Journal of Trace Elements in Medicine and Biology 76 (2023) 127118

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

# Antimicrobial and bone repair effects of boric acid in a rat model of dry socket (alveolar osteitis) following dental extraction

Renan R. da Costa [a], Raquel D.S. Freitas [b,c], Gabriela da Cunha [a], Sílvia D. de Oliveira [a], João B.B. Weber [a,*]

[a] Pontifical Catholic University of Rio Grande do Sul, PUCRS, School of Health and Life Sciences, Post-Graduate Program in Dentistry, Porto Alegre, RS, Brazil
[b] Pontifical Catholic University of Rio Grande do Sul, PUCRS, School of Medicine, Post-Graduate Program in Medicine and Health Sciences, Porto Alegre, RS, Brazil
[c] Nutrition Thinking® Co., Tecnopuc, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil

ARTICLE INFO

*Keywords:*
Alveolar osteitis
Boric acid
Wound healing
Dry socket
MicroCT
Bone tissue

ABSTRACT

*Background:* Alveolitis occurs after dental extraction without blood clot formation, leading to an inflammatory process and bacterial contamination. Boric acid (BA) demonstrates anti-inflammatory, antimicrobial, and osteogenic properties. This study aims to evaluate the possible antimicrobial effects and bone repair of BA in a rat model of alveolitis (dry socket).
*Methods:* 33 male Wistar rats were submitted to the extraction of the upper right incisor and dry socket induction. They were first divided into two groups: dry socket (n = 17) and dry socket + 0.75 % BA (n = 16). Samples for the microbiological analysis were collected immediately after dental extraction, at the detection of clinical alveolitis, 7, and 14 days after BA application. For microCT and histological analysis, samples from euthanized rats were used in 14 and 28 days after alveolitis detection.
*Results:* Higher bacterial counts were found in 4–5 days after alveolitis induction, compared to the baseline in both experimental groups, decreasing significantly after 7 and 14 days of treatment with BA ($P < 0.05$). The microCT evaluation displayed increased bone volume, bone volume fraction, trabecular thickness, and bone mineral density in a time-dependent manner, regardless of BA treatment. On the other hand, the number of trabeculae and total bone porosity decreased over the 28 days of the experiment in the dry-socket group and both groups, respectively ($P < 0.05$). Histological analysis did not differ on bone repair in both experimental groups.
*Conclusion:* This was the first report investigating the effects of BA in a rat model of alveolitis regarding microbiological and bone repair aspects. The BA local application decreased the total aerobic and facultative bacteria counts and does not seem to benefit the bone repair after alveolitis development. This study paves the way for more studies involving alveolitis and different BA applications.

## 1. Introduction

Alveolar osteitis, or dry socket, is characterized by an acute inflammation caused by the absence of blood clots within the dental alveolus following dental extraction [1–3]. Dry socket is the most common complication following dental extraction and develops from two to four days after procedure [2]. It is characterized by alveolar bone exposure, yellow-grey necrotic tissue with mucosal erythema in the vicinity, intense radiating pain, and fetid odor [4–7].

Alveolitis begins with the fibrin meshwork degradation of the alveolar blood clot. Fibrinolysis occurs due to the inflammatory process, by the physiologically formed plasmin or by bacterial products, such as streptokinases and staphylokinases [8]. Microbial activity has been proposed as the primary activator of fibrinolysis; however, external factors affect this condition, such as smoking, oral contraceptives, trauma, and the anatomy of the mandibular region [3,6].

Following the alveolitis onset, pain evolves from moderate to severe, resolving its course after 5–10 days [5]. Despite the controversies regarding treatment protocols [9], it has been established that oral cavity irrigation and local application of antimicrobial and analgesic agents are the patient's best options until the healing process is completed [5].

* Correspondence to: School of Health and Life Sciences, Pontifical Catholic University of Rio Grande do Sul, PUCRS, Avenida Ipiranga, 6681, Partenon, Porto Alegre, RS 90619-900, Brazil.
*E-mail address:* jbweber@pucrs.br (J.B.B. Weber).

https://doi.org/10.1016/j.jtemb.2022.127118
Received 27 June 2022; Received in revised form 20 November 2022; Accepted 30 November 2022
Available online 9 December 2022

Boric acid (BA) is derived from boron and has in its composition AN0128 [10], a substance with anti-inflammatory and antibacterial properties [11–13]. BA inhibits the lipopolysaccharides (LPS)-induced release of tumor necrosis factor (TNF) by human monocytes [10]. Also, BA acts on the osteogenesis process, stimulating the expression of gene markers related to the proliferation and differentiation of bone marrow-derived stromal cells [14]. Furthermore, BA can act in conjunction with osteoblasts over the osteoclastic activity [15]. In this context, the present study aimed to investigate the antimicrobial and bone repair effects of BA local application in a rat model of dry socket.

## 2. Material and methods

### 2.1. Animal care

The study protocol followed the Brazilian guidelines for the care and use of animals for scientific and didactic procedures from the National Council for the Control of Animal Experimentation (CONCEA/Brazil). It was also approved by the local Animal Ethics Committee (CEUA/PUCRS 9820/2020). The animal studies report complies with the ARRIVE (Animals in Research: Reporting In Vivo Experiments) guidelines [16].

Male Wistar-specific pathogen-free rats (250–320 g; 8 weeks-old; n = 40) were obtained from the Center for Experimental Biological Models (CeMBE/PUCRS). The initial sample (n) was reduced to 33 rats due to losses throughout the experimental procedures (during anesthesia, post-anesthesia period, dental extraction procedure, irreversible root fracture, and/or doubtful alveolitis).

The animals were housed under standard conditions of temperature (22 ± 2ºC), light (12-h light-dark cycle), and humidity (50–70 %), in ventilated cages, with autoclaved wood chips bedding. They received a standard rat chow diet (Nuvilab®), with free access to filtered water and food. However, when rats were submitted to dental extraction, chow was offered only 3 h after the procedure.

### 2.2. Experimental protocol

At T1, rats were submitted to the extraction of the upper right incisor and dry socket induction. At the exact moment, samples were collected for microbiological analysis, considered as baseline. At T2, which was from 4 to 5 days after the dental procedure, clinical alveolitis was observed, confirmed, and another sample was collected for microbiological analysis. Afterwards, animals were randomly assigned to two experimental groups: dry socket and dry socket + BA local application. BA was applied at T2 directly into the alveolus. At T3, which was 7 days following dry socket development, another sample was collected for microbiological analysis. The same occurred at T4, which corresponds to 14 days after the dry socket was developed; however, euthanasia by isoflurane inhalation was performed in randomly assigned rats from both experimental groups. Twenty-eight days after the dry socket, the remaining animals were euthanized by the method mentioned above, and the maxillae were collected for microCT analysis. At the end of the study, the total n was divided into four experimental groups: dry socket 14 days, dry socket 28 days, dry socket 14 days + BA, and dry socket 28 days + BA.

The timeline of the experimental procedures can be observed in Fig. 1.

#### 2.2.1. Dry socket induction

After a 40-min period of habituation, the animals were slightly anesthetized with isoflurane (Isoflurane®, Cristália, Brasil 100 % - 1 mL/L) in 5 % oxygen (2.4 mL/min). This was performed as an anesthesia induction before the injection with ketamine (Ketalar®, Pfizer, USA, 50 mg/mL) and xylazine (Rompun®, Bayer, Canada, 20 mg/mL), administered intraperitoneally, in order to reduce the stress. During the whole surgical procedure, rats were stabilized over a heated table to maintain core temperature.

As proposed formerly by Carvalho et al. [17], the dry socket induction occurred as follows. An incision with a 15 C blade was made between the interdental and intrasulcular papillae of the upper right incisor. The rupture of the periodontal fibers was performed using a Hollemback 3 S spatula, and dental dislocation movements were initiated. Afterwards, dental extraction was performed using pediatric forceps when observed the dental mobility and the presence of a diastema between the upper incisors no. 1. With the intent to evoke the alveolus ischemia, a gaze embedded with adrenaline (Adren®, Hipolabor, Brasil, 1 g/1.000 mL; 1:1000) was applied directly into the dental alveolus. After the reduction of hemorrhage, absorbent paper points embedded with adrenaline were placed into the alveolus during 20–30 min, until the alveolus ischemia was finally completed and observed.

Subsequently to the dental procedure, the animals received a subcutaneous administration of 1 % butorphanol (Butorfin®, Vetnil, Brasil, 5 mL) and were transferred to another heated surface until complete recovery. The animals received analgesic medication every 8 h or according to signs of suffering and/or pain throughout the 4 consecutive

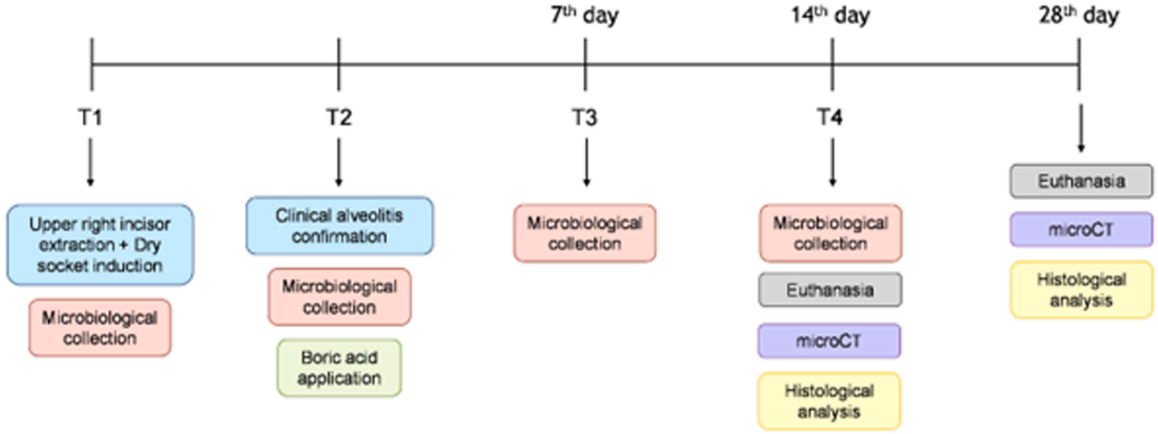


**Fig. 1.** Timeline of the experimental procedures. At T1, the upper right incisor was extracted, and dry socket was induced, along with sample collection for baseline microbiological analysis. At T2, clinical alveolitis was confirmed, boric acid was applied in the alveolus from rats of the intervention group, as well as sample collection for microbiological analysis (before boric acid application). At T3 (7 days after clinical alveolitis confirmation) only microbiological samples were collected. At T4 (14 days after clinical alveolitis confirmation) the last samples for the microbiological analysis were collected and rats (n = 18) were euthanized for maxillae collection for histological analysis. 28 days after the clinical confirmation of alveolitis, euthanasia was performed (n = 17) and maxillae were removed for microCT and histological analyses.

days after the dental procedures. After this first post-surgery period, the rats were constantly accompanied and, if necessary, received analgesic medications until remission of all painful symptoms. None of the rats in the study were excluded due to signs of suffering.

#### 2.2.2. Clinical alveolitis and BA treatment

After 4–5 days of the upper right incisor extraction, the animals were evaluated regarding a clinical condition compatible with a dry socket. The criteria for dry socket detection were: (I) the presence of edema and local erythema, (II) absence of blood clot, (III) granulation tissue in the alveolus, and the (IV) existence or not of purulent secretion in the alveolus cavity. After this, only two rats failed to develop alveolitis, as previously described.

Rats were randomly allocated in two experimental groups, dry socket (n = 17) and dry socket + BA (n = 16). The dry socket group did not receive any treatment in the alveoli, only the pain management protocol when necessary and until the full completion of the clinical state. The animals included in the dry socket + BA group were again anesthetized with ketamine (Ketalar®, Pfizer, USA, 50 mg/mL) and xylazine (Rompun®, Bayer, Canada, 20 mg/mL), administered intraperitoneally, for BA application.

The preparation of the 0.75 % BA solution was made by 0.75 g of boric acid (pure) sterile bottle powder (Dinâmica Química Contemporânea LTDA, Brazil) diluted and homogenized in vortex stirrer for 1 min in 100 mL of sterile 0.9 %-saline solution. This concentration was chosen based on previous reports [10–12,19]. The dental alveolus was filled with the BA (0.75 %) solution, using a pipette, until alveolar cavity was full (average volume of 100 microliters), followed by suture of the cavity with a simple 5–0 mononylon thread. Subsequently, this experimental group received 1 % butorphanol for pain management during the first post-surgery day and/or when necessary, or while the pain symptoms were perceived. Importantly, the dental socket did not suffer any type of intervention before the BA application.

The rats were euthanized after 14 (n = 18, randomly assigned from both experimental groups) and 28 days (n = 15, randomly assigned from both experimental groups). At the euthanasia of the 28th day, maxillae were collected for microCT and histological analysis.

### 2.3. Microbiological analysis

The microbiological analysis was performed from the collection in situ and sample cultivation at four different time points. Samples collected with a sterilized absorbent paper point were inoculated in 0.85 %-saline solution and homogenized. Afterward, the collected material was again homogenized and spread, in triplicate, on the surface of two different types of growth medium. To investigate total aerobic and facultative bacteria, samples were spread on Brain Heart Infusion (BHI) agar, and for the investigation of the aerobic and facultative Gram-negative bacteria, samples were spread on MacConkey (MAC) agar. Samples were collected in four different time points: pre-alveolitis, immediately after dental extraction (T1), alveolitis, before treatment (T2), samples collected when clinical alveolitis was observed, 7 days after BA application (T3), 14 days after BA application (T4). Cultures were aerobically grown at 37 °C for 24 h. Afterward, colony-forming units (CFU) were counted and registered.

### 2.4. MicroCT analysis

With the intent to evaluate the bone repair of the alveoli, microCT analysis was performed in samples from the euthanized rats (Fig. 2). The methodology used followed the guidelines' recommendations for bone microstructures analysis from rats, as previously described by Bouxsein et al. [18]. A desktop microCT system (SkyScan 1174v2; Bruker microCT, Kontich, Belgium) was used to evaluate the bone mineral density (gr/cm$^3$) and microarchitecture parameters of the rat socket. Scanning of the specimens was placed in a 15 mm diameter tube filled with 2.5 mL of a solution containing 4 % paraformaldehyde and 0.1 % glutaraldehyde and was carried out at 50 kV and 800 mA. Images were captured and reconstituted by the CTan program (version 1.16.4.1 +, Bruker MicroCT), and used to determine region of interest (ROI). The images of the alveolar socket were used to standardize the region of interest, using a cylinder measuring 1 mm of diameter, covering the beginning of the palatine bone (anterior limit) and the beginning of the contralateral incisor dental root (posterior limit). The analysis of bone volume (in mm$^3$), bone volume fraction (BV/TV in percentage), trabecular thickness (Tb.Th in mm), trabecular separation (Tb.Sp in mm), number of trabeculae (Tb.N per mm), total bone porosity (Po (tot) in %), and bone mineral density (BMD in Hounsfield unit [HU]) were performed using the same software.

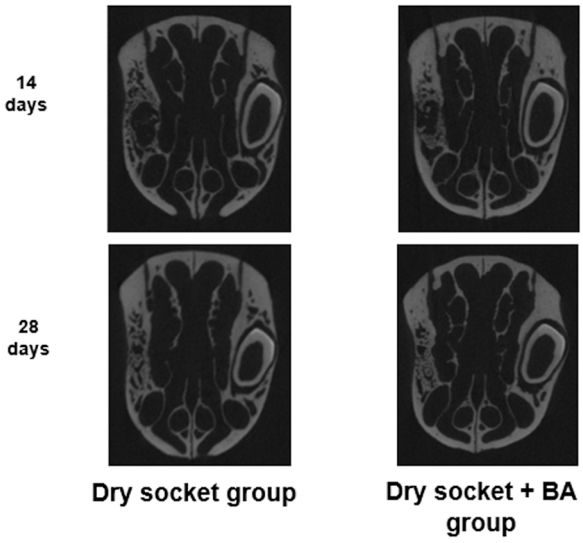


**Fig. 2.** Bone microCT images on 14 and 28 days after alveolitis detection in right socket, with or without BA treatment.

### 2.5. Histological analysis

Following the microCT, bone samples were forwarded to process and histological analysis. The maxillae were decalcified with a 10 % ethylenediaminetetraacetic acid (EDTA) solution for 15 days and embedded in paraffin. Five-μm thick slices were made in longitudinal sections of the dental alveolus and submitted to hematoxylin-eosin (H&E) staining. The histological images were obtained using a microscope (Nikon E200) with the 100x magnification, followed by the morphological qualitative analysis.

### 2.6. Statistics analysis

The data obtained were submitted to Shapiro-Wilk tests to confirm normal distribution among the final four experimental groups, resulting in a normal distribution in the microCT samples and a non-normal distribution in the microbiological analysis. For the microCT analysis, a one-way analysis of variance (One-way ANOVA) was performed first to evaluate significance among groups. When group interactions were statistically significant ($P < 0.05$), Sidak's post hoc test was utilized. The confidence interval was 95 %. As for the microbiological analysis, a two-way analysis of variance (Two-way ANOVA) followed by Tukey's post hoc test was performed. As for two analyses, the Kolmogorov-Smirnoff normality test was used, proving the homogeneity of the samples. Statistical tests and graphs designs were held using GraphPad Prism Software 9.2.0 (GraphPad Software Inc. San Diego, CA).

## 3. Results

### 3.1. Microbiological analysis

Rats were submitted to alveolar sampling throughout the experimental protocol for 14 days. Alveolitis has been established and a significant increase of total aerobic and facultative bacteria loads was detected at T2 compared to the baseline ($P < 0.05$) in both experimental groups (Table 1-A). However, it appears that the aerobic and facultative Gram-negative bacteria demonstrated a significant growth in T2, in comparison to baseline, in the BA-treated rats, and this growth reduced in a significant time-dependent manner (Table 1-B).

### 3.2. Histological analysis

On the 14th day after dry socket induction, both experimental groups (dry socket and dry socket + BA) demonstrated similar histological morphology. It was possible to observe an abundance of connective tissue, small areas of inflammatory infiltrate, and the presence of blood clots. Neo-formed bone tissue displayed decreased organization regarding its physiological process, with thin trabeculae in development. Additionally, it was possible to see wide open spaces with no residual BA.

Following 28 days of the experiment, both groups remained histologically similar. There was a reduction of connective tissue and inflammatory infiltrate, as well as blood leakage and angiogenesis. The neo-formed bone tissue remained like what was observed in 14 days of the experiment. The open spaces became evident due to the fragility of the osteoid deposition. Representative images of the histological evaluation can be seen in Fig. 3.

### 3.3. MicroCT analysis

The development of alveolitis affected bone repair differently as observed by the microCT analysis (Fig. 4 and 5). Bone volume, bone volume fraction, and the trabecular thickness displayed a significant increase along the experimental period, regardless of BA application (Fig. 4A, B, C). However, the trabecular separation did not differ among experimental groups (Fig. 4D). On the other hand, the number of trabeculae diminished in a significant manner in the dry socket group, between 14 and 28 days after clinical alveolitis detection (Fig. 4E). Additionally, the total bone porosity significantly decreased after 28 days of dry socket development, regardless of BA treatment (Fig. 4F). Finally, the dry socket groups (without BA) demonstrated a significant increase of bone mineral density in 28 days of experimental protocol, regardless of BA application (Fig. 4G).

## 4. Discussion

In this study, the authors aimed to test the effects of BA as a therapeutic approach for alveolitis because of its antimicrobial and bone remodeling properties. After 14 and 28 days of clinical detection of alveolitis, histological and microCT analysis were performed, and after 7 and 14 days, bacterial growth was evaluated. Herein, it was observed that BA affected bacteria growth over different time points but did not influence bone repair. Notably, this study is the first to test the effect of a local application of BA in a rat model of alveolitis. Although the treatment of alveolitis is already well established in the literature, curettage of the alveolus should be avoided and the application of local substances that reduce contamination combined with analgesic medication has shown satisfactory results. Thus, the BA is an interesting agent for local application.

Firstly, the BA formula used in this study was a 0.75 % BA with 0.9 % saline solution. It was previously demonstrated that 0.75 % BA with a water-soluble tetrazolium salt assay did not develop cell toxicity in the periodontal tissue [12]. Also, other studies used BA in gel, demonstrating beneficial results and being easier to manipulate; however, none of these studies were carried out in a condition such as alveolitis [10,11,

**Table 1**
Mean ± SD do number of colony-forming units (CFUs) observed in rats with dry socket, treated or non-treated with boric acid (BA). After extraction of the upper right incisors, clinical alveolitis was detected by the absence of blood clot or granulation tissue and presence of edema. The microbiological samples were collected at T1 (before tooth extraction), T2 (alveolitis induced and collected immediately before BA treatment), T3 (7 days after BA local application and dry socket confirmation), and T4 (14 days after BA local application and dry socket confirmation). Total aerobic and facultative bacteria (A) and aerobic and facultative Gram-negative bacteria (B) were counted throughout the different time points.

(A) Total bacteria

| | Dry socket | | | | | Dry socket + BA | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean ± SD | Median | Minimum values | Maximum values | P-values | Mean ± SD | Median | Minimum values | Maximum values | P-values | P-values$^{\Delta}$ |
| T1 | 2900 ± 7922 | 667,0 | 0,000 | 33300 | – | 3369 ± 9220 | 683,5 | 0,000 | 37700 | – | > 0,9999 |
| T2 | 294449 ± 173559 | 310000 | 333,0 | 560000 | 0,6621$^{\alpha}$ | 1248250 ± 1682160 | 401500 | 16000 | 5500000 | < 0,0001$^{\alpha}$ | < 00001 |
| T3 | 163088 ± 288100 | 53300 | 2530 | 1180000 | 0,9893$^{\beta}$ | 186094 ± 297950 | 41200 | 2130 | 1170000 | < 00001$^{\beta}$ | > 0,9999 |
| T4 | 53344 ± 53473 | 40700 | 1870 | 237000 | 0,9959$^{\gamma}$ | 76581 ± 159306 | 14700 | 1300 | 637000 | 0,9966$^{\gamma}$ | > 0,9999 |

(B) Gram-positive bacteria

| | Dry socket | | | | | Dry socket + BA | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean ± SD | Median | Minimum values | Maximum values | P-values | Mean ± SD | Median | Minimum values | Maximum values | P-values | P-values$^{\Delta}$ |
| T1 | 9782 ± 32,76 | 0000 | 0000 | 133,0 | | 31,27 ± 62,67 | 0000 | 0000 | 200,0 | | > 0,9999 |
| T2 | 8155 ± 11665 | 2670 | 0000 | 46000 | 0,9559$^{\alpha}$ | 24251 ± 78476 | 2250 | 66,70 | 317000 | 0,1008$^{\alpha}$ | 0,3639 |
| T3 | 9275 ± 17091 | 1000 | 0000 | 56667 | > 0,9999$^{\beta}$ | 4396 ± 7633 | 1167 | 0000 | 23667 | 0,2680$^{\beta}$ | 0,9799 |
| T4 | 159,0 ± 302,0 | 0000 | 0000 | 1170 | 0,9261$^{\gamma}$ | 1396 ± 4532 | 16,65 | 0000 | 18300 | 0,9998$^{\gamma}$ | > 0,9999 |

$^{\alpha}$ denotes the comparison between T1 vs. T2; $^{\beta}$ denotes the comparison between T2 vs. T3; $^{\gamma}$ denotes the comparison between T3 vs. T4; $^{\Delta}$ denotes comparison between the experimental groups in each time point evaluated.

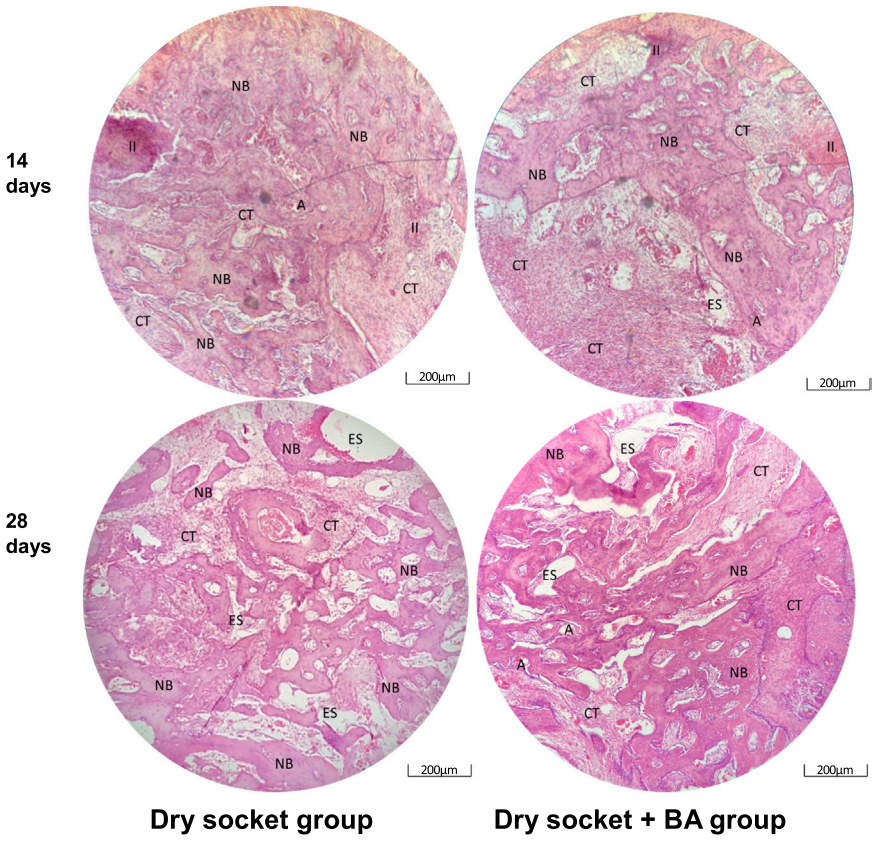


**Fig. 3.** Histological images from rats 14 and 28 days after clinical alveolitis confirmation, with and without boric acid (BA) application. II, inflammation infiltrate; CT, connective tissue; A, angiogenesis; NB, neoformed bone; ES, empty space. Images were obtained in 10x magnification.

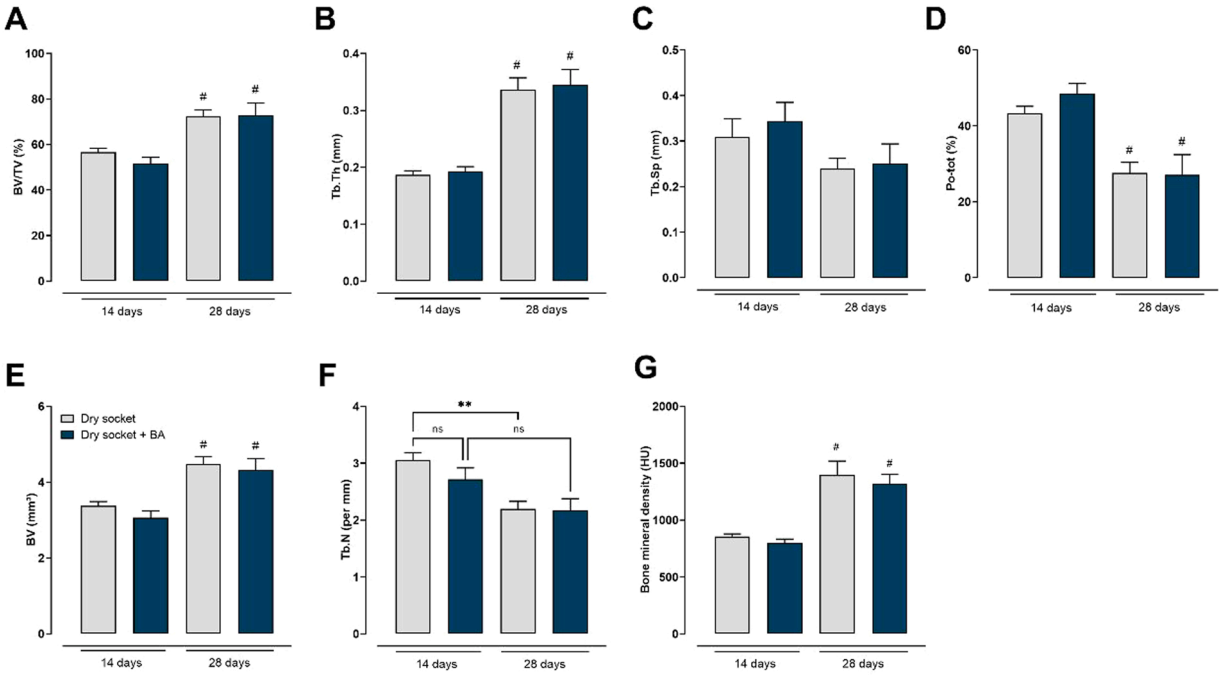


**Fig. 4.** Bone microCT evaluation in rats on 14 and 28 days after alveolitis detection, with or without BA treatment. (A) Bone volume (in $mm^3$), (B) Bone volume fraction (BV/TV in percentage), (C) trabecular thickness (Tb.Th in mm), (D) trabecular separation (Tb.Sp in mm), (E) number of trabeculae (per mm), (F) total bone porosity (%) and (G) bone mineral density (HU). The data are represented in mean ± SEM and #P < 0.05 shows dry socket 14 days vs. dry socket 28 days.

19]. In the current study, a BA solution was more useful taking into account that the BA application site is a bone cavity and it is easier to manipulate. The hermit suture of the alveolus, using mainly palatine soft tissue, guaranteed total occlusion of the filled cavity. Also, diet and water restriction in the first hours after the procedure aimed to increase exposure to the substance.

Microbiological evaluation allowed us to detect a significant growth of total aerobic and facultative bacteria, after the alveolitis induction as expected. Rodrigues et al. [20] found prevalence in alveolitis of anaerobic Gram-negative bacteria and facultative Gram-positive bacteria common in pericoronitis and periodontal diseases, such as Capnocytophaga ochracea, Fusobacterium nucleatum subsp. nucleatum, Prevotella melaninogenica, Streptococcus anginosus, Treponema socranskii, and Streptococcus sanguinis (former S. sanguis). On the other hand, Soni et al. [21] found normal members of the microbiota such as Streptococcus spp., Staphylococcus spp., and Enterococcus spp. in alveolar osteitis using a culture method similar to that employed in the present study.

Fibrinolytic activity can result in premature loss of the intra-alveolar blood clot after extraction [22]. Thus, the presence of facultative anaerobic Streptococcus spp. and Staphylococcus spp. producing activators of plasminogen pathways like streptokinase and staphylokinase are of special concern. Specially Streptococcus spp. are found in large proportions in the oral microbiota, even in a homeostasis state [21], and are initial colonizers of the biofilm that are main source of bacteria to alveoli [23]. The data from total aerobic and facultative bacteria reflect the natural course of alveolitis, with a high count after 4–5 days of induction and, in the end, at T4, the total reduction of counts in the dry socket + BA group, suggesting the involution or complete resolution of the local bacteria contamination after BA application. Thus, considering these results is suggested the efficiency of BA in the presence of total aerobic and facultative bacteria. Furthermore, other studies displayed an antibacterial effect of BA application in periodontal alterations that have a similar microbiota in your early stage [10,11,19].

As for the microCT analysis, we observed that the bone repair occurred in a time-dependent manner, regardless of BA treatment. Hassumi et al. [24] observed the bone repair in rats' alveoli in 7, 14, 21, and 28 days and concluded that microCT is the best tool to evaluate the quality and quantity of neo-formed bone. Notably, this study is the first to evaluate bone remodeling in alveolitis through microCT, combined with the participation of BA local treatment. Herein, the data obtained demonstrated that both groups (dry socket and dry socket + BA) gained bone quantity and quality during the bone remodeling process. The increase of bone volume, bone volume fraction, and thickness of trabeculae show an increased bone neoformation between 14 and 28 days in both experimental groups, regardless of BA application. On the other hand, the number of trabeculae and total porosity displayed an important decrease of these parameters. These data corroborate with the observed by Hassumi et al. [24]; nevertheless, regardless of BA application, the bone repair was successfully resolved in 28 days.

Regarding the effects of BA in bone repair, Hadidi et al. [25] evaluated the BA injection in femur fractures in mice and displayed a significant increase in bone volume and trabecular thickness after 28 days of bone remodeling. The authors suggest that the experimental period for the femur fracture model should be studied for a more extended period since BA demonstrated a significant effect only at the last experimental time point. Furthermore, Gölge et al. [26] used radiographic images to evaluate the actions of BA in femur fractures in rats after BA local application (8 mg/kg), BA oral administration (4 and 8 mg/kg/day), and the combination of both administration routes (8 mg/kg). The authors concluded that the best results were from the groups that received the local application and the combination of both administration routes [26]. In general, these results contradict the findings of this current study. Nonetheless, the BA dosage, administration routes, the experimental model and the intervention proposed were utterly different. So, leading to believe that, maybe, a higher concentration of BA could benefit the alveolitis-associated bone repair.

Concerning the histological data obtained, it was possible to detect a delayed healing process. Somewhat corroborating with the results obtained here, Rodrigues et al. [20] conclude that this significant delay of alveolus repair affected by alveolitis occurs due to bacterial infection and inflammatory processes compared to normal conditions. Also, these same authors claim that an intense osteoclastic activity might precede this delay in bone repair. Regarding the data obtained in this current study, an intense ossification was expected in 28 days following alveolitis development, mainly in the dry socket + BA group. Nonetheless, this information goes against the data obtained from the microCT analysis, possibly because of the histological slides and size. During microCT analysis, it is possible to select a region of interest that exclusively involves the interior of the dental alveolus and obtain a more precise quantity of mineralized tissue. As for the histological evaluation, the selection regions where it is possible to observe the alveolus are based on unstained microscopic images, with a certain possibility of positioning errors, resulting in imprecise outcomes since bone neoformation occurs differently and unevenly in the interior of the alveolus. In that case, the result can demonstrate a lesser proportion of neo-formed bone tissue since the repair and bone deposition initiates in the apical portion of the alveolus [20,27]. Concerning the BA local application, this agent does not influence the bone repair in dry sockets situations. Further studies regarding your osteogenic activity in alveolitis are needed to improve knowledge about this issue and to stimulate new research with this boron compound.

The main limitations of this investigation are related to the histological analysis. The difficulty encountered during the confection of histological slides that involve the whole dental alveolus through its longest axis limited the standardization of the analysis. Also, another limitation was the presence of contaminants and the communication of the alveolus to the oral cavity, which can impair the bone repair, initially in the medium and apical portions of the alveolus, interfering in the microCT and histological data. Lastly, since alveolitis has its origin in blood ischemia, this condition could affect the cellular response over the osteogenic effect of BA, which acts on osteoblast and osteoclast activities.

In conclusion, the BA local application seems to affect the total aerobic and facultative bacteria counts throughout the time course of the disease. However, BA application failed to benefit the bone repair after alveolitis development. It is worth mentioning that this is the first report that investigates the effect of local BA application in a rat model of alveolitis. Nevertheless, this investigation paves the way for future research regarding different BA applications in alveolitis.

## Funding

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – [Finance Code 001].

## Authors declaration

We declare that all reviewers' considerations have been appreciated. The revised manuscripts (clean and marked changes) and the figures are attached.

## References

[1] J. Bosco, S. Oliveira, Á. Bosco, C. Schweitzert, E. Jardim Júnior, Influence of local tetracycline on the microbioita of alveolar osteitis in rats, Braz. Dent. J. 19 (2008) 119–123.

[2] S. Chandran, M. Alaguvelrajan, A. Karthikeyan, K. Ganesan, M.K. Faiz, S.S. Krishna , 2016.Vallabhaneni, Incidence of Dry Socket in South Chennai Population: A Retrospective Study, 2016.

[3] M.Ç. Burdurlu, F. Cabbar, V.Ç. Dagasan, N.S. Işıksaçan, V. Olgaç, The preventive effect of medicinal herbal extract and gelatin sponge on alveolar osteitis: An in vivo micro-computed tomography and immunohistochemical analysis in rats, Arch. Oral. Biol. 122 (2021), https://doi.org/10.1016/j.archoralbio.2020.105002.

[4] M.A. Khalifah, Major autohemotherapy as a treatment modality for alveolar osteitis, J. Ozone Ther. 2 (2018), https://doi.org/10.7203/jo3t.2.3.2018.11292.
[5] M.P. Keshini, S.K. Shetty, S. Sundar, S.N. Chandan, S. Manjula, Assessment of healing using alvogyl and platelet rich fibrin in patients with dry socket - an evaluative study, Ann. Maxillofac. Surg. 10 (2020) 320–324, https://doi.org/10.4103/ams.ams_259_19.
[6] O. Chow, R. Wang, D. Ku, W. Huang, Alveolar osteitis: a review of current concepts, J. Oral. Maxillofac. Surg. 78 (2020) 1288–1296, https://doi.org/10.1016/j.joms.2020.03.026.
[7] J. Crawford, Dry sockets after extraction, Dent. Cosm. 38 (1896) 929–931.
[8] M.A. Sheikh, A. Kiyan, Pathogenesis and management of dry socket (alveolar osteitis), Pak. Oral. Dent. J. 30 (2010).
[9] A. Kolokythas, E. Olech, M. Miloro, Alveolar osteitis: a comprehensive review of concepts and controversies, Int. J. Dent. 2010 (2010) 1–10, https://doi.org/10.1155/2010/249073.
[10] A. Mamajiwala, K. Sethi, S. Mahale, C. Raut, P. Karde, A. Kale, Comparative evaluation of boric acid gel versus chlorhexidine gel in the treatment of chronic periodontitis: randomized, placebo-controlled clinical study, Contemp. Clin. Dent. 10 (2019) 457–464, https://doi.org/10.4103/ccd.ccd_752_18.
[11] S. Singhal, A.R. Pradeep, D. Kanoriya, S. Garg, V. Garg, Boric acid gel as local drug delivery in the treatment of class II furcation defects in chronic periodontitis: a randomized, controlled clinical trial, J. Invest. Clin. Dent. 9 (2018), https://doi.org/10.1111/jicd.12279.
[12] M. Sağlam, U. Arslan, Ş. Buket Bozkurt, S.S. Hakki, Boric acid irrigation as an adjunct to mechanical periodontal therapy in patients with chronic periodontitis: a randomized clinical trial, J. Periodontol. 84 (2013) 1297–1308, https://doi.org/10.1902/jop.2012.120467.
[13] S. Argin, M. Gülerim, F. Şahin, Development of antimicrobial gelatin films with boron derivatives, Turk. J. Biol. 43 (2019) 47–57, https://doi.org/10.3906/biy-1807-181.
[14] X. Ying, S. Cheng, W. Wang, Z. Lin, Q. Chen, W. Zhang, D. Kou, Y. Shen, X. Cheng, F.A. Rompis, L. Peng, C.Z. Lu, Effect of boron on osteogenic differentiation of human bone marrow stromal cells, Biol. Trace Elem. Res. 144 (2011) 306–315, https://doi.org/10.1007/s12011-011-9094-x.
[15] A.A. Gorustovich, T. Steimetz, F.H. Nielsen, M.B. Guglielmotti, A histomorphometric study of alveolar bone modelling and remodelling in mice fed a boron-deficient diet, Arch. Oral. Biol. 53 (2008), https://doi.org/10.1016/j.archoralbio.2008.01.011.
[16] C. Kilkenny, W.J. Browne, I.C. Cuthill, M. Emerson, D.G. Altman, Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research, PLoS Biol. 8 (2010) 6–10, https://doi.org/10.1371/journal.pbio.1000412.
[17] P.S. de Carvalho, R.C. Mariano, T. Okamoto, Treatment of fibrinolytic alveolitis with rifamycin B diethylamide associated with gelfoam: a histological study, Braz. Dent. J. 8 (1997).
[18] M.L. Bouxsein, S.K. Boyd, B.A. Christiansen, R.E. Guldberg, K.J. Jepsen, R. Müller, Guidelines for assessment of bone microstructure in rodents using micro-computed tomography, J. Bone Miner. Res. 25 (2010) 1468–1486, https://doi.org/10.1002/jbmr.141.
[19] D. Kanoriya, S. Singhal, V. Garg, A.R. Pradeep, S. Garg, A. Kumar, Clinical efficacy of subgingivally-delivered 0.75 % boric acid gel as an adjunct to mechanotherapy in chronic periodontitis: a randomized, controlled clinical trial, J. Investig. Clin. Dent. 9 (2018), https://doi.org/10.1111/jicd.12271.
[20] M.T.V. Rodrigues, C.L. Cardoso, P.S.P. de Carvalho, T.M. Cestari, M. Feres, G. P. Garlet, O. Ferreira Júnior, Experimental alveolitis in rats: microbiological, acute phase response and histometric characterization of delayed alveolar healing, J. Appl. Oral. Sci. 19 (2011), https://doi.org/10.1590/S1678-77572011000300015.
[21] N. Soni, V. Singh, S. Mohammad, R. Singh, U. Pal, R. Singh, J. Aggrwal, M. Pal, Effects of honey in the management of alveolar osteitis: a study, Natl. J. Maxillofac. Surg. 7 (2016) 136, https://doi.org/10.4103/0975-5950.201354.
[22] H. Birn, Etiology and pathogenesis of fibrinolytic alveolitis ("dry socket"), Int. J. Oral. Surg. 2 (1973) 211–263, https://doi.org/10.1016/S0300-9785(73)80045-6.
[23] P.E. Kolenbrander, R.J. Palmer, A.H. Rickard, N.S. Jakubovics, N.I. Chalmers, P. I. Diaz, Bacterial interactions and successions during plaque development, Periodontology 2000 42 (2006) 47–79, https://doi.org/10.1111/j.1600-0757.2006.00187.x.
[24] J.S. Hassumi, G. Mulinari-Santos, A.L. da, S. Fabris, R.G.M. Jacob, A. Gonçalves, A. C. Rossi, A.R. Freire, L.P. Faverani, R. Okamoto, Alveolar bone healing in rats: micro-CT, immunohistochemical and molecular analysis, J. Appl. Oral. Sci.: Rev. FOB 26 (2018), e20170326, https://doi.org/10.1590/1678-7757-2017-0326.
[25] L. Hadidi, S. Ge, M. Comeau-Gauthier, J. Ramirez-Garcia Luna, E.J. Harvey, G. Merle, Local delivery of therapeutic boron for bone healing enhancement, J. Orthop. Trauma 35 (2021) e165–e170, https://doi.org/10.1097/BOT.0000000000001974.
[26] U.H. Gölge, B. Kaymaz, R. Arpaci, E. Kömürcü, F. Göksel, M. Güven, Y. Güzel, S. Cevizci, Effects of boric acid on fracture healing: an experimental study, Biol. Trace Elem. Res. 167 (2015) 264–271, https://doi.org/10.1007/s12011-015-0326-3.
[27] R.M. Macedo, S.A. Lacerda, R. Okamoto, S. Shahdad, L.G. Brentegani, Vital bone formation after grafting of autogenous bone and biphasic calcium phosphate bioceramic in extraction sockets of rats: Histological, histometric, and immunohistochemical evaluation, Implant Dent. 27 (2018) 615–622, https://doi.org/10.1097/ID.0000000000000815.