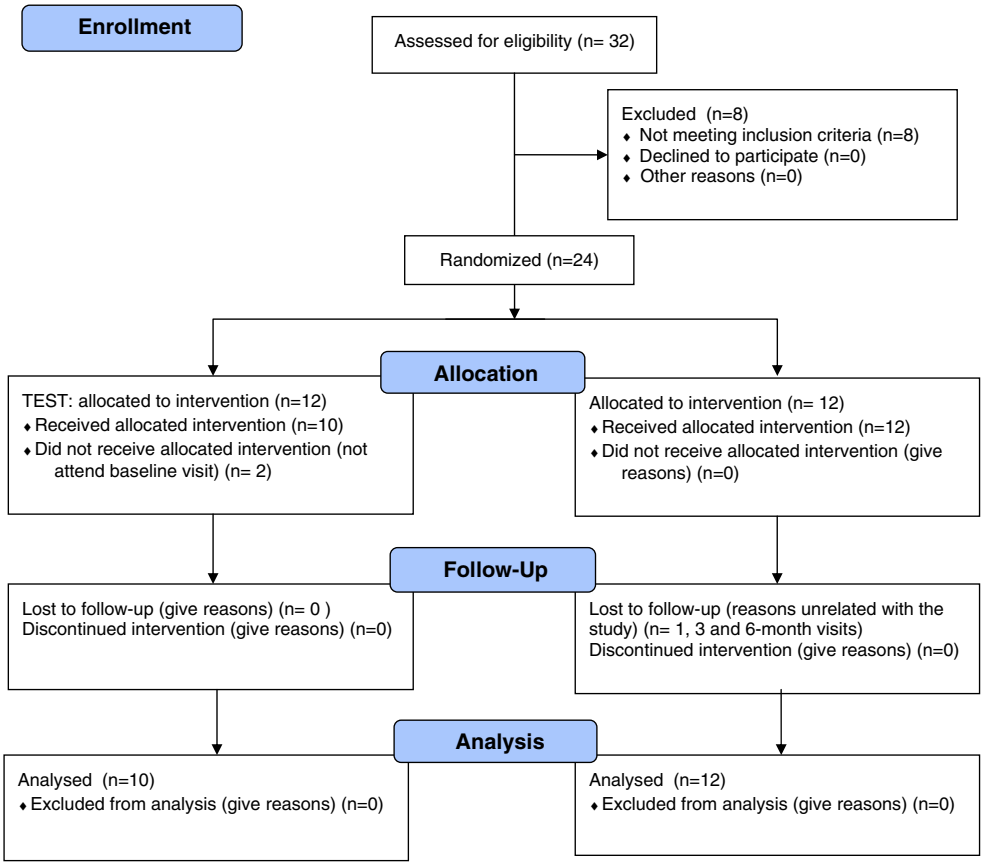
Study population

A total of 24 patients recruited between October 2005 and January 2007 participated in the study, but 22 were included in the analyses since two patients were randomized and received a study number, but did not attend the baseline visit (Fig. 1, flow chart). Twenty-one patients completed the study reaching the end of the follow-up period at 6 months. One patient in the placebo group dropped out between the 3- and 6-month visits due to reasons unrelated to the study. All the follow-up visits were completed by October 2007.

Subject characteristics at baseline

The patient's demographic characteristics are shown in Table 1. The mean age was 50 years in both groups. In the test group, three patients were smokers (≥9 cigarettes/day) versus two in the placebo group. Gender distribution included nine women in the placebo and five in the test group. No significant differences were detected between groups.

Fig. 1 Flow diagram



Changes in clinical outcome variables

The baseline examination revealed that both study groups demonstrated similar values for PlI, CAL, and PPD. The mean BOP percentage, however, was significantly higher ($p<0.028$) in the test group than in the placebo (Table 2). The frequency distributions by PPD categories, shallow (1–3 mm), intermediate (4–6 mm) and deep (≥6 mm), at baseline, were not significantly different between both groups.

Baseline values of PPD at the treatment sites were 3.58 and 3.72 mm in test and control, respectively ($p<0.449$). Reductions in mean PPD were registered at every follow-up visit. This PPD reduction was higher in the test group, although the differences were not statistically significant when compared with the control group at any visit (Table 2). Overall, the mean reduction in PPD after 6 months was 0.32 mm (±0.26 mm) in the test group versus 0.22 mm (±0.52 mm) in the placebo ($p<0.147$).

No change in CAL was observed in the control group at the end of the follow-up. In the test group, however, there was a mean CAL gain from baseline to 6 months of 0.30 mm. These differences, however, were not statistically different ($p<0.380$) (Table 2).

Mean BOP at baseline was 0.56 in the test and 0.37 in the control group ($p<0.028$). After treatment, a significant decrease between baseline and 3 months was observed in the test group ($p<0.039$) (Table 3). BOP reductions in the control group were not statistically significant.

The mean percentages of sites with different PPD categories are shown in Table 4. The percentage of pockets with PPD≥6 decreased in the test group from 5% to 1%, while only minor changes were observed in the placebo group. These differences, however, were not statistically significant. In moderate pockets, no relevant changes were detected between or within groups. In shallow pockets (1–3 mm), significant changes were observed only in the test group ($p<0.038$), corresponding to an increase in the percentage of these pockets between baseline and 6 months. In the control group, these changes were not statistically significant.

Table 1 Demographic data at baseline

	Placebo	Test
Mean age	50.2	50.0
Standard deviation	9.6	8.3
Range	36–71	36–59
N	12	10
Smokers, *n*	2	3
Non-smokers, *n*	10	7
Female, *n*	9	5
Male, *n*	3	5

Table 2 Mean probing pocket depth (PPD) and clinical attachment level (CAL) with standard error (St. error) and 95% confidence intervals (CI), at each visit and in changes between visits

PPD						
Group	Visit	*n*	Mean	St. error	CI	
Placebo	Baseline	12	3.73	0.13	3.46	3.99
	1 m	12	3.59	0.13	3.33	3.86
	3 m	12	3.54	0.13	3.27	3.80
	6 m	11	3.51	0.14	3.23	3.78
Test	Baseline	10	3.58	0.15	3.28	3.88
	1 m	10	3.30	0.15	3.00	3.60
	3 m	10	3.32	0.15	3.02	3.62
	6 m	10	3.26	0.15	2.96	3.56
Changes	Group	*n*	Mean	St. error	CI	
Baseline–1 month	Placebo	12	−0.11	0.11	−0.34	0.11
	Test	10	−0.30	0.12	−0.55	−0.06
Baseline–3 months	Placebo	12	−0.17	0.11	−0.39	0.06
	Test	10	−0.29	0.12	−0.53	−0.04
Baseline–6 months	Placebo	11	−0.20	0.12	−0.46	0.06
	Test	10	−0.34	0.13	−0.61	−0.06
CAL						
Group	Visit	*n*	Mean	St. error	CI	
Placebo	Baseline	12	4.72	0.36	4.00	5.44
	1 m	12	4.76	0.36	4.04	5.47
	3 m	12	4.60	0.36	3.88	5.32
	6 m	11	4.73	0.37	3.98	5.48
Test	Baseline	10	4.31	0.31	3.67	4.94
	1 m	10	4.10	0.31	3.46	4.73
	3 m	10	4.12	0.31	3.49	4.76
	6 m	10	4.01	0.31	3.38	4.65
Changes	Group	*n*	Mean	St. error	CI	
Baseline–1 month	Placebo	12	0.01	0.21	−0.43	0.45
	Test	10	−0.18	0.23	−0.67	0.31
Baseline–3 months	Placebo	12	−0.14	0.13	−0.42	0.14
	Test	10	−0.16	0.15	−0.47	0.15
Baseline–6 months	Placebo	11	−0.04	0.21	−0.48	0.40
	Test	10	−0.23	0.22	−0.70	0.23

Changes in microbiological outcome variables

From 12 patients randomized to the placebo group, one sample could not be processed at baseline, 1-month, and 3-month visits. At the 6-month visit, three samples could not be evaluated. From the ten patients randomized to the test group, only one sample at the 6-month visit could not be assessed.

Total anaerobic counts Mean total counts were similar in both groups at baseline. In both groups, a reduction was observed after 1 month, while minor changes were observed at 3 and 6 months (Table 5). No statistically significant differences were observed in either the intragroup or the intergroup comparisons.

Detection of pathogens Overall, minor changes were observed (Table 6). *P. intermedia* was reduced in the test group from 100% to 66.7%, while in the placebo group the percentages remained unchanged (from 90.9% to 89.9%). The same tendency was observed for *F. nucleatum* (no changes in the placebo group, with both visits 100%, and from 100% to 77.8% in the test group). *P. micra* demonstrated clear reductions in both groups.

Table 3 Bleeding on probing (BOP) and plaque index (PlI) with standard error (St. error) and 95% confidence intervals (CI), at each visit and in changes between visits

BOP						
Group	Visit	*n*	Mean	St. error	CI	
Placebo	Baseline	12	0,37	0,06	0.25	0.50
	1 m	12	0.28	0.06	0.16	0.41
	3 m	12	0.25	0.06	0.12	0.38
	6 m	11	0.22	0.07	0.09	0.36
Test	Baseline	10	0.56	0.06	0.45	0.67
	1 m	10	0.40	0.06	0.29	0.51
	3 m	10	0.33	0.06	0.22	0.44
	6 m	10	0.38	0.06	0.27	0.50
Changes	Group	*n*	Mean	St. error	CI	
Baseline–1 month	Placebo	12	−0.09	0.06	−0.21	0.03
	Test	10	−0.16	0.06	−0.29	−0.03
Baseline–3 months	Placebo	12	−0.13	0.05	−0.23	−0.03
	Test	10	−0.22	0.05	−0.34	−0.11
Baseline–6 months	Placebo	11	−0.17	0.05	−0.29	−0.06
	Test	10	−0.14	0.06	−0.26	−0.02
PlI						
Group	Visit	*n*	Mean	St. error	CI	
Placebo	Baseline	12	0.37	0.07	0.23	0.51
	1 m	12	0.25	0.07	0.11	0.38
	3 m	12	0.27	0.07	0.13	0.41
	6 m	11	0.16	0.07	0.02	0.31
Test	Baseline	10	0.26	0.08	0.10	0.41
	1 m	10	0.24	0.08	0.09	0.40
	3 m	10	0.31	0.08	0.16	0.47
	6 m	10	0.24	0.08	0.09	0.40
Changes	Group	*n*	Mean	St. error	CI	
Baseline–1 month	Placebo	12	−0.10	0.05	−0.19	0.00
	Test	10	−0.05	0.05	−0.15	0.06
Baseline–3 months	Placebo	12	−0.08	0.07	−0.23	0.06
	Test	10	0.03	0.08	−0.13	0.19
Baseline–6 months	Placebo	11	−0.15	0.06	−0.28	−0.01
	Test	10	−0.03	0.07	−0.17	0.11

Quantitative changes in the selected pathogens (Table 7) Both treatment groups showed similar counts of putative pathogens at baseline, with the exception of *A. actinomycetemcomitans* (3.77 in the placebo group, versus 0.00 in the test group). These differences, however, were not statistically significant. After treatment, an increase in *P. intermedia* (baseline–6 months) was observed in the test group and a decrease in *Eubacterium* sp. (baseline–1 month). A reduction in *Capnocytophaga* sp. (baseline–3 months) was observed in both groups. No statistically significant intergroup differences or intragroup changes were detected.

Qualitative changes of the selected pathogens in respect to the total flora (Table 7) No significant differences were observed at baseline. In the test group, a reduction in *P. micra* (baseline–3 months) was noted, while in the placebo group, there was a decrease in *F. nucleatum* (baseline–3 months) and an increase in *P. intermedia* (baseline–6 months). No statistically significant intergroup or intragroup differences were detected.

Table 4 Mean percentage of sites with different probing depth at each visit and in changes between visits

Group	Visit	*n*	Mean% sites >6 mm	Mean% sites 4–6 mm	Mean% sites 1–3 mm
Placebo	Baseline	12	2	47	49
	1 m	12	3	39	56
	3 m	12	2	37	58
	6 m	11	3	35	58
Test	Baseline	10	5	38	51
	1 m	10	1	35	63
	3 m	10	1	32	66
	6 m	10	1	28	70
Changes	Group	*n*	Mean	Mean	Mean
Baseline–1 month	Placebo	12	0	−5	6
	Test	10	−2	−7	13
Baseline–3 months	Placebo	12	−1	−7	9
	Test	10	−3	−9	15
Baseline–6 months	Placebo	11	0	−9	9
	Test	10	−3	−12	19

Adverse events

No adverse effects or events were reported or observed, either in the test or in the placebo groups.

Discussion

The present study aimed to investigate the efficacy of the adjunctive use of the subgingival local application of a CHX–xanthan gel in the treatment of chronic periodontitis patients. Twenty-four patients affected by moderate to advanced localized chronic periodontitis, were consecutively recruited for this study. In order to enter in the study, each subject had to demonstrate the presence of four to ten sites with PDD> 4 mm after conventional non-surgical periodontal therapy ("residual" pockets) or during a visit of supportive periodontal therapy ("relapsing" pockets). The justification for selecting these sites to evaluate the efficacy of this antimicrobial formulation was based on the fact that the presence of residual and/or relapsing sites is the main indication for localized subgingival application of antimicrobial agents [12, 25, 35]. In this randomized parallel controlled clinical trial, we have evaluated the efficacy of applying subgingivally a gel containing two CHX formulations linked with xanthan as adjuncts to mechanical root debridement in the treatment of "residual" and "relapsing" pockets, in comparison to the application of a placebo gel also containing xanthan. Six months after this treatment, these comparisons yielded no significant differences between the treatment groups, both in the clinical and the microbiological outcome measures evaluated. The clinical improvements, however, were higher in the test group, with significant reductions in BOP (between baseline and 3 months) and a significant increase in the proportion of shallow pockets (1–3 mm) at 6 months. The microbiological impact of both therapies was limited and similar in the two treatment groups.

Table 5 Mean log of total anaerobic colony-forming units, with standard error (SE) and 95% confidence interval (CI)

Group	Visit	*n*	Mean	SE	CI	
Placebo	Baseline	11	6.78	0.16	6.45	7.10
	1 month	11	6.67	0.16	6.34	6.99
	3 months	11	6.72	0.16	6.40	7.05
	6 months	9	6.44	0.18	6.09	6.80
Test	Baseline	10	6.79	0.19	6.39	7.18
	1 month	10	6.71	0.19	6.32	7.11
	3 months	10	6.60	0.19	6.20	6.99
	6 months	9	6.82	0.21	6.40	7.24

When comparing these results from other similar studies using adjunctive antimicrobials, it is important to emphasize the clinical indication of their usage, since adjunctive antimicrobials have been applied both locally and systemically and have proven efficacy when combined with SRP [35–39]. The efficacy of the subgingival application of local antimicrobials in the management of pockets in chronic periodontitis has been recently evaluated in systematic reviews [8, 40] and despite the heterogeneity among the studies and products evaluated, this adjunctive therapy provide an overall additional benefit over SRP, with a mean probing pocket depth reduction of about 0.5 mm, irrespective of the antimicrobial product evaluated [8]. What still remains unclear is whether these results, even

Table 6 Frequency of detection of target bacterial species, per group and visit

Group	Visit		Aa	Pg	Pi	Tf	Pm	Cr	Fn	Eu.	Cap.	Ec
Placebo	Base	*n*	11	11	11	11	11	11	11	11	11	11
		Positive	4	7	10	4	7	2	11	2	3	2
	1 m	*n*	11	11	11	11	11	11	11	11	11	11
		positive	3	9	9	3	3	0	11	2	2	2
	3 m	*n*	11	11	11	11	11	11	11	11	11	11
		Positive	1	7	10	2	4	2	11	1	0	0
	6 m	*n*	9	9	9	9	9	9	9	9	9	9
		Positive	3	7	8	1	4	3	9	1	0	1
Test	Base	*n*	10	10	10	10	10	10	10	10	10	10
		Positive	0	7	10	2	7	0	10	0	3	3
	1 m	*n*	10	10	10	10	10	10	10	10	10	10
		Positive	1	6	9	2	4	2	8	3	3	3
	3 m	*n*	10	10	10	10	10	10	10	10	10	10
		Positive	1	8	8	1	4	0	8	1	0	1
	6 m	*n*	9	9	9	9	9	9	9	9	9	9
		Positive	1	7	6	0	3	1	7	1	1	2

Aa A. actinomycetemcomitans, Pg P. gingivalis, Pi P. intermedia, Tf T. forsythia, Pm P. micra, Cr Campylobacter rectus, Fn F. nucleatum, Eu. Eubacterium sp., *Cap. Capnocytophaga* sp., *Ec E. corrodens*

statistically significant, are clinically meaningful over time and therefore are the cost–benefit ratio of these therapies.

With this aim of attaining a significant added clinical benefit in localized disease sites and at the same time reducing the chance of some of the well-known side effects of the use of antibiotics, the research attention has focused on the local application of CHX-based formulations. Formulated as a 1% CHX gel and applied subgingivally, it resulted in similar outcomes when compared with the use of a 1% metronidazole gel [21]. The formulation as subgingival chips or dental varnishes when applied subgingivally has resulted in diverse results [15, 41, 42]. While some studies have reported clinically relevant and statistically significant findings when applied subgingivally as andjunct to conventional therapy (SRP) [43], others failed to demonstrate significance [41]. The best results with the use of subgingival CHX chips reported a mean PPD reduction after 6 months of 0.78 mm, which is clearly higher than what achieved in the present study (0.34 mm). These

Table 7 Mean counts ("counts", in log of colony-forming units) and proportions of total flora ("proport", in percentage), per group and visit

Group	Visit		Aa	Pg	Pi	Tf	Pm	Cr	Fn	Eu.	Cap.	Ec
Placebo	Base	Counts	3.77	6.66	5.56	5.06	5.19	5.72	5.49	5.49	4.38	4.27
		Proport	0.46	17.59	2.93	0.60	0.52	0.80	3.91	0.52	0.41	0.21
	1 m	Counts	3.47	5.66	5.77	5.13	5.08	na	5.36	4.58	4.12	3.48
		Proport	0.25	10.02	8.33	1.01	1.29	0.00	3.73	0.93	0.14	0.09
	3 m	Counts	3.26	6.03	5.82	4.66	5.01	4.23	5.18	5.39	na	na
		Proport	0.01	8.82	8.19	0.71	0.99	0.27	2.60	1.04	0.00	0.00
	6 m	Counts	4.27	5.73	6.00	4.17	4.64	4.27	5.36	4.17	na	3.77
		Proport	0.53	4.94	14.92	1.27	0.67	0.33	3.67	0.38	0.00	0.26
Test	Base	Counts	na	6.20	6.06	5.00	5.08	na	5.48	na	4.56	4.02
		Proport	0.00	11.30	7.25	0.58	1.40	0.00	4.01	0.00	0.31	0.75
	1 m	Counts	3.65	6.01	6.02	4.68	4.68	3.90	5.44	4.35	3.72	3.52
		Proport	0.35	6.25	8.06	0.46	1.12	0.30	2.88	0.53	0.32	0.20
	3 m	Counts	2.60	6.71	6.07	3.82	5.13	na	5.45	3.42	na	4.12
		Proport	0.01	18.50	8.26	0.11	0.53	0.00	3.73	0.04	0.00	0.03
	6 m	Counts	3.51	6.59	6.27	na	4.92	2.87	5.68	3.87	4.17	4.47
		Proport	0.32	12.10	8.56	0.00	3.10	0.05	4.47	0.46	0.32	0.21

Aa A. actinomycetemcomitans, Pg P. gingivalis, Pi P. intermedia, Tf T. forsythia, Pm P. micra, Cr Campylobacter rectus, Fn F. nucleatum, Eu. Eubacterium sp., *Cap. Capnocytophaga* sp., *Ec E. corrodens*

differences can be explained by the different initial PPD (6.64 versus 3.58 mm, respectively), since deeper pockets would always have a higher potential for improvement than shallower ones [44].

More recently, xanthan-based gel formulations containing 1.5% CHX have been made available for clinical investigation. The addition of a xanthan gum to the gel aims to increase the bio-availability of the CHX formulation [29], since this antimicrobial has limited subgingival sustantivity [7]. When used subgingivally as an adjunct to SRP and compared with a 10% doxycycline gel and a placebo gel, both antimicrobial formulations yielded statistically significant clinical benefit compared with the placebo gel [24]. Similarly in a multicenter study, this (Xan–CHX) formulation applied subgingivally was compared with SRP alone and significant differences were reported when compared with SRP alone [30]. xBoth studies used split-mouth designs with sample populations consisting on patients with untreated moderate to severe chronic periodontitis. The comparison of these results to those reported in the present investigation is difficult since the patients selected are different (untreated versus patients with "residual" or "relapsing" sites), the clinical study design utilized is different (split-mouth versus parallel), and the treatments provided are different (test versus no treatment, instead of test versus a blind placebo). We purposely selected "residual" and "relapsing" sites since, according to the scientific literature, this is the clearest indication of the subgingival application of antimicrobial agents, rather than in untreated generalized moderate to advanced chronic periodontitis patients [12, 25]. It is also clear that the use of a split-mouth experimental design is not the most appropriate for the evaluation of antimicrobial agents in the treatment of periodontitis and, moreover, it is crucial in any clinical trial the inclusion of a proper placebo for appropriate assessment of the treatment effect. The magnitude of the clinical changes detected in the present study was small, but it must be taken into account that the selected patients harbored either "residual" or "relapsing" pockets. In other words, a poorer response can be expected in sites already treated and following SPT or in non-responding sites after basic periodontal therapy [45, 46]. The lack of significant clinical benefits of this (Xan–CHX) formulation when compared with the placebo gel reported in this study can also explained mainly by the limited sample size utilized and also by the good plaque control levels in both treatment groups what improves the outcome in the control group. Other possible explanation for the minor changes observed is the partial inactivation that CHX molecules may suffer when they contact with proteins from saliva, blood, or pus. Given the big amount of this kind of proteins that can be found in the crevicular fluid, it may be possible that the part of applied CHX will not be active [47]. Another limitation of the study design includes the lack of the evaluation of the host response.

The inclusion of patients with "residual" or "relapsing" pockets may also represent a difficult therapeutic target, since there are situations that in spite a meticulous mechanical debridement, removal of retentive factors, and meticulous self-performed dental cleaning [4, 44], the expected clinical outcomes are not attained [48–51] and these situations of unresponsive sites or recurrent disease are the ones which could benefit the most with the adjunctive use of a topically applied antimicrobial that could support in the control of the pathogenic subgingival biofilm [52, 53].

The microbiological impact observed in the present study was also limited. Minor changes in the frequency of detection of *P. intermedia*, *F. nucleatum*, and *P. micra* were observed in the group treated with the (Xan–CHX) formulation, but significant differences in reducing the counts of selected pathogens or in the changing the proportions of these bacteria were not encountered. These results are somehow different from previously reported microbiological results using the same antimicrobial. When the (Xan–CHX) formulation was compared with a 1% CHX gel [21] both groups reported significant reductions in total bacterial counts, without demonstrating intergroup differences. When this formulation was compared with SRP alone in the treatment of chronic periodontitis patients, significant reductions in total bacterial counts were reported in both treatment groups, but the tested product resulted in significant reductions at 3 months, compared with SRP alone [30].

One of the most important factors when assessing adjunctive antimicrobials in the treatment of periodontitis is the evaluation of adverse effects. In the present study, none of the patients suffered from local or systemic side effects in any group. Conversely, previous publications had registered local complications after the placement of other vehicles for subgingivally delivered CHX in up to 50% of the sample [54]. In addition, no microbiological side effects were observed, namely overgrowth of opportunistic bacteria.

Conclusions

Within the limitations of the present investigation, the reported results show that SRP with adjunctive subgingival application of a xanthan-based 1.5% CHX gel may improve, although to a limited extent, the clinical outcomes (as shown by the significant reductions in BOP and the increase in the proportion of 1–3 mm pockets), in chronic periodontitis patients with "residual" or "relapsing" pockets, although intergroup differences were not statistically significant. No side effects, neither clinical nor microbiological, were detected after the use of this local antimicrobial formulation.

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Conflict of interest They authors declare that they have no conflict of interest.

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