Proposal for a new site to insertion of dental implants for osseointegration test: Research methodology

Alex Semenoff-Segundo¹, Natalino Francisco da Silva¹, Vinicius Canavarros Palma², Evanie Menezes Marçal Vieira¹, Álvaro Henrique Borges¹, Tereza Aparecida Delle Vedove Semenoff¹

¹Department of Post-graduate Program, School of Dentistry, University of Cuiabá, Cuiabá, Mato Grosso, Brazil, ²Department of Improvement Course in Minor Oral Surgery, Brazilian Association of Dentistry, ABO/MT, Cuiabá, Mato Grosso, Brazil

Abstract

Background: The objective of this paper was to present a methodology for osseointegration in rat mandibles.

Materials and Methods: A total of 16 Wistar male rats with 400 g were undergone to anesthesia, and then blunt instruments were used to expose the alveolar inferior nerve. Once the nerve canal was emptied, one implant (2.2 mm × 4 mm) was installed near the mandibular canal. The animals were sacrificed within the experimental period of 15 (n = 8) and 30 (n = 8) days. The histomorphometric analysis included both bone-implant contact and bone area.

Results: In a comparison of the variables between 15 and 30 days, there were differences in bone maturation (Student’s parametric test for independent samples - P < 0.05). In addition, the technique was shown to be reproducible and performed at a lower cost compared with histological studies involving other animals.

Conclusion: This specific technique of placing implants into the mandibles of rats is a viable alternative in studies of this nature.

Keywords
Dental implants, methods, rats

Correspondence
Alex Semenoff-Segundo, Universidade de Cuiabá, Av. Manoel José de Arruda n° 3.100, Jardim Europa, Cuiabá, MT, Brazil. Phone: +55-65-3623-3009 or +55-65-9983-8030. Email: semenoff@uol.com.br.

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Introduction

The field of implantology has experienced extensive growth and development. For two decades, the most significant aspects of implantology that were studied were related to osseointegration and the stability of marginal ridges.[1,2] These factors are still widely studied today, but biomechanics and function,[3] surface and anatomy of the implants,[4,5] esthetics,[6] and knowledge of risk factors,[7] are also studied. These features, in addition to increasing the quality of life for people[8] in need of dental implants, have delineated a new horizon in this promising area of research.

Specifically, knowledge of systemic factors and implant surfaces has been derived from studies in animals, especially in rats.[9,10] The rat is a mammal with response patterns similar to humans, especially in their biological responses to external substances. Rats serve as suitable models in studies that would be impossible to conduct in humans; these studies are therefore deemed “preclinical.”[9,11]

For osseointegration studies in rats, researchers have often sought to integrate an interface with the tibia, femur and, to a lesser extent, other types of bones.[9,10] This is an appropriate model system and is well-cited in the recent literature,[9,10] but it is also interesting to use this model for implants into the jaw because there is such a similarity in the anatomical response between rats and humans.

However, the difficulty of the technique and the small size of the region make it challenging to select a sufficient area for analysis. The literature describes implants placed in the region of the upper first molars,[13,14] but that technique requires great precision and produces little material (i.e., bone tissue) to analyze. Given the above evidence, the objective of this study is to present a methodology for osseointegration in rat mandibles.
Materials and Methods

These experiments were approved by the Ethics Committee on Animal Research under protocol number 77/05.

Totally 16 male Wistar rats weighing approximately 400 g each were selected. The animals originated from the vivarium of the School of Dentistry (University of Cuiabá, UNIC, State of Mato Grosso, Brazil). Before the experiments began, all animals were subjected to an adaptation period and were placed in housing cages that were composed of plastic and that also contained sterilized shredded paper on the floor.

Their diet consisted of standard rat chow (Presence-Nutrição Animal, Paulínia, SP, Brazil) and water, both available *ad libitum* throughout the study period. The animals were subjected to automated 12 hr light/dark cycle and a controlled temperature and humidity of approximately 24°C and 60%, respectively. Prior to the experimental surgical protocol, the animals were anesthetized by an intramuscular (IM) injection of a combination of 0.1 ml ketamine hydrochloride (50 mg/ml; Dopalen; Agribrands Saúde Animal, Paulínia, SP, Brazil) and 0.05 ml of xylazine hydrochloride (2 g/100 ml; Rompun®, Bayer Saúde Animal, São Paulo, SP, Brazil) per 100 g of body weight. Initially, the surgical procedures were performed via an extraoral route on the lateral surface of the mandible. After the skin was shaved and disinfected with 2% chlorhexidine (Riohex, São José do Rio Preto, SP, Brazil) a 3 cm incision was made on the lateral skin surface of the mandibular body using No. 28 and No. 15 scalpel blades (Swann-Morton-Sheffield, South Yorkshire, England). This process was followed by a plane-by-plane dissection until the mental foramen, and neurovascular bundle were located [Figure 1].

The mandibular canal was subsequently emptied, and a cavity 5 mm long was prepared in the canal lumen with spear drills, followed by a 2.0 mm twist drill [Figure 2] both from the Neodent® surgical kit (Neodent®, Curitiba, PR, Brazil) under profuse irrigation with 0.9% sterile saline solution throughout the procedure.

To produce these perforations, drills adapted to a Kavo® reductor contra-angle 20:1 (KaVo do Brasil, Joinville, SC, Brazil) and connected to an NSK® electric motor at 1200 rpm (NSK®, Tochigi, Japan) were used. The implant (Neodent®, Curitiba, PR, Brazil) [Figure 3] was manually inserted using a 1.2 mm digital key for the placement of implants with a 2.2 mm diameter and a 4 mm length [Figure 4]. The implants were completely inserted into the bone tissue. Shortly after each surgery, a single dose of IM benzathine penicillin G (Pentabiotico Veterinário, Pequeno Porte [small size], Fort Dodge, Campinas, SP, Brazil) was administered (20,000 U/kg IM), which was prepared and adjusted to the weight of each animal.

The rats were monitored daily during the post-operative period. 15 days post-operatively, eight rats were euthanized by an anesthetic overdose. Thirty days post-operatively, the remainder...
of the animals were euthanized according to the same procedure. Prior to euthanasia by an anesthetic overdose, the final weight of each animal was recorded.

The mandibles were then fixed and stored in a container with 10% formalin. After histological processing, the polymerized resin blocks containing the specimens were sectioned along the implant axis using the Exakt System (Exakt Apparatebau, Nordestedt, Hamburg, Germany). The exposed surface of each section had an approximate thickness of 70 μm and was subsequently stained with 1% toluidine blue [Figure 5]. A histometric analysis was performed using a Leica DMLB Microscope (Leica Microsystems Wetzlar GmbH, Germany) and the LAS-4.1.0 image analysis software (version-image processing and analysis system-Leica Microsystems Wetzlar GmbH, Germany).

The bone-to-implant contact (BIC) surface and bone area (BA) variables were measured within the limits of the implant threads. Both of the histometric variables were evaluated only for the first four implant threads, measuring this and subtracting the non-BIC areas of this same region.

Initially, the data normality were tested using the Kolmogorov–Smirnov test, which has a Gaussian normal distribution curve. Student’s parametric test for independent samples was then selected (IBM SPSS Statistics version 20, Armonk NY, United States). The individual who examined the statistical data was trained to appreciate the histological structures involved.

**Results**

The results of the study show that dental implants inserted into the mandibular canal demonstrated adequate osseointegration. Despite the absence of innervation in the region and consequent partial paraesthesia, there was no weight loss, death or indications of post-operative pain in any of the test animals.

At 15 days the contact surface between the implant and the bone BIC, and the amount of new bone formation between the turns of the implants (BA), showed waves of forming bone still slightly mineralized. At 30 days, a more mature and corticated bone was observed in BIC and BA. Significant differences were found in the BIC and BA in the comparisons between the periods of 15 days and 30 days (P < 0.05) [Table 1].

**Discussion**

The technique we describe for the placement of an implant near the mandibular canal of rats was successful. The results infer that this technique is reproducible both from a clinical and from a laboratory perspective, as it was possible to analyze the histology of the loop areas of the implants and the contact between the bone and the implant, differences in the rate of healing at 15 and 30 days, and finally the placement of implants following the use of spear drills and a 2.0 mm twist drill.

Some of the criteria used to characterize a successful implant placement were derived from a classic study.[1] The material of the implant used in this study is the same as that used in implants sold by major manufacturers. Another important point is that the designs of the implant in addition to the treated surface were also the same as those that are available in the market. During the drilling procedure, the engine speed remained the same as in the milling of implants in humans. A third important point is that during wound healing the implant remained empty with no contamination by oral and extra-oral substances and was covered by a tissue layer in the upper half of the implant.

According to the literature, the techniques that are most commonly used in studies of osseointegration in rats are performed in the tibia and femur.[10] Other studies are performed at other sites, but many of them do not involve conventional dental implants.[10]

The embryonic origin of the tibia and the femur bones is the same as that of the jaws. However, the amounts of cortical bone and cancellous bone are different.[14,16] It is interesting to emphasize that the placement of dental implants in bone for studies of this type is widely accepted, and it appears that the placement of implants in the mandible of rats can assist and improve the accuracy of the current information on the pathogenesis of certain diseases[17] and on the osseointegration of dental implants.

The literature features a number of studies where dental implants were placed in the maxilla of rats. In this manuscript, we attempted to reproduce the same technique, but some issues...
hampered our efforts at this particular method. Among them were the extraction of the upper first molar and the possible loss of implants during animal feeding.

It is important to highlight that before start of the study, four animals that would be discarding were used in a pilot study. On that occasion two animals were euthanized with excess anesthetic, and immediately thereafter, we proceeded to remove tissues for the anatomical and morphometric studies to observe the feasibility of the methodology for the placement of dental implants in the mandibular canal region [Figure 6]. After this phase was completed, the other two animals underwent the surgical process. In this phase, the animals were observed their behavior as well as their ability to consume food and water during the post-operative period. This pilot study lasted 30 days.

The company that manufactured the implants that were used in this project and that have been subjected to scientific analysis reported the necessity that the implants be larger to improve some points. Thus, subjects would receive a treated surface used in current implants, better precision characteristics of screw threads and standard sizes for the normal use of keys. It was clarified that the insertion technique used to place the implants into the maxilla is efficient and reproducible; however, we believe it is a challenging method due to the absence of a natural surface treatment and a lower amount of surface area to measure between the bone and the implant surface and between the screw threads of the implants. An advantage of the technique highlighted in this manuscript is that the implants were positioned in a closed environment without any dental occlusion in the animals, which helped to avoid influences of mechanical force.

Despite many relevant aspects, there were also some limitations to our study. During surgical drilling, the operator observed the presence of bone Type I, and in two instances, observed a fragmentation of the apical sagittal crest jaw in three animals; thus, the use of the 2 mm twist drill was always necessary. During the incident, it was decided to continue the implant placement procedure and to keep the animals under clinical observation. After histological processing, nothing remarkable was observed in the tissue of these animals in comparison with the others. An anatomical limitation combined with the difficulty of this surgical technique means that the implants should be placed exactly between the space of the molars and the central incisors.

In the rat, the incisor passes across the bottom edge of the base of the jaw, and thus, surgical care must be taken. Another aspect to consider is that in the most apical portions of the implants were nearby teeth central incisor [Figure 5]. Connective tissue also formed in this region, which is not necessarily a limitation because the area of analysis in this study was standardized to the region of five turns of the implant in the cervico-apical direction.

For the histometric analysis, there were no technical difficulties in cutting the implants. One advantage of this model system is that for studies in rats, less material is used for the preparation compared with the amount used for studies in rabbits and dogs. However, it is clear that the objects of study are quite different from humans, yet, as others have mentioned, this model allows for clinical data collection that would be impossible in a mouse model.

The image capture technique was simple, and the BIC and BA were reproducible in a similar manner to previous research in this area. In this manuscript, two test times were selected, and as the results show, it seems that even at 15 days the formation of immature bone can be observed and studied. At 30 days, the bone appears to already be formed, with contact between the implant and bone observed as in other studies.

According to the above data, it appears that the technique of implant placement in the mandible is feasible, reproducible and inexpensive.

Conclusion

The technique of placing implants in the mandible of rats is an alternative in such studies.

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