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Brief Report

Comparison of 0.12% Chlorhexidine and a New Bone Bioactive Liquid, BBL, Mouthrinses on Oral Wound Healing: A Randomized, Double Blind Clinical Human Trial

Eduard Ferrés-Amat [1,2 #], Ashraf Al Madhoun [3, #], Elvira Ferrés-Amat [1,2], Neus Carreo [4], Miguel Barrajas [5,6], Areej Said Al-Madhoun [6], Eduard. Ferrés-Padró [1,6], Carles Marti [6,7] and Maher Atari [6,8,*]

1. Oral and Maxillofacial Surgery Service, Hospital HM Nens, HM Hospitales, 08009 Barcelona, Spain.
2. Oral and Maxillofacial Surgery Department, Universitat Internacional de Catalunya, St Josep Trueta s/n, Sant Cugat del Vallès, 08195 Barcelona, Spain.
3. Department of Animal and Imaging Core Facilities, Dasman Diabetes Institute, Dasman, 15462, Kuwait.
4. Department of Periodontology, Universitat Internacional de Catalunya (UIC), C/Josep Trueta s/n, 08195, Sant Cugat del Valles, Barcelona, Spain.
5. Biochemistry and Molecular Biology Department, Universidad Pública de Navarra, Pamplona, Spain.
6. Biointelligent Technology Systems SL, Diputaccion 316, 3D, 08009 Barcelona, Spain
7. Oral and Maxillofacial Surgery Department, Hospital Clinic de Barcelona, 08036 Barcelona, Spain
8. Ziacom Medical SL, C. Buhos, 2, 28320 Madrid, Spain

* Correspondence: matari@biointelligentsl.com. MA.

# Equal contribution.

Abstract: After surgery, oral cavity healing occurs in a hostile environment and requires proper oral care and hygiene to accelerate recovery. The aim of the current study is to investigate and compare the bioreactivity characteristics of chlorhexidine based (CHX) mouth rinse and a novel bone bioactive liquid (BBL) mouth rinse on oral healing within seven days application post-surgery. A randomized, double blind clinical trial conducted in 81 patients. The mouth rinses were applied twice a day for a period of 7 days. The visual analog scale (VAS) protocol was applied to measure pain index. Early wound healing score (EHI) was determined in evaluate the oral cavity healing progress. No adverse effects were observed using the mouth washes, but CHX resulted in teeth staining. CHX and BBL were sufficient to reduce pain over a period of 7 days. However, the BBL group demonstrated a statistically significant reduction in VAS stating day 4. Relative to CHX group, the EHI scores were significantly higher in the BBL group, independent from the tooth location. No gender differences were observed in both VAS and EHI scores. Relative to the commercially available CHX, BBL mouth rinse reduced pain and accelerated oral cavity healing. Suggesting an improvements of oral cavity microenvironment at the wound site that mediates soft tissue regeneration.

Keywords: chlorhexidine; bioactive liquid; oral wound healing; pain index score; early wound healing score

1. Introduction

Wound healing requires a chronological sequence of complex biological processes (1). All tissues follow an essentially the same pattern to promote healing with focuses on quick recovery (2). Nevertheless, these processes are dependent on an intact hemostatic and inflammatory mechanisms that are widely influenced by genetic and environmental factors, in particular wound healing of the oral cavity with a remarkable hostile environment containing residential microbiome (3).

After oral surgery or tooth extraction, the sequential healing processes initiate momently. The periodontal pocket will be blocked by blood coagulation (4), and a re-epithelization mechanism will be initiated followed by a granulation tissue generation (5). After one week of the tissue remodeling, bones replenishment occurs, and cavity closure completes within a period of eight weeks after tooth extraction (6, 7).

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Notably, several factors interfere with healthy oral healing prosses including the tooth location, smoking, and mouth caring attitudes (8, 9). Therefore, oral care and hygiene are crucial after surgery to minimize pain, inflammation and dental plaque formation (10). Nevertheless, maintaining high hygienic conditions can be challenging to patients (11). Thus, efficient would healing detergents are necessary to sustain and accelerate recovery after oral surgery.

Non-prescription dental hygiene's are available and sufficient to prevent common oral health problems. Chlorhexidine (CHX) is the most common anti-plaque and anti-gingivitis agent (12). CHX is a cationic bisbiguanide compound with a broad-spectrum anti-microbial property. It binds to the microbe cell and precipitates cell contents (13-15)., CHX-gluconate is widely used in dentistry and is available as an oral rinse, gel, spray, and dental varnish. In a recent review article, Rajendiran et.al summarizes the current developments in antiplaque, anti-gingivitis, and anti-periodontitis properties of CHX and other compounds (16).

The bone bioactive liquid (BBL) comprising a saline solution containing calcium chloride ($CaCl_2$) and magnesium dichloride hexahydrate ($MgCl_2$-$6H_2O$) with a net negative charge that promotes healing and soft and hard tissue regeneration at wounded periodontal cavity (17). Furthermore, BBL significantly intensifies hydroxyl groups at the wound surface and improves hydration significantly in comparison to other mouthwashes (unpublished data). BBL creates hydrophilic environment and allows active ionic interaction with blood plasma, progenitor endothelial and epithelial cells, consequently, the coordination and communication between the cells are significantly improved the wound site (17).

The aim of the present study was to compare the efficacy of BBL and CHX (0.12%) mouth rinses in improving clinical parameters and soft tissue healing after tooth extraction. The wound healing properties of BBL may support its usage as a new pharmaceutical product with good physical, chemical and biological stabilities.

2. Materials and Methods

2.1. Study population Inclusion and exclusion criteria

The study cohort comprised 81 patients from all genders at age above 14 years old, who accepted to participated voluntarily in clinical trial. The sociodemographic characteristics of the patient's cohort were described in the Supplementary Table S1. Written informed consents were obtained from all study participants following the ethical guidelines of the Declaration of Helsinki and approved by the ethics committee at Complejo Hospitalario de Toledo and institutional review board, Spain (CEIm HM Hospitales 21.03.1786-GHM; protocol ID: V01-2021; date: 16/04/2021; Clinical Trial Registry Platform: Clinical Trial Gov. Press). Participants' inclusion criteria: systemically healthy, full mouth plaque and bleeding scores <20%, healthy periodontium, no local or systemic antibiotic or antiseptic treatments for 3 months prior involvement in the study. Exclusion criteria included the use of medications that cause gingival enlargement or the presence of gingival idiopathic overgrowth; smokers; patients with systemic diseases or conditions that could interfere with routine periodontal therapy such as pregnancy or lactating females, uncontrolled periodontal disease, previous or current history of bisphosphonate treatment, immune deficiencies, uncontrolled diabetes, rheumatoid disease, radiotherapy, chemotherapy, infectious diseases.

2.2. Removal of patients from therapy or assessment

Participants were free to withdraw from the study at any time, without any prejudice or justifications. If the patient discontinued prematurely from the study, any relevant evaluations and observations and reasons for study discontinuation were recorded in the Case Report Form (CRF). Participants discontinuing due to infection or medical reasons, were monitored until complete recovery.

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2.3. Study Design

The study was designed as a one week randomized, prospective, double-blinded pilot clinical trial. This prospective study included patients who required two trans alveolar surgical extractions of inferior or superior third molar or any simple or surgical tooth extraction. 171 dental extractions in 81 patients were randomized to two groups: control group (CG, 20 male and 22 female patients) received Perio-Aid Intensive care mouthwash made of 0.12% CHX- di-gluconate (Dentaid, Barcelona, Spain); and test group (TG, 19 male and 20 female patients) received BBL mouthwash, a bioactive solution generated in our laboratory, comprising a phosphate buffer saline (PBS) solution containing 1.35 mM $CaCl_2$ and 0.75 mM $MgCl_2$-$6H_2O$ with a net negative charge. Mouth rinses were administered twice a day for 7 days and no eating or drinking was permitted for a period of 1 hour after the treatment. After a period of 7 days, clinical parameter data were analyzed to determine clinical changes during the treatment. Patients' follow-ups were done twice via phone calls at days 2 and 4 to find out the degree of postoperative pain as described in **Table 1**. To respect patient's data confidentiality, data management system was applied as described in (18).

Table 1. Study design.

Criteria	Visit 1 (Surgery day)	Phone calls		Visit 2
		Day 2	Day 4	Day 7
Informed consent	X			
Inclusion/exclusion criteria	X			
Collection of clinical data	X			X
Oral examination	X			X
Patients' dairy: Pain VAS scale 0-10		X	X	X
Patients' dairy: clinical healing measurements	X			X
Tolerability and post-treatment side effects				X

2.4. *Surgery assessments*

Three independent dentists participated in clinical data measurements and registrations. The study participants received a diagnostic workup including clinical examinations, oral photographs, and standardized periapical radiographs to evaluate the proposed surgical sites. Before the surgical procedure, patients underwent periodontal therapy and received extensive oral hygiene instructions to provide a better oral environment. The protocols for full mouth plaque scores (FMPS), and full mouth bleeding scores (FMBS) were implements exactly as described by T.J. O'Leary *et al*. (19) and J. Ainamo *et al*. (20), respectively, and were recorded after the hygienic phase of the periodontal therapy. No surgery was performed until patients reached an FMPS <20% and an FMBS <20%. Each patient received both surgeries on both bilateral areas at different days. The surgical extractions of tooth were carried out with local anesthetic, raising a mucoperiosteal flap with osteotomy and no periodontal dressing was applied postoperatively. Unless otherwise required, dental extractions were performed without stitches in both control and test groups to evaluate the healing capacity of both liquids

2.5. Post-surgical Procedures

All patients received 600 mg ibuprofen every 8 hours for 4 days and 500 mg amoxicillin every 8 hours for 7 days, or 100 mg doxycycline every 24 hours for 5 days for patients with allergy against the amoxicillin. Patients were instructed to do mouthwash with 15 ml twice a day after their regular homecare practice for a period of 7 days. The use of ice packs was recommended for at least 3 hours post-surgery. All patients were instructed to discontinue tooth brushing at the surgical sites for 7 days. After 30 days, a professional

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prophylaxis was performed to remove stains caused by Perio-Aid Intensive care mouth-wash.

2.6. Wound healing measurement procedures

After a period of 7 days, patients were examined for healing evaluation. The early wound healing-index (EHI) (21) was assessed by two blinded clinical examiners. The five different degree scale was applied and scores 5 to 1 were applied gradually based on the observations: Complete flap closure without fibrin line; Complete flap closure with fibrin line; Complete flap closure with small fibrin clot(s); Incomplete flap closure with partial necrosis; and Incomplete flap closure with complete necrosis (more than 50% of the former flap is involved). In addition, EHI was assessed using a healing index Landry *et al.* (22) which grades the wound on a scale 1-5 as described in Supplementary Table S2. The wound area was classified as either partially or fully keratinized. In case of partial keratinization, the wound area was further classified as partially or fully keratinized for examination after an additional 7 days.

2.7. The post-surgical pain, safety, and discomfort measurement procedures

Efficacy measurements were assessed by pain scale evaluation post-surgery at days 2, 4 and 7 though a phone call contacts with the patients in accordance with their subjective pain feeling. A modified visual analog scale (VAS) was applied as described in (23). No pain scale = 0, moderate pain scale = 5 and maximum pain scale = 10. Furthermore, safety measurements were evaluated by the incidence of adverse events (AEs) and serious adverse events (SAEs) that could be detected by the investigator or patients' communications throughout the entire study.

2.8. Statistical and analytical methods

Shapiro-Wilk normality test was performed to assess the normality of the distribution. Data were reported as mean and standard deviation or median and interquartile range (IQR) based on data distribution. Differences between treatments and gender were assessed using the Mann-Whitney test or t-test based on data distribution. A two-tailed test with a *P*-value < 0.05 was considered the cut-off level for statistical significance. The Statistical Package for Social Sciences software (SPSS, version 23, Chicago. Inc, US) was used for data analysis.

3. Results

In this study, no incidence of adverse events was observed, and no post operatory compilations were reported. Patients did not present statistically significant differences on infection prevention between the CG and TG ($p = 0.96$).

3.1. BBL mouth rinse dramatically reduces VAS:

In general, patients from both treatment groups showed a progressive decrease in pain over surgery consecutive week (**Figure 1A).** Nevertheless, statistical data analysis revealed that the CHX group showed a significant reduction in VAS only at day 7 ($p < 1x10^{-5}$). Alternatively, the BBL group demonstrated a significant reduction in VAS stating day 4 ($p < 1x10^{-4}$) which was further reduced at day 7 ($p < 1x10^{-8}$, **Figure 1A**).

AT day 2 the VAS was tentatively lower in the BBL group, relative to the CHX group but was not significant ($p = 0.084$). However, significant differences in VAS scores were observed at days 4 and 7, with a notable 50-70% lower values were scored in the BBL group relative to that of the CHX group (**Figure 1A**).

Since the study compromises patients from different genders, we were interested to evaluate the VAS in each separately. VAS was comparable between males and females and no significant differences were recorded ($p = 0.78$). In all genders, time course study indicated that the VAS has identical trend with a significant reduction starting at day 4 for the BBL group and starting at day 7 for the CHX group (**Figure 1B and C)**.

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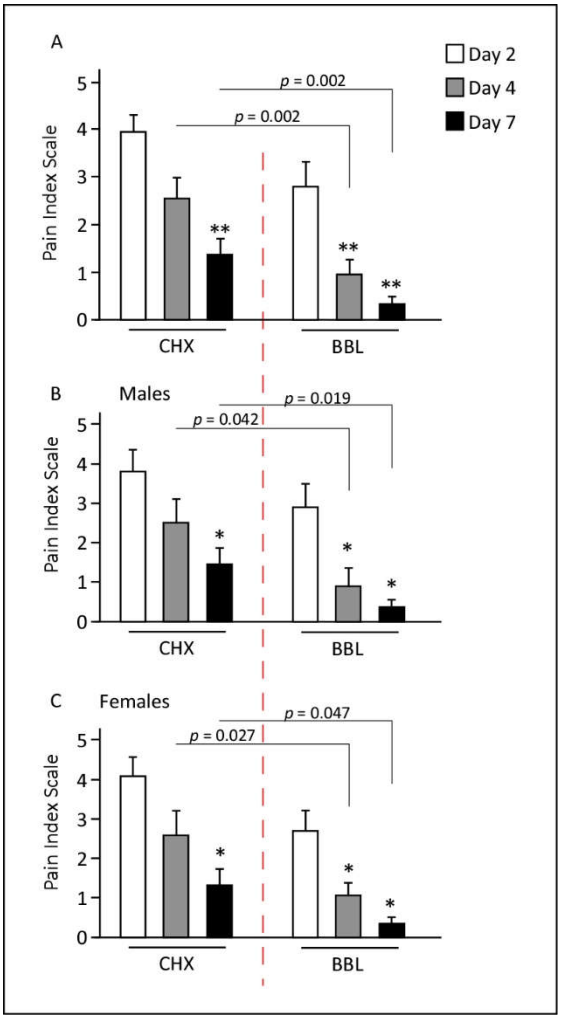

Figure 1. Pain visual analog scale (VAS) scores were reposted at days 2, 4 and 7 from the day of surgery. A. Both CHX and BBL groups showed significant improvements in pain scores, yet the BBL group VAS scores were significantly improved relative to the CHX group. **B. and C.** Comparable study between both genders. Males and females responded similarly for both treatments, and the VAS response trend was comparable. *$p < 1x10^{-4}$.

3.2. BBL mouth rinse improves EHI

The total extracted teeth were 89 and 82 for the BBL and CHX groups, respectively. As observed in **Figure 2**, both CHX and BBL treatments improved oral wound healing at day 7 post surgery, independent from the number of extracted teeth. Nevertheless, wound closure was notably enhanced in response to BBL treatment. Table 2 shows that EHI scores given by Landry *et al*. (22) classification for a total of 171 dental extracts performed in the patients cohort. In general and independently from the dental extract position, the EHI scores were remarkably higher after BBL treatments, relative to that after CHX treatment. Together, these results indicate that BBL treatment enhanced gingival tissue healing.

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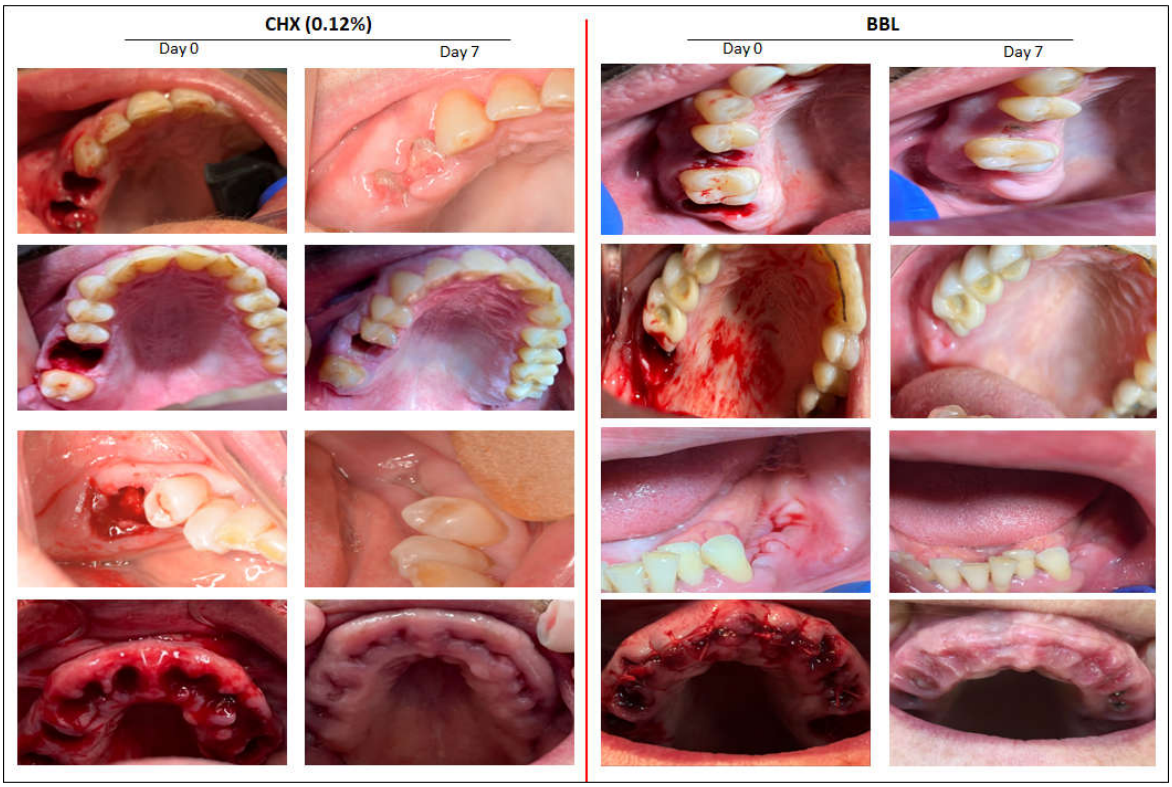

Figure 2. Representative images for patients at the surgery day 0 and after 7 days mouth wash with CHX or BBL. A notable wound healing improvements were detected in patients used BBL mouth wash.

Table 2. The early wound healing score (EHI) was determined as described by Landry et.al. 1988.

Healing index	Total 171 Dental Extractions operated in 81 Patients	
	BBL Treatments	0.12% Chlorhexidine Treatments
1—very poor	0	0
2—poor	0	4
3—good	5	64
4—very good	49	9
5—excellent	38	2

Then, we evaluated the EHI scores for both treatments. Data analysis revealed a statistically significant differences in EHI scores between the two treatments. The average of the total EHI score for the BBL group was 4.40 ± 0.56 and for the CHX group was 3.1 ± 0.57, indicating a remarkable healing process in the BBL group.

Since the tooth location influences the EHI score, we classified the extracted teeth into pre-molars, centrals and molars and determined the EHI score accordingly. As shown in **Figure 3A**, the EHI score for each teeth segment was significantly higher in BBL group relative to that of the CHX group. Comparisons between the teeth segments, molars scored the lowest EHI, indicated a delayed healing process relative to the other teeth locations (**Figure 3A)**.

No significant differences in EHI scores were detected between genders (p = 0.645). Both males and females showed identical patterns with respect to the teeth location segments, where a significant improvement in EHI score was observed in the BBL group relative to that of the CHX group (**Figure 3B and C**).

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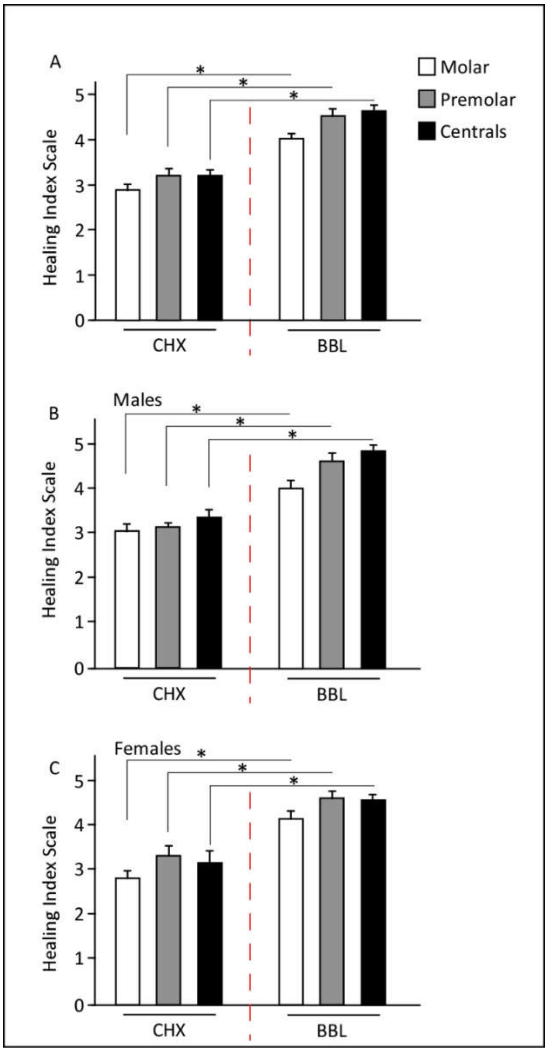

Figure 3. Early wound healing-index (EHI) at day 7. A. EHI scores were independent from the tooth position and were significantly higher in BBL treatments. **B. and C.** Comparable study between both genders. Males and females responded similarly for both treatments, with a significant improving in EHI post BBL treatment. *$p < 1x10^{-4}$.

4. Discussion

In our previous preclinical study, we applied pre-treated bone level tapered (BLT)-titanium implants with BBL into foxhound dogs. The data indicated that BBL improves histological and histomorphometric characteristics of the implants, reduces titanium surface roughness, improves wettability, and promotes healing and soft and hard tissue regeneration at the implant site (17). In the current study, BBL was applied as a mouth wash to human patients who had teeth extraction surgeries and its prospective clinical properties were compared to that of the CHX mouth wash. Overall, the human data support the previously reported animal study and indicate significant improvements in wound healing and soft tissue regeneration. Notably, the study patient cohort consisted of a heterogenous population with different sociodemographic background that were randomly distributed in this double blinded study.

CHX is a gold standard mouth wash with the anti-plaque and anti-gingivitis properties (24, 25). Nevertheless, it has negative side effect profile precludes long-term use that causes poor patient compliance (25). The adverse-effects of CHX include burning sensation, tartar and calculus formation, soft-tissue trauma and allergy, taste alteration, and teeth staining (26-29). Therefore, studies are directed toward the use of different concentration of CHX and/or alternative product usage. Several studies compared the use of different concertation of CHX, best reviewed in (29-31). In general, studies indicate that there

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are no statistically significant differences in the efficacy of 0.12% and 0.2% CHX mouthwashes, and that concentrations above 0.2% will unnecessarily increase the unwanted side effects. CHX alternatives including cetylpyridinium chloride, oxidizing mouthwashes, povidone-iodine (PVP-I). Although, some of these products shows less adverse effects, yet the absence of clinical research such as randomized clinical trials and systematic meta-analysis reviews, or the absence of commercially available formulations for intra-oral use limits their applications.

The bioactive BBL is a new commercial mouth rinse solution is basically a negatively charged liquid saline containing Ca^{+2} and Mg^{+2} salts. BBL has no taste, no odor and does not induce allergic reactions. Comparing the efficacy of CHX and BBL, in human patients, revealed that the latter reduces pain dramatically within a period of 4 days and promote complete oral healing with 7 days. The current randomized- double blind clinical trial indicates that BBL is an efficient mouth rinse solution for prospective clinical applications. Nevertheless, further longitudinal studies are required to delineate its capacity as ani-plaque and antiseptic property.

5. Conclusions

Clinical data collected in patients' diary revealed a statistically significant positive effect for the BBL mouth wash in improving post-operatory quality of wound healing when compared to CHX mouth wash during and after 7 days of application. Both mouth rinses showed a differential progressive reduction in pain over the surgery consecutive week, however, BBL should a significant pain relief starting day 4. No gender differences associated with pain or would healing were observed in response to the applied mouth washes. Together, the wound healing properties of BBL may support its usage as a new pharmaceutical product with good physical, chemical and biological stabilities.

Study Limitation:

On days 2 and 4 of the study, patients' evaluation follow-ups were done based on phone calls, which may raise some bias concerns regarding early health status post-surgery. Although, the clinical observations were performed by several specialized dentist who were blinded for the treatment type, VAS analysis may raise some bias concerns. In addition, as a general clinical practice, all patients were treated with antibiotics besides the study treatment, which may suggest that the observed study outcome is due to effect of both the treatment and the antibiotic.

Authors' contributions: Ed.F-A and El.F-A; supervised and conducted the patients meetings and evaluation. N.C., M.B., Ed.F-P. and C.M.; Data analysis and interpretation. A.S.A-M and A.A-M, Statistic analysis and were blinded for the outcome assessment and measurements; M.A., Conception and design and assembly of data; A.A-M and M.A., data analysis, outcome assessment, interpretation, and manuscript writing. All authors participate in revision and approval of the final version of the Manuscript.

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Ethics approval and consent to participate: The human study approvals and the obtained written informed consents, from all participants or guardians of patients below 16 years old, were in accordance with the ethical guidelines of the Declaration of Helsinki and approved by the ethics committee at Complejo Hospitalario de Toledo and institutional review board, Spain (CEIm HM Hospitales 21.03.1786-GHM; protocol ID: V01-2021; date: 16/04/2021; Clinical Trial Registry Platform: Clinical Trial Gov. Press).

Availability of data and materials: The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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