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EFFECT OF CALCIUM PHOSPHATE COMPOSITE WITH DOXYCYCLINE AS A GRAFT FOR BONE DEFECT

Bruno S. B. Lopes [1], Larissa C. R. de Souza [2], Richard A. Fills-Cerchar [1], Guilherme L. Campos [1], Soraia Macari [1], Leonardo A. Lanza [1], Alfonso Gala-Garcia [1, 3], Carlos A. Tagliati [2] and Maria E. Cortés [* 1]

Department of Restorative Dentistry [1], Faculty of Dentistry, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.
Department of Clinical and Toxicological Analysis [2], Faculty of Pharmacy, UFMG, Belo Horizonte, Minas Gerais, Brazil.
Faculty of Dentistry and Health [3], Universidade Federal da Bahia, Brazil.

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Correspondence to Author:
Maria Esperanza Cortés Segura

Professor,
Department of Restorative Dentistry, Faculty of Dentistry, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

E-mail: mecortes@ufmg.br

ABSTRACT: The objective was to determine the effect of calcium phosphate composite with doxycycline molecularly included as a graft on bone defect after tooth extraction for slow delivery of drug. **Methods:** animal were distributedinto four groups: Group 1 (Blood Clot), Group 2 (TCP), Group 3 (TCP/PCL/PLGA), and Group 4 (TCP/PCL/ PLGA/ DOX/CD). An alveolar bone defect was created, and the analysis was conducted at preset intervals of T1 (14 days) and T2 (28 days). **Results:** The results of the histological study using H&E staining demonstrated the existence of newly created bone in all experimental groups. Group 4 exhibited the biggest area of newly formed bone at T1, and this observation was consistent across all time points examined. Group 4 exhibited a consistent volume across the measured time intervals, as evidenced by the existence of more advanced and structured trabeculated bone in T2 in comparison to T1. Even more Group 4, notably T2, displayed the highest proportion (47%) of newly formed bone area in comparison to Group 1 (36%), Group 2 (33%), and Group 3 (31%). The statistical analysis indicated that there were not statistically significant differences at recorded times both within the same groups and between the different groups ($p>0.05$). The number of osteoclasts observed in Group 4 of T2 exhibited a reduction compared to Group 4 of T1, suggesting that DOX has an inhibitory effect on the formation of osteoclasts. Therefore, the doxycycline/CD combination promotes the production of new bone during the crucial early phases of bone repair (T1).

INTRODUCTION: After an exodontia procedure, the alveolar repair process involves the formation of bone tissue that remodels and regenerates the defect region [1]. This process of bone repair may be influenced, sped up, or slowed down by vascular supply, local microbial conditions, and systemic factors.

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In this context, the proliferation and differentiation of bone cells directly involved in the repair process [2] play an important role, with osteoblasts responsible for bone matrix deposition and osteoclasts for its resorption [3].

Biomaterials with different compositions and uses have been talked about a lot in terms of how to improve the process of alveolar bone regeneration after extractions. In terms of the tensile strength and modulus of elasticity of the neoformed tissue, the osteoconductive properties of bioceramics and polymers forming biocomposites and hybrid materials are generally satisfactory for use in bone reconstructions [4].

Together with cyclodextrin, biodegradable polymers are still widely used as controlled drug delivery systems, which seek to control the release of the drug in question, thereby maintaining its therapeutic level for a longer period of time. In this case, local application of Doxycycline (DOX) [5]. DOX belongs to the bacteriostatic antibiotic family, specifically the tetracycline class, and its anti-resorptive activity for bone tissue [6] has been studied. *In-vitro* investigations [7] have indicated that DOX may inhibit osteoclastogenesis. In addition, studies demonstrate the osteogenic efficacy of DOX, making its use in reconstructive bone procedures [8] desirable. The extended duration of doxycycline exposure did not have any detrimental effects on the microarchitecture or biomechanical properties of the bones in male DBA/2J mice that were in a healthy state. Even the use of doxycycline did not demonstrate efficacy in preventing or mitigating the adverse alterations in trabecular microarchitecture, cortical structure, and biomechanical properties of bone that were induced by prolonged diabetes [9]. Therefore, the purpose of this study is to examine bone regeneration stimulated by the composite of biodegradable polymers co-glycolic polylactic acid (PLGA) and polycaprolactone (PCL), beta-tricalcium phosphate (TCP), and cyclodextrin (CD), with the incorporation of Doxycycline (DOX) in a controlled drug release system, in alveolar defects in the jaws of rats.

MATERIALS AND METHODS: Forty-four male Wistar rats (10 to 12 weeks of life at the beginning of the study) with an average weight of 250g (±50g) were used for this study. The Federal University of Minas Gerais (CEUA/UFMG) Ethics Committee on the use of Animals (CEUA/UFMG) approved the ethics protocols for animal experimentation in research under the number 223/2018. The maintenance conditions followed the feeding standards and temperature *ad libitum* between 23 and 25 °C and a light/dark cycle every 12 hours.

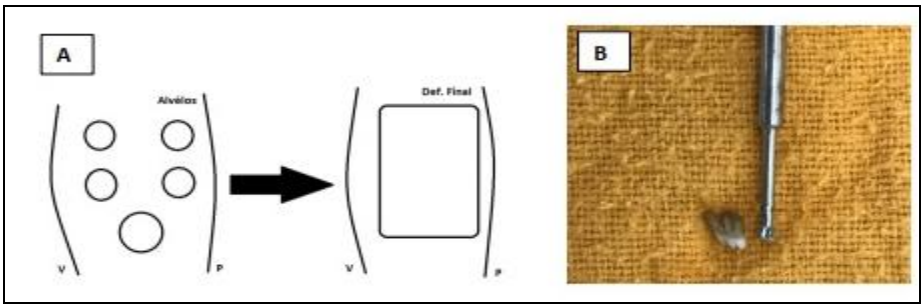

FIG. 1: SCHEMATIC DRAWING OF THE PRODUCTION OF THE ALVEOLAR DEFECT, STARTING FROM THE FIVE REMAINING SOCKETS AFTER EXTRACTION (A). THE PRINTING MARK ON THE DRILL USED FOR MAKING THE DEFECT SHOWS THE DEPTH OF PENETRATION ACCORDING TO THE AVERAGE LENGTH OF THE ROOT OF THE MAXILLARY FIRST MOLAR (B)

Under general anesthesia, the first left upper molar was carefully extracted from all animals via intraperitoneal injection of a solution containing Xylazine Hydrochloride 2% (Xilazin- SyntecTM) and Ketamine Hydrochloride 10% (Cetamin-SyntecTM) in a volume of 0.135mL/100g of body weight. After extraction, with the aid of a spherical drill under low rotation and profuse irrigation using a saline solution, it created a bone defect, expanding the alveolar bone remaining to have as limits the vestibular, palatine, mesial, and distal cortical **Fig. 1** and **2**. There were no antibiotics or other medications used.

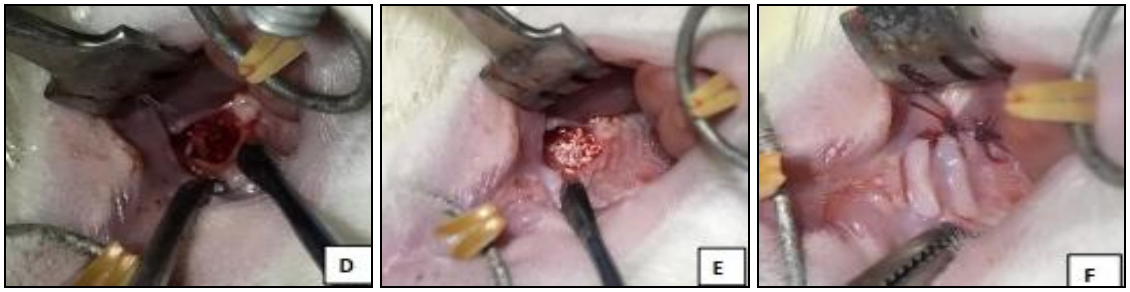

FIG. 2: SURGICAL SEQUENCE FOR THE PREPARATION OF AN ALVEOLAR DEFECT: SOFT TISSUE REMOVAL AND EXPOSURE OF THE OPERATIVE FIELD (A), DEMONSTRATION OF THE DISLOCATION POSITION OF THE LEFT UPPER FIRST MOLAR (B), INCISION AND DETACHMENT OF THE TISSUES (C), BONE DEFECT ALREADY PREPARED (D), FILLING WITH BIOMATERIAL (E), AND SUTURE (F)

Following the extraction of a molar, the animals were subsequently and randomly allocated into the subsequent groups: the socket of Group 1 was permitted to undergo natural recovery without any intervention, such as treatment, and was filled solely by a blood clot. This group served as the control group. Group 2 employed bioceramics composed of tricalcium phosphate (TCP) particles with a size range of 80–100mesh.

Group 3 consists of TCP bioceramics and polymers, specifically polycaprolactone (PCL) and poly (coglycolic lactic acid) (PLGA 60,000 g/mol, 50:50). In Group 4, a composite consisting of TCP/PCL/PLGA/DOX/CD was utilized as the test group. This composite was modified by incorporating Doxycycline (DOX) molecularly included into the composite that had been manufactured beforehand. This study looks into how cyclodextrin (CD) can be used as a molecular encapsulating agent to create a DOX delayed release system. Specifically, a 1:1 molar ratio inclusion compound of cyclodextrin and DOX was prepared, with a final DOX content of 5%. This approach aims to control the release of DOX over time.

All animals were euthanized at 2 or 4 weeks denoted (T1) and (T2) after tooth extraction, respectively, with an overdose of anesthetic solution (6 animals per group, per experimental period). The rodents' jaws were then taken off and cut open in the sagittal plane. The extra soft tissue was cut away from each animal's sagittal sections with a portable handsaw, revealing the area where the extraction took place.

The samples were fixed in 10% formaldehyde, demineralized in a 10% EDTA solution with a pH of 7.3, and then embedded in paraffin (Histosec tablets, Merck, GermanyTM). We fixed the samples in 10% formaldehyde, took out the minerals in a 10% EDTA solution with a pH of 7.3, and then put them in paraffin (Histosec tablets, Merck, GermanyTM). Hematoxylin and eosin (HE), Masson's trichrome, and TRAP immunohistochemical staining were applied to the specimens. The area of bone neoformation selected for histological and histomorphometric analysis was the central region (middle third) of the socket defect created by the extraction of the first molar, alluding to the first upper molar on the opposite side **Fig. 3.**

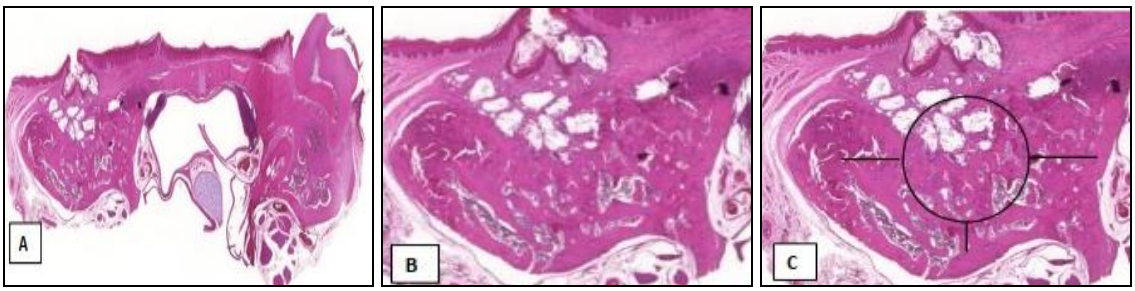

FIG. 3: SCHEMATIC REPRESENTATION OF THE STRATEGY FOR SELECTING THE AREA ELECTED FOR HISTOMORPHOMETRIC ANALYSIS: COMPLETE CORONAL SECTION (A), GREATER ENLARGEMENT FOR THE REGION OF THE BONE DEFECTAFTER EXTRACTION OF THE LEFT UPPER FIRST MOLAR (B), ANALYZED AREA SURROUNDED IN THE CENTER OF THE DEFECT, DISREGARDING THE ALVEOLAR CORTICES (C).

All animals were euthanized at 2 or 4 weeks denoted (T1) and (T2) after tooth extraction, respectively, with an overdose of anesthetic solution (6 animals per group, per experimental period). The jaws of the rodents were then removed and dissected in the sagittal plane, and the sagittal sections of each animal were separated from the excess soft tissue using a portable handsaw, isolating the region containing the extraction site. The samples were fixed in 10% formaldehyde, demineralized in a 10% EDTA solution with a pH of 7.3, and then embedded in paraffin (Histosec tablets, Merck, GermanyTM). The pieces were sectioned perpendicular to the long axis of the alveolar process using a microtome to acquire 5 μm-thick slices, using the first upper molar on the opposite side as a reference. Hematoxylin and eosin (HE), Masson's trichrome, and TRAP immunohistochemical staining were applied to the specimens. The bone neoformation area chosen for histological and histomorphometric study was the middle third of the socket defect left by the removal of the first molar, which refers to the upper first molar on the other side **Fig. 3.**

Statistical Analysis: The collected data were analyzed by the R software (R Core Team, 2016). Descriptive statistics were analyzed. The normality of the data was verified by the Shapiro-Wilk test. To check if there was a difference in the neoformation between the groups, the results were submitted to the variance (ANOVA), in the factorial model (4x2), which is used to compare the means of the treatments. The level of significance of 95% ($p<0.05$)

RESULTS:

Histological Analysis - HE: After hematoxylin and eosin staining, a qualitative analysis of the histological sections was conducted for each group, and its characteristics (from the closure of the surgical wound to the presence of inflammatory infiltrate) were observed separately for both T1 and T2 **Fig. 4 A-H.**

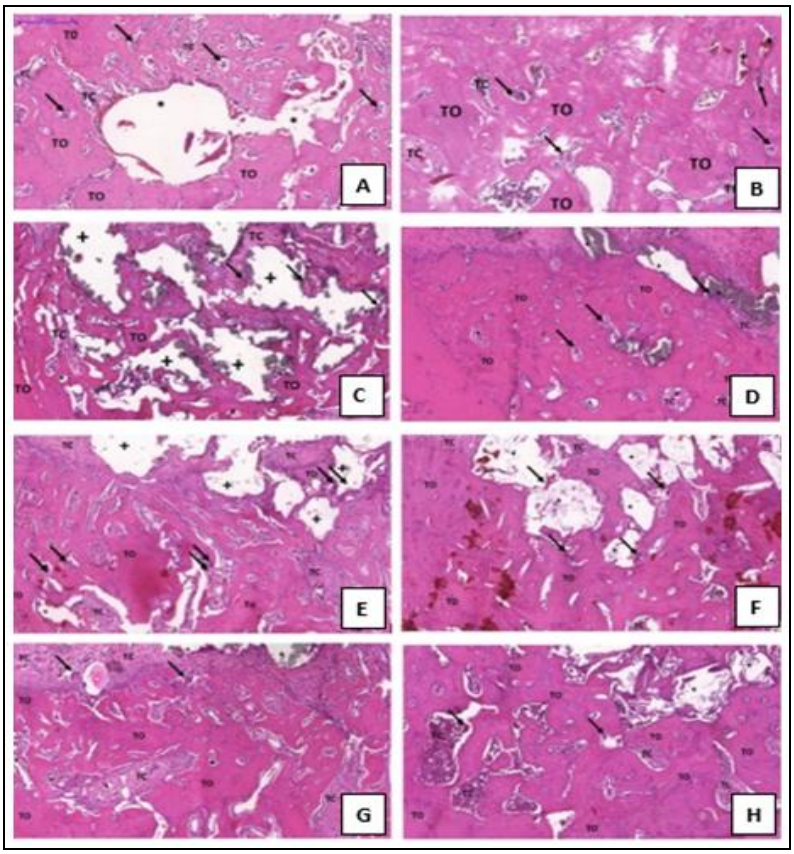

FIG. 4: REPRESENTATIVE PHOTOMICROGRAPHS OF ALVEOLAR BONE TISSUE (TO) 14 DAYS AFTER EXTRACTION, CONNECTIVE TISSUE (CT), REMAINING BIOMATERIAL (+), AND OSTEOCLASTS (ARROW). GROUP 1: BLOOD CLOTT1 (A) AND T2 (B), GROUP 2:TCPT1 (C) AND T2 (D), GROUP 3: TCP/PCL/PLGA -T1 (E) AND T2 (F), AND GROUP 4 TCP/PCL/PLGA/DOX/CD- T1 (G) AND T2 (H) (HE STAINING, × 400, 10X MAGNIFICATION, SCALE200MM).

In all parts of the orthoceratinized, stratified pavement with a noticeable granular layer, lining epithelium was found. Presence of blade-thick, cellularized connective tissue beneath the epithelium (nutrition epithelial layer). Additionally, the formation of new bone tissue was observed in varying degrees and according to time examined. In certain cases, the biomaterial did not degrade completely **Fig. 4 C-H.**

For Group 4 containing Doxycycline, at T1 **Fig. 4G**, it is possible to observe a significant volume of neoformed bone combined with a small presence of osteoclastic cells and residual biomaterial in the analyzed field, and all of these characteristics positively overlap when compared to Groups 1, 2, and 3. There was a small amount of inflammatory infiltrate (mostly mononuclear cells and lymphocytes and macrophages, with a volume appropriate for trauma) in Group 4 at both T1 and T2, but it was seen less often. 1 and T2, but was observed less frequently.

Histological Analysis:

Masson's Trichrome: Staining with Masson's trichrome (with aniline) is primarily useful for staining connective tissue, including bone tissue. The present investigation reveals that the bone trabeculate blushes more strongly in bluish tones as this tissue matures **Fig. 5A-D**. As the tissue is more immature and/or neoformed, it appears in various hues of red.

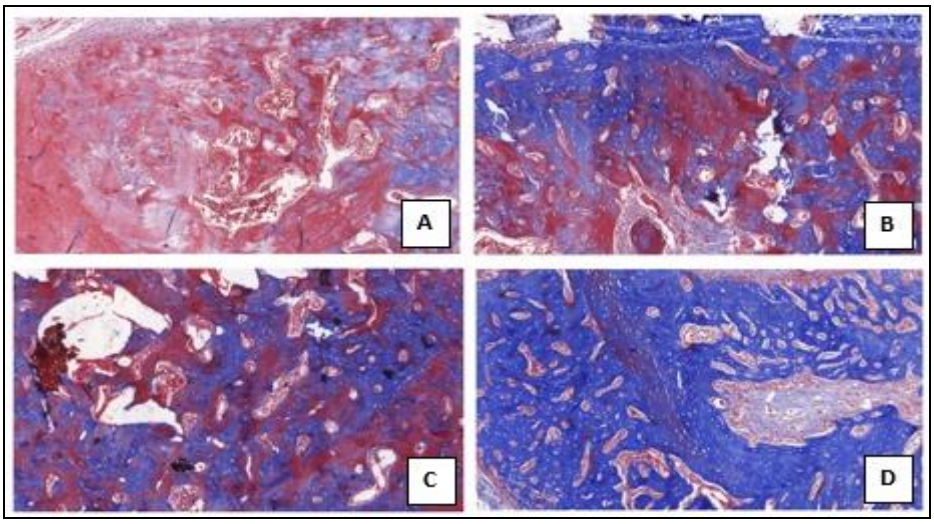

FIG. 5: PHOTOMICROGRAPH OF T2 MASSON TRICHROME STAINING. GROUP 1: BLOOD CLOT, (A), GROUP 2 (TCP), (B), GROUP 3: TCP/PCL/PLGA(C) AND GROUP 4: TCP/PCL/PLGA/DOX/CD(D) (10X INCREASE, 200MM SCALE).

According to the literature, the bone neoformation in the animal model used in the study, rats, does not appear to undergo significant alterations after 14 days. Therefore, we chose to analyze slides stained at 28 days (T2) in order to obtain stable findings for the tested groups. When observing the distinct groups for T2 (bone neoformation stability), Group 4's trabeculae were significantly more mature than those of the other groups.

Histomorphometric Analysis (Bone Neoformation): The standard for the histomorphometric analysis was the average of 1/3 of the central area of the bone defect after extraction, which measured approximately 200.000 m2. Group 4 had greater bone neoformation than the other groups, particularly in T1, but this difference was not statistically significant. According to Masson's trichrome, this portion of T2 remained essentially unchanged, but the trabeculated clearly organized and matured as T2 progressed. The only significant difference at the 0.05 significance level is between Groups 1 and 2 in T2 ($p<0.05$) **Table 1.**

TABLE 1: MEASURES OF THE AREA OF NEOFORMED BONEIN MM² OF BONE AFTER TOOTH EXTRACTION

Treatment (By Group)	Average, standard deviation (SD),and percentage of total formed bone area (µm²) by time					
	T1	SD	%	T2	SD	%
BloodClot	72024.3	32858.9	36%	151851.9	25668.6	76%

βTCP	65341.6	22330.0	33%	89347.3 *	73042.2	45%
βTCP/PCL/PLGA	60929.3	30939.5	30%	105450.5	31649.4	53%
βTCP/PCL/PLGA/DOX/βCD	91066.1	390001.0	46%	92643.2**	46963.2	46%

*p ≤ 0.006; **p ≤ 0.069

Importantly, although there was no statistically significant difference between the other differences, there was a difference in the area of bone neoformation, and Group 4 had the greatest result in this regard in T1. Considering the area of neoformed bone for all groups, bone neoformation was greater at T2 than at T1 for all groups.

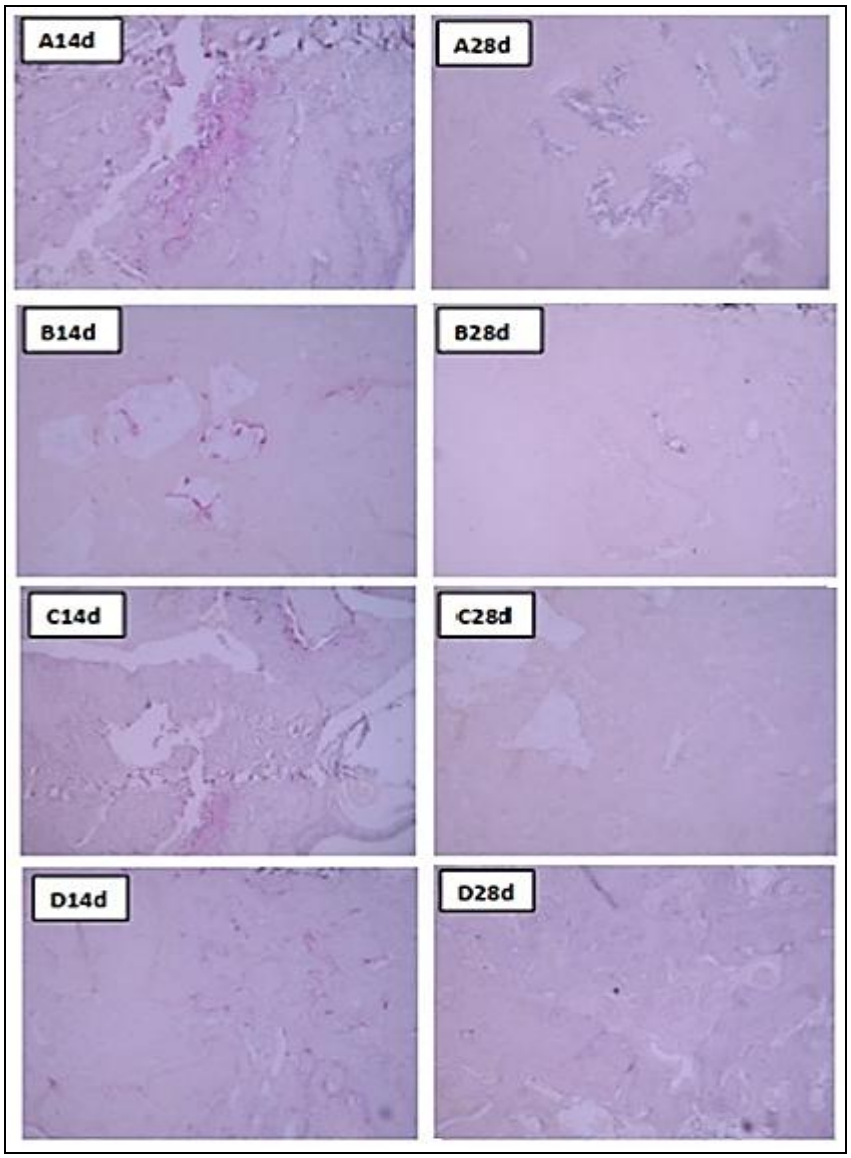

FIG. 6: THE TARTRATE-RESISTANT ACID PHOSPHATASE (TRAP) AFTER DIFFERENT TREATMENTS AND TIMES (T1 AND T2). GROUP 1: BLOOD CLOT (A), GROUP 2: TCP, (B), GROUP 3: TCP/PCL/PLGA(C) AND GROUP 4: TCP/PCL/PLGA/DOX/CD(D)

DISCUSSION: In this research, it was hypothesized that the composite containing DOX added to a polymer and a mineral matrix would promote bone neo-formation in rats following the extraction of their upper molars. Alveoli are filled with blood clot and the repair process begins immediately after extraction. In the subsequent phases, morphologically, there are four major stages: the phase of cell proliferation, the phase of development and maturation of the connective tissue, and the phase of bone maturation [10], which concludes the process. When analyzing bone neoformation at various times, we observed that the sample presented, primarily for Group 4

(TCP/PCL/PLGA/DOX/CD), at 14 days had a larger area of bone filling the defect, with dense and mature trabeculate in its large area, in contrast to newly placed areas of bone, such as those that showed Masson's coloring. In another study [11] related to the effect of DOX on the same animal model, it was found that the 14th day after surgery is characterized by extensive areas of bone neoformation, surrounded by fibrous tissue, inflammatory infiltration, and numerous osteoblasts. In the present study, it was also possible to observe the presence of numerous osteoblasts surrounding the neoformed bone trabeculate, in addition to moderate inflammatory infiltrate of mononuclear predominance.

At 14 days, mature bone can already be found in the apical third of the socket, while embryonic bone fills the socket's coronal portion [12]. This operative time was also observed in the test group (Group 4: TCP/PCL/PLGA/DOX/CD) of the current investigation.

The quantified bone neoformation in post-exodontia maxillary defects in rodents and reported elsewhere [3] that there was no significant difference in the percentage of neoformation between the second and fourth weeks. This information is added to the findings of our study, in which there was no significant difference in the area of bone neoformation between T1 and T2 (14 and 28 days) for Group 4 with DOX, indicating that the controlled drug delivery system may be more effective in the first days of bone repair. However, when we analyzed the other control groups, we found differences, albeit not statistically significant, between the operative durations, including for the clot group, which contradicts what the aforementioned author reported.

According to the literature the phase of bone repair begins during the first week and is characterized by a decrease in cell expression, transient inflammation, and the differentiation of pluripotent cells into fibroblasts and osteoblasts. In the alveoli of the treated rodents, an activity of DOX following events that contribute to the repair process of the dental alveoli following tooth work was confirmed. Regarding the presence, type, and intensity of inflammatory infiltrate in Group 1 (Clot), a mixed leukocyte infiltrate was observed, but predominantly mononuclear cells (lymphocytes and macrophages) with mild to moderate intensity. While treatment with DOX marginally reduced the infiltrate of inflammatory leukocytes, histologically characterized as a mild infiltrate, the infiltrate retained the same characteristics. It is conceivable that DOX will collaborate in the process of bone repair because the modulation of the inflammatory process accelerates bone remodeling after tooth loss [13].

In fact, it has been demonstrated that the phase of bone repair begins during the first week and is characterized by a decrease in cell expression, transient inflammation, and the differentiation of pluripotent cells into fibroblasts and osteoblasts[13]. In the alveoli of the treated rodents, an activity of DOX following events that contribute to the repair process of the dental alveoli following tooth work was confirmed. Regarding the presence, type, and intensity of inflammatory infiltrate in Group 1 (Clot), a mixed leukocyte infiltrate was observed, but predominantly mononuclear cells (lymphocytes and macrophages) with mild to moderate intensity. While treatment with DOX marginally reduced the infiltrate of inflammatory leukocytes, histologically characterized as a mild infiltrate, the infiltrate retained the same characteristics. It is conceivable that DOX will collaborate in the process of bone repair because the modulation of the inflammatory process accelerates bone remodeling after tooth loss.

Several works of literature refer to the effects of DOX on the manifestation of numerous factors that impede the development of the alveolar bone repair process [13]. It was also demonstrated that DOX can inhibit the expression of BMP-2, modulate the angiogenesis process, and promote osteogenic differentiation both *in-vitro* and *in-vivo*, indicating that this tetracycline analog has an effect on bone formation and regeneration [14].

In a rat calvaria model [15] confirmed the effectiveness of DOX on RANKL-marked osteoclastogenesis by inhibiting the action of the MMP-9 enzyme. DOX modulates the mRNA expression of functional markers of osteoclasts, such as tartrate-resistant acid phosphatase (TRAP) and Cathepsin K, as observed in the same study.

In the present study, we observed that the higher peak of neo-formation related to the presence of DOX took place until 14 days and remained, in percentage, in the 28-day analysis. Such an incident may be justified by the fact that the drug release system employed during this first period has been more effective at keeping DOX available locally. Thus, inflammation decreased, promoting osteogenesis, as was related when treatedwith a controlled drug release system, in particular with the use of DOX, which affects bone neo-formation rates in the post-exodontic repair of alveoli in a rat model [16]. Increasing the quantity of comparison control groups for composites may enhance the statistical outcomes and may provide further insights into the osteogenic effects of the investigated drug, even if it contradicts prevailing experimental principles. It is important to consider the size of the particles in the biomaterial. It is advisable to choose smaller particles, it is advisable to choose smaller particles, as this may reduce the likelihood of partial closure of the epithelium in animals and maybe decrease the duration of resorption.

CONCLUSION: Based on the findings presented, this study demonstrates that the incorporation of the inclusion compound of doxycycline had a beneficial impact on alveolar healing and bone neoformation after a 14-day period, as compared to the use of bioceramics alone or a blood clot, which required 28 days to achieve similar results. Nevertheless, further investigation is necessary to assess the action of DOX on alveolar abnormalities on a more extensive scale. The implementation of accurate defect standardization does not seem to have yielded substantial effects on the outcomes. However, it did provide a constraint due to the challenges encountered within a constrained operative field. The possible impact of DOX on bone neo-formation in animal models other than bioceramics warrants further investigation in order to fully understand its favorable assembly.

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CONFLICT OF INTEREST: The authors declare they do not have any conflict of interest

REFERENCES:

1. Cardaropoli D, Tamagnone L, Roffredo A and Gaveglio L: Relationship between the buccal bone plate thickness and the healing of postextraction sockets with/without ridge preservation. Int J Periodo Restor Dent 2014; 34(2): 211-7.
2. Suárez-López Del Amo F and Monje A: J Efficacy of biologics for alveolar ridge preservation/reconstruction and implant site development: An American Academy of Periodontology best evidence systematic review. Periodontol 2022; 93(12): 1827-1847.
3. Shahabooei M, Razavi SM, Minaiyan M, Birang R, Behfarnia P and Yaghini J: A histomorphometric study of the effect of doxycycline and erythromycin on bone formation in the dental alveolar socket of rat. Adv Biomed Res 2015; 4: 71.
4. Razazpour F, Najafi F, Moshaverinia A, Fatemi SM and Sima S: Synthesis and characterization of a photo-cross-linked bioactive polycaprolactone-based osteoconductive biocomposite. JBMRA 2021; 109(10): 1858-1868.
5. Chen Y and Dong CM: pH-sensitive supramolecular polypeptide-based micelles and reverse micelles mediated by hydrogen-bonding interactions or host-guest chemistry: characterization and *in-vitro* controlled drug release. J Phys Chem B 2010; 114(22): 7461-7468
6. Biewer B, Rompen E, Mittelbronn M, Hammer GP, Quatresooz P and Borgmann FK: Effects of minocycline hydrochloride as an adjuvant therapy for a guided bone augmentation procedure in the rat calvarium. Dent J (Basel) 2023; 11(4): 92.
7. Holmes SG, Still K, Buttle DJ, Bishop NJ and Grabowski PS: Chemically modified tetracyclines act through multiple mechanisms directly on osteoclast precursors. Bone 2004; 35: 471-8.
8. Yagan A, Kesim S and Liman N: Effect of low-dose doxycycline on serumoxidative status, gingival antioxidant levels, and alveolar bone loss in experimental periodontitis in rats. J Periodontol 2014; 85: 478-489.
9. Fowlkes JL, Nyman JS, Bunn RC, Cockrell GE, Wahl EC and Rettiganti MR: Effects of long-term doxycycline on bone quality and strength in diabetic male DBA/2J mice. Bone Reports 2015; 1: 16-19.
10. Vieira AE: Intramembranous Bone Healing Process Subsequent to Tooth Extraction in Mice: Micro-Computed Tomography, Histomorphometric and Molecular Characterization. PloS One 2015; 10(5).
11. Toledano-Osorio and Manuel: Dexamethasone and Doxycycline Doped Nanoparticles Increase the Differentiation Potential of Human Bone Marrow Stem Cells. Pharmaceutics 2022; 14(9): 1865.
12. Yoneda T: Application of Coenzyme Q10 for Accelerating Soft Tissue Wound Healing after Tooth Extraction in Rats. Nutrients 2014; 6(12): 5756-5769.
13. Rubin: Rubin pathology: clinicopathological bases of medicine. Guanabara Koogan/GEN, 2006. Philadelphia Ed: Wolters Kluwer/Lippincott Williams & Wilkins, 2008
14. Lucateli RL: DOX and Autogenous Bone in Repair of Critical Size Defects. Implant Dentistry 2018; 27(4): 1-6.
15. Kim KY, Kim Y, Lee J, Shin H and Lee WRS: Tetracycline Analogs Inhibit Osteoclast Differentiation by Suppressing MMP-9-Mediated Histone H3 Cleavage. Int J Mol Sci 2019; 20: 4038.
16. Bonetto LM Corso PFCL, Kuchar GG, Gerber JT, Cunha LF and Mohammed Elsalanty M: Effect of Age and Sodium Alendronate on Femoral Fracture Repair: Biochemical and Biomechanical Study in Rats. Front Cell Dev Biol 2021, Sec. Cell Growth and Division 2021; 9.

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