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Novel Model of Mono Cortical Bone Defect in Rat Mandible: An Interesting Tool for Osseous Investigations

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Abstract

Introduction: biomaterials are worldwide tested in healthy sciences disciplines to be further applied in human patients and these tests ought to be conducted in small animals before they can be safely applied in human clinical research. **Objectives:** describe in detail a novel model of a mandible defect in Wister rats to provide the researchers the possibility to easily test their biomaterials when the mandible is the fundamental object of study. **Materials and Methods:** eighteen adults' male Wister rats were operated for the creation of this mandible defect to locally apply a medication used as a complementary treatment of odontogenic tumor surgery. **Results:** if properly executed, the vestibular aspect of mandible body of Wister rats is the unique region possible to create a real monocortical defect, preserving the lingual table. Bottom surface and space between internal margins of the defect allow placement of substances in a consistency of cements, powders, and liquids. **Conclusions:** this novel model will allow the researchers to easily test their biomaterials at the peculiar mandible region, obtain the results in short periods under laboratory conditions, and use less animals, applying the principle of 3 Rs in animal research (replacement, reduction and refinement).

Keywords: Rats, Wister. Mandible, Bone defects, Bone repair, Biocompatible material.

Introduction

New drugs and biomaterials are worldwide tested in healthy sciences disciplines such as medicine and dentistry, among others, to be further applied in human patients. These tests ought to be conducted in living biological environments before they can be safely applied in human clinical research and finally commercialized (1). In general, rats are a homogenous group of rodents extensively used for these purposes due to they are easy to deal, maintain and their organism responds quite similar to the human being (2).

Specifically in oral and maxillofacial region, traumas and removal of aggressive pathologies are very common in children and adults, creating complex hard tissue defects. One of the most expectations of these patients is the final rehabilitation to correct residual deformities (2); other example is volume recovery of maxilla and/or mandible of elderly patients if oral rehabilitation with implants and prosthodontics is scheduled. Autogenously grafts are yet the best options to overcome such problems; nonetheless, creation of a substitute to it is a theme of interest between researchers and surgeons (3, 4, 5).

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For this, non-critical and critical size bone defects are surgically simulated in animal model as a platform to applicator various biomaterials in order to investigate its osteoinduction and osteoconduction potential. In rats, calvarias region is the most common used site (5), but it may respond different to maxilla or mandible because distinct anatomic aspects, abundant vascularization from pericardium and increased animal mortality secondary to intraoperative brain injuries. As far as the experiment can approximate to structure of interest, probably better the results will be (1). On the other hand, mandible defects are frequently created in ramus region, a very thin structure that make difficult the creation of a monocortical thickness defect, a fundamental characteristic to maintain the tested substance in contact and supported solely by hard tissue (1,2,6,7). The present work aims to propose a novel model of a mandible defect in Wister rats to provide the researchers, possibility to easily test their medications and biomaterials when mandible is a fundamental object of study.

Materials and Methods

Ethic Committee in Animal Research of Dentistry Faculty of University of São Paulo approved the present study (protocol 01/2015), which mainly intended to histological evaluate Carnoy's solution effects at mandible of Wister rats. Eighteen male healthy animals of *Rattus Norvegicus* specie, 60 days old and weighting around 250 to 350 grams were allocated for surgeries, equally separated in groups A, B and C and sacrificed to specimen preparation for histological analysis 0, 3 and 10 days after the procedures, respectively. From beginning to end of experiments all animals were treated ethically: had free access to food and water, stayed in a cage of polycarbonate with forced air ventilation located in a room of 12 hours light/12 hours dark cycles and 24º Celsius temperature.

Technically, each animal was manually immobilized and weighted to calculate general anesthesia dose that was intraperitoneally injected with an insulin needle in a rate of 100 mg/kg ketamine and 10 mg/kg xylazine mixture. After the animal was under profound sedation, Wistar rat is carefully placed in dorsal decubitus onto aseptic table and the submandibular region is cleaned with antiseptic solution. Then, 0,6 ml of 2% lidocaine/1:200.000 epinephrine is injected bilaterally along where the incisions will be placed; this is crucial to painless manipulate animal's soft and hard tissues. Then, operator localizes the mandible angle, grasps upward the cetaceous tissue of this region with an Adson forceps, and performs a stab incision with an iris scissor aligned to inferior border of mandible. This scissor is closed introduced within this stab incision, interiorly pushed under skin tangent to inferior border; when resistance occurs, the scissor is wide opened to dissect subcutaneous plane and closed again to be totally removed from this pathway of dissection.

Certainly, operator will know how much dissection is enough; it means that this maneuver can be repeated. Once dissection is satisfactory, one scissor blade is introduced through pathway of dissection, tangent to mandibular basal region, and skin is full-length is incised about 15 mm. An assistant retracts it with two senn-muller retractors and a bright region can be seen at the most anterior region of inferior border. A freer elevator placed exactly at this point will detach posterior periosteum and massager muscle towards to begging of ramous and they are incised. Now, senn-muller retracts together skin, masseter muscle, and periosteum to expose a line crossing obliquely the vestibular aspect of mandibular body. With one hand, operator firmly grabs this oblique line and lingual cortical to stabilize the mandible and with other hand, perforates the vestibular aspect in a depth of 0,5 millimeters or less, right above the line whilst assistant retracts superiorly the anterior portion of massager muscle and irrigates with saline solution. This perforation is performed with a 3 millimeter diameter and 1 millimeter thickness gross granulation diamond point in a disk format, commonly used for dental procedure. Velocity of perforation is 1.500 rpm with a subtle pressure movement until a half or less of diamond point penetrates the bone. After debris removal and homeostasis, researchers may insert biomaterials or medicaments of interest within the monocortical defect or cover it with barriers. To finish the procedure, massager muscle is repositioned and closed with a continuous absorbable suture and skin may be closed with a continuous 4-0 nylon suture.

Surgeries were performed in the morning. For immediate postoperative care, animals which were sacrificed 3 and 10 days after surgery received a sweet analgesic solution (0,15 ml of dipyrone) when they returned from general anesthesia and had swallow and gag reflex. Caregiver may give additional doses of 0,15 ml each 6 hours for the first day; this scheme is enough to adequately control animal's pain. Polluted ration must also be available in powder consistency because diminished bite force secondary to partial massager muscle detachment may difficult rodent to feed only by solid food.

Submandibular swelling may alert researchers for some postoperative infection in course; however, this swelling is only inflammatory and no animal of our study had local/systemic infection; therefore, we do not recommend administration of antibiotics after surgery.

All postoperative sacrifices were accomplished under general anesthesia. After animal is under profound sedation, recommended technique of euthanasia is cervical dislocation. It is easy and fast to perform as well as permits a quick specimen collection and fixation. All sutures are removed and operatory field exposed; depending on the researcher objectives, masseter muscle may be left in place covering the monocortical defect, maintaining periosteum preserved throughout preparation to histological analysis. To remove the specimen, now, is interesting to expose the whole mandible and partially resect it about 2 or 3 millimeters away from monocortical defect, or even more, with a small cylindrical carbide burr (Figure 1 A, B, C and D show all the process). Specimen must be delicately manipulated and immediately transferred to fixative solution.

Results

Submandibular approaches and creation of monocortical defects lasted an average of 7 minutes and no animal died neither intraoperatively nor postoperatively. Wistar rats of group B and C lost weight, but animals of group C gained some grams from eighth to tenth day after surgery (graph 1). Figure 2 shows the histologic architecture of monocortical mandibular defect 10 days after its confection. It is fulfilled with granulation tissue composed mainly by fibroblasts; secondary bone became to ossify from periphery of concavity. It is possible to see reparative tissue/native bone interface.

Discution

Different techniques were tried in a pilot rat model before we find this possibility. Firstly, molars were removed to soak alveolus with Carnoy's solution, but dental roots easily fracture, alveolus is rapid filled with blood clot and operatory field is extremely limited. Secondly, we tried mandibular ramus; however, perforating this region will always exposes medial pterygoid muscle. Inferior border of mandible is difficult to use due to intimate relationship with inferior incisive tooth.

If properly executed, we believe that this region is the unique site possible to create a real monocortical aperture in mandible of Wistar rat, preserving lingual table; unfortunately, a perforation more than a half of diamond point thickness may perforate roots of molars and inferior incisive. This is troublesome because dental pulp, especially dental papilla from incisive will proliferate inside the defect, promoting bias to histological analysis. Limit diamond point with auto-cured resin and perform a very careful perforation may restrain this problem. Wrong placed skin incision may difficult operatory field access; bear in mind that its direction must be similar to the contour of inferior border of mandible. Periosteum and masseter muscle must be incised touching inferior border and their detachment must not perforate oral mucosa to prevent contamination of biomaterial. At this moment, some vessels that penetrate masseter may be injured; if abundant hemorrhage occurs, a firm compression with a cotton or a piece of gauze for 1 minute is enough to promote hemostasis. Copious saline irrigation when perforating the bone is imperative to diminish heat and necrosis. We strongly recommend performing the surgery in two people from beginning to end of procedure.

Bottom of defect and space between margins allows placement of substances in a consistency of cements, powders, liquids, autologous scaffolds, as well as permits coverage with membranes without dislocation. Intraoperative control is very calm and time of surgery do not extend beyond animal recovery from general anesthesia; as mandible is used bilaterally per animal, total number of rats could be diminished. An important point to discuss is whether the defect we propose reaches prerequisites in terms of size. To a biomaterial shows its capacity of osteoinduction and osteoconduction, defect cannot heal alone; this is called critical size, a defect that do not completely repairs in a lifetime of the animal. Specifically to rats, this size is suggested to have 5-8 mm diameter (1,4); however, other authors found similar results with a 4 mm diameter defect that did not completely heal at sixteenth week (8) and 2 mm diameter defect that did not completely heal at twelfth week (6), both in mandible of rats. This concept must be sometimes questioned because it is infrequent the researches to expect animals die of old age (9) and then, verify if defect once created healed. Howsoever, if 3 mm diameter defect proposed in the present work is doubtful regarding the possibility to mask the effects of a biomaterial, other regions are available (8,10).

Femur of Wistar rats is also possible to be used (11) but its core is very medullar and if biomaterial is proposed to be applied in human mandible, femur should be discouraged. Metabolic rate of Wistar rat must also be considered; the smaller the animal, the higher the metabolic rate compared with that of a human: 30 days of man's life correspond to one day of rat's life (9). It means that shorter observation period to obtain data sampling are usually required when small animals are used instead of larger ones, simply because they heal faster. Long periods of observations would potentially demonstrate both test and control groups with the defects advanced/complete healed, failing to disclose the beneficial potential of a biomaterial (1)

The main characteristic that differentiates our defect is the real monocortical bed that will contact with the substance in test. This bed is composed solely by osseous tissue, in contrast with dura-mater and muscle, when calvarial and mandibular ramus are used, respectively. Direct contact of bone grafts and biomaterials with soft tissues and muscle is very interesting, but graft/native bone interface and biomaterial/native bone interface is of utmost importance to answer many clinical doubts in surgeries of oral and maxillofacial region (1,4,12). A full-thickness calvaria defect in Wistar rat shows more bone formation at inner than outer surface and periphery of the defect (5) and isolating periosteum may prevent tissue ingrowth to reduce bias of analysis (13,14); if this theory is extrapolated to defect proposed here, osteogenic and osteoinductive potential of the biomaterial will purely influence bone matrix. Furthermore, histological analysis perpendicular to the vestibular cortical will allow visibility of cells proliferation at any local of the whole defect.

Perhaps, dental implants inserted at this very region, perpendicularly through vestibular and lingual corticals, permits to visualize osseointegration of some threads.

Conclusions

This novel model will allow the researchers to easily test their biomaterials at the peculiar mandibular region, obtain results in short periods under laboratory conditions and use less animals, applying the principle of 3 Rs in animal research (replacement, reduction and refinement).

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Conflict Of Interest

No potential conflict of interest relevant to this article was reported.

References

Stavropoulos A, Sculean A, Bosshardt DD, Buser D, Klinge B. Pre-clinical in vivo models for the screening of bone biomaterials for oral/craniofacial indications: focus on small-animal models. Periodontol 2000. 2015 Jun;68(1):55-65. doi: 10.1111/prd.12065.

Saadeh PB et al. Repair of a critical size defect in the rat mandible using allogenic type I collagen. J Craniofac Surg. 2001 Nov;12(6):573-9.

DeConde AS et a. Defining the critical-sized defect in a rat segmental mandibulectomy model. JAMA Otolaryngol Head Neck Surg. 2014 Jan;140(1):58-65. doi: 10.1001/jamaoto.2013.5669.

Gallego L et al. Repair of rat mandibular bone defects by alveolar osteoblasts in a novel plasma-derived albumin scaffold. Tissue Eng Part A. 2010 Apr;16(4):1179-87. doi: 10.1089/ten.TEA.2009.0517.

Honma T et al. Bone formation in rat calvaria ceases within a limited period regardless of completion of defect repair. Oral Dis. 2008 Jul;14(5):457-64.

Chin VK, Shinagawa A, Naclério-Homem Mda G. Bone healing of mandibular critical-size defects in spontaneously hypertensive rats. Braz Oral Res. 2013 Sep-Oct;27(5):423-30. doi: 10.1590/S1806-83242013000500006.

Zellin G, Gritli-Linde A, Linde A. Healing of mandibular defects with different biodegradable and non-biodegradable membranes: an experimental study in rats. Biomaterials. 1995 May;16(8):601-9.

Kaban LB, Glowacki J, Murray JE. Repair of experimental mandibular bony defects in rats. Surg Forum. 1979;30:519-21.

Andreollo NA, Santos EF, Araújo MR, Lopes LR. Rat's age versus human's age: what is the relationship? Arq Bras Cir Dig. 2012 Jan-Mar;25(1):49-51.

Spicer PP, Kretlow JD, Young S, Jansen JA, Kasper FK, Mikos AG. Evaluation of bone regeneration using the rat critical size calvarial defect. Nat Protoc. 2012 Oct;7(10):1918-29. doi: 10.1038/nprot.2012.113.

Hoerth RM et al. Mechanical and structural properties of bone in non-critical and critical healing in rat. Acta Biomater. 2014 Sep;10(9):4009-19. doi: 10.1016/j.actbio.2014.06.003.

Alsberg E, Hill EE, Mooney DJ. Craniofacial Tissue engineering. Crit Ver Oral Biol Med. 2001; 12(1):64-75.

van Leeuwen AC, Huddleston Slater JJ, Gielkens PF, de Jong JR, Grijpma DW, Bos RR. Guided bone regeneration in rat mandibular defects using resorbable poly(trimethylene carbonate) barrier membranes. Acta Biomater. 2012 Apr;8(4):1422-9. doi: 10.1016/j.actbio.2011.12.004.

Kim J, Kim H. Rat Defect Models for Bone Grafts and Tissue Engineered Bone Constructs. Tissue Engineering and Regenerative Medicine. 2013; 10(6):310-16. Doi 10.1007/s13770-013-1093-x.

Figure 1: A - Diamond point that will perform the monocortical defect. Remember that the perforation will be accomplished by friction, then, copious saline irrigation is imperative.

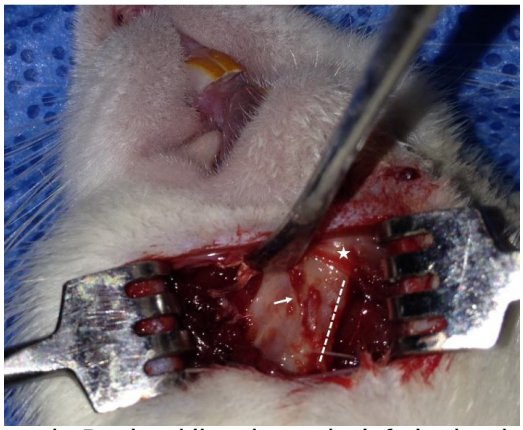


Figure 1 B - Submandibular approach. Depicted line shows the inferior border of mandible that will guide the operator during all procedure. Star demonstrates the bright point located at the most anterior region of inferior border. After the incision of periosteum and masseter, their retraction will expose an oblique line that will determine the position of monocortical defect. Observe that the ramus was not exposed.

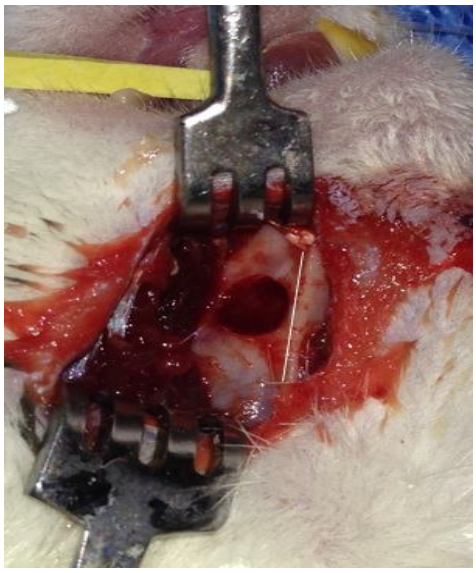


Figure 1 C - Monocortical mandibular defect. The white continuous line is parallel to oblique line and the defect is right above it. If this anatomic reference is respected, the operator will be able to adequately perform the defect.

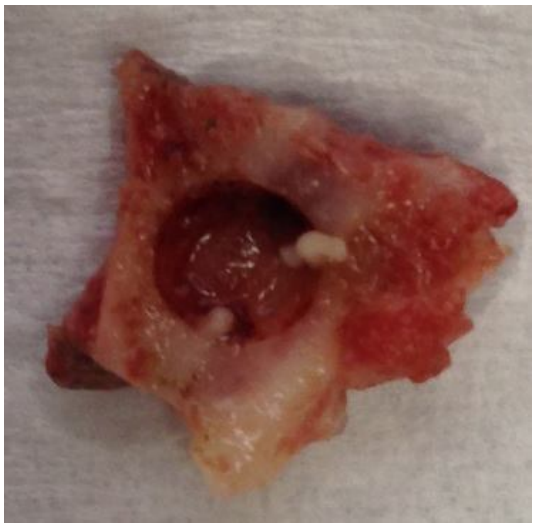
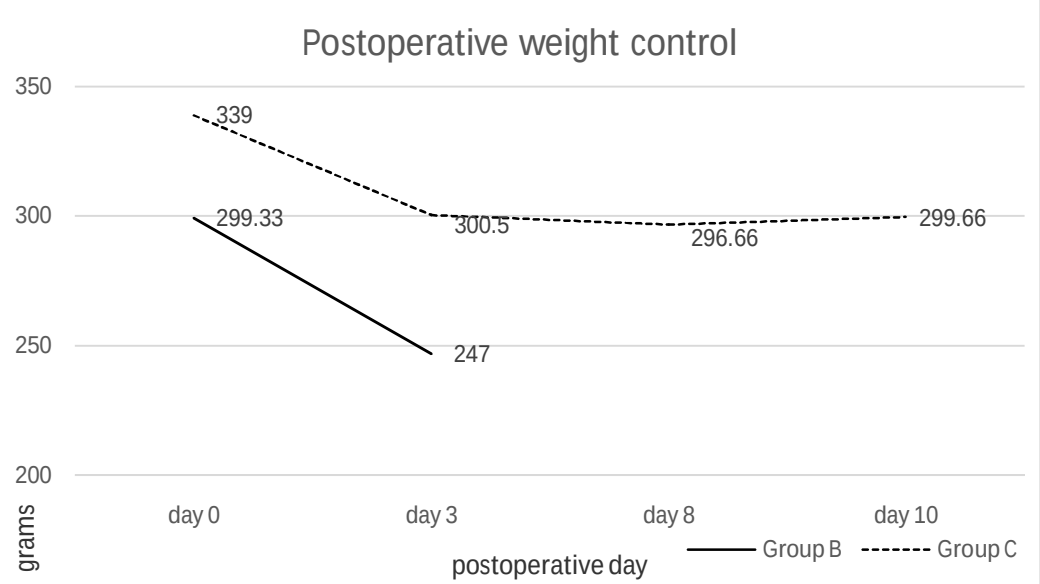


Figure 1 D - Specimen immediately after collection.



Graph 1: Postoperative weight control.

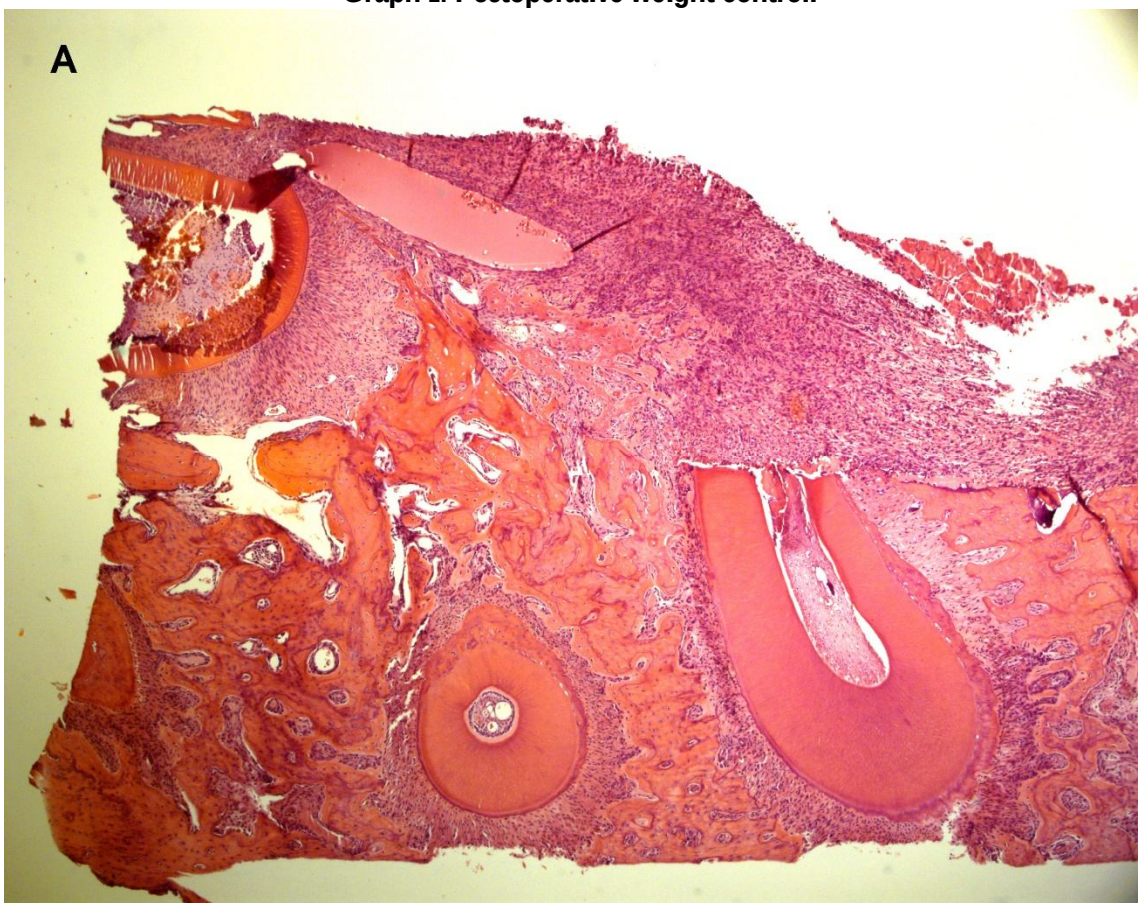


Figure 2 - x4 H&E of control group C. A - Right axial view of defect fulfilled with reparative tissue. Note that, unfortunately, one root was injured; however, osteoid tissue and medullary spaces prevail.

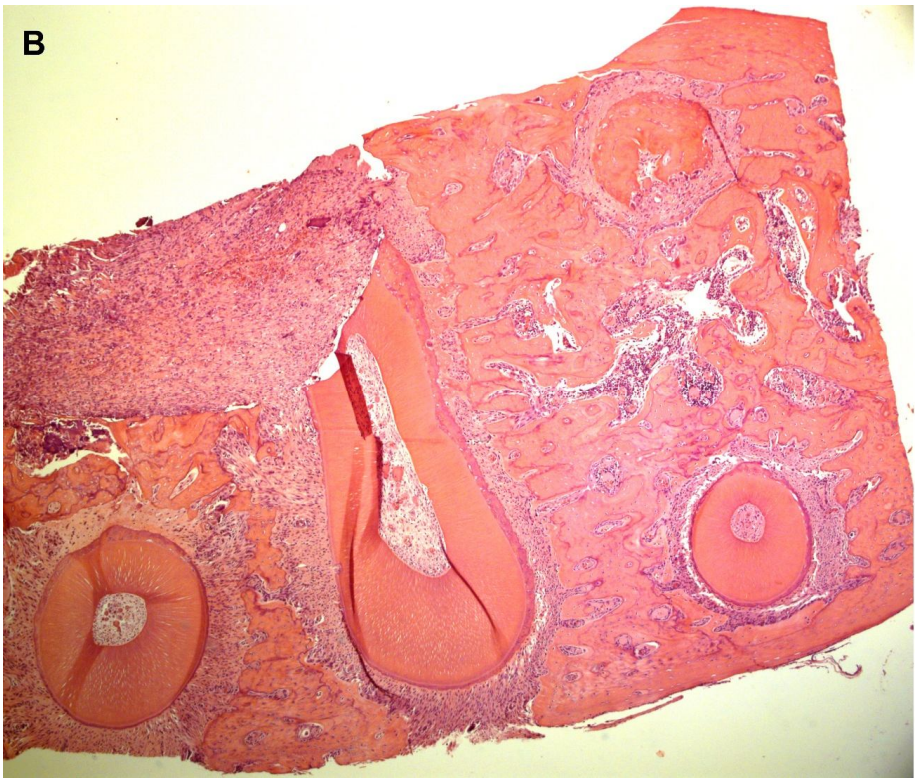


Figure 2 - x4 H & E of control group C. B – Left axial view of same defect as A; interface between reparative tissue and native tissue can be seen.