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# THE DEVELOPMENT OF A PRECLINICAL MODEL FOR OSTEOINTEGRATION OF DENTAL IMPLANTS - A PILOT STUDY

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***Abstract***

*Functional tooth replacement and bone regeneration are areas of interest in modern dentistry and dental implant research involves increased attention to osteointegration. The aim of the study was to develop a small, inexpensive and reproducible animal model for testing dental implants. Fifteen male Wistar rats, 20 weeks old, average weight of 400 grams were included in the study. They were subjected to a rigorous bone support preparation protocol so that the maxillary first premolar was extracted from the left half arch. After a period of 30 days, necessary for the bone refilling of the dental alveolus, the radiological examination was performed. Then a surgical intervention was performed to mount the titanium implants of an adapted size. Clinically, the evolution was favorable, with no signs of discomfort or oral infection. At the radiological evaluation, optimal bone regeneration could be observed. necessary to ensure a suitable place for implant mounting. The implantation procedure was laborious due to the limited working area. However, rats are proving to be suitable animal models for implant-related studies or innovative treatments administered under pathological conditions.*

***Key words****: implant, osteointegration, rat, tooth extraction.*

## INTRODUCTION

Dental implants, in recent times, represent the life-saving solution for patients with compromised oral health, tooth decay or other conditions that make an alternative of tooth replacement impossible. The demand is increasing, which makes the producers become competitive and offer an increasingly effective product, with high quality in terms of osteointegration or its acceptance by the human body. For this, the implants must pass two big thresholds, before being used in the dental clinic: *in vitro* and *in vivo* tests (Pilawski, 2020). Through the latter, the safety and effectiveness of implants in a living organism is evaluated. For researchers, choosing a suitable animal model is still difficult because regulatory agencies require the validation of a preclinical animal model (Stadlinger, 2012), and the ISO 7405:2018 standard requires that dental implants be tested in their human form. Consequently, the testing of dental implants would be justified only on large animals, but the choice of an experimental animal model is essential to be able to obtain justifiable preclinical results in subsequent clinical research (Spicer, 2012). Therefore, the animal model must guarantee the reproducibility of the clinical condition for which an implant is tested (Li, 2015).

When we talk about dental implants, we can think that their most appropriate testing would be at the level of the oral cavity, but the segmental mandibular defects potentially created at this level represent the biggest challenge, due to their poor intrinsic healing capacity. Researchers in the field of implant testing prefer the choice of small animal models for the well-known economic reasons (housing, care), easy maneuverability and the many possibilities of surgical intervention (da Silva

Morais, 2018). The results of the experiments can be influenced if attention is not paid to the fact that there are species-specific differences related to remodeling, composition, and the process of bone regeneration (Pearce, 2007). In terms of bone remodeling, humans, pigs, dogs, sheep, and goats are moderately similar, while the rabbit is the least comparable. Bone composition, mechanical abilities and bone density have shown interspecies differences (Aerssens, 1998).

Some researchers provide evidence that bone remodeling in rodents is similar to humans, which represents an advantage in choosing a model for studying implants (Baron, 1984). Cellular and molecular indices, regulation of the growth process, and expressed chemokines or cytokines are comparable to humans (Vieira, 2015). Moreover, the morphology of the alveolar bone of rodents does not differ from that of the pig, an animal considered to be the closest to humans, histological and immunohistochemical data highlighting this fact in a comparative study between species (Pilawski et al., 2020).

The aim of the study was to develop an animal model for the study of dental implants. We considered that rats represent the appropriate animal model, considering the morpho-functional similarities of the alveolar bone, the economic advantages, the manipulation and the surgical approach, even if the size requires the adaptation of the size of the implant to be tested.

## MATERIALS AND METHODS

The animal experiments were carried out at the Baneasa Animal Facility (BAF) of the Bucharest National Medical-Military Institute for Research and Development (IC). The study was approved by the Ethics Committee of the Faculty of Veterinary Medicine Bucharest and by the veterinary health authority, in accordance with EU Directive 63/2010 on the care, use and protection of animals used for scientific purposes.

The procedures developed to create the animal model for testing dental implants were performed on 15 Wistar rats, aged 20 weeks, from the SPF (Specific Pathogen Free) kennel of BAF. Throughout the experiment, the animals were housed, in groups of 5, in conventional conditions at a temperature of 20-22°C, a 12 hours light: 12 hours dark cycle and received water and feed *ad libitum*. The general health status of all animals was checked daily and the specific clinical status and body weight monitoring, were evaluated every 2 weeks after surgery. The exclusion criteria were established before the start of the experiment and included as a condition, weight loss of 20% or more at any time of the experiment, which would require the immediate euthanasia of the animal.

### The experimental procedure

1. Extraction of the maxillary molar

Under general anesthesia with a mixture consisting of IP Ketamine (0,5mg/kg, Pasteur, Romania) and Medetomidine (0,5mg/kg, Biotur, Romania), the animals were positioned on the operating table in dorso-ventral decubitus. A spacer was positioned between the upper and lower incisors. With a dental take-off for human use, the gingiva near the left maxillary first molar was separated from the tooth, and by rotational movements in the axis, it was extracted. The roots that broke and remained attached to the alveolus after the extraction were also removed with surgical forceps so that the extraction site remained free of any dental remains. The gingiva was sutured in a single point with a 4/0 resorbable multifilament thread (Novosyn Quick). At the end of the operation, the animals received an antidote (Atipamezole SC, 0.02 mg/kg, Biotur, Romania) an antibiotic (Enrofloxacin SC, 5mg/kg, Pasteur, Romania), and an anti-inflammatory (Ketoprofen SC, 5 mg/kg, Dopharma, Romania) for 3 days. After 4 weeks of healing of the extraction socket, radiological analysis by the high-sensitivity bioluminescence technique (IVIS Lumina XRMS, Werner ROEDL–PerkinElmer, Austria) was performed to check the level of bone regeneration. The experimental extraction operation in rats is shown in Figure 1.

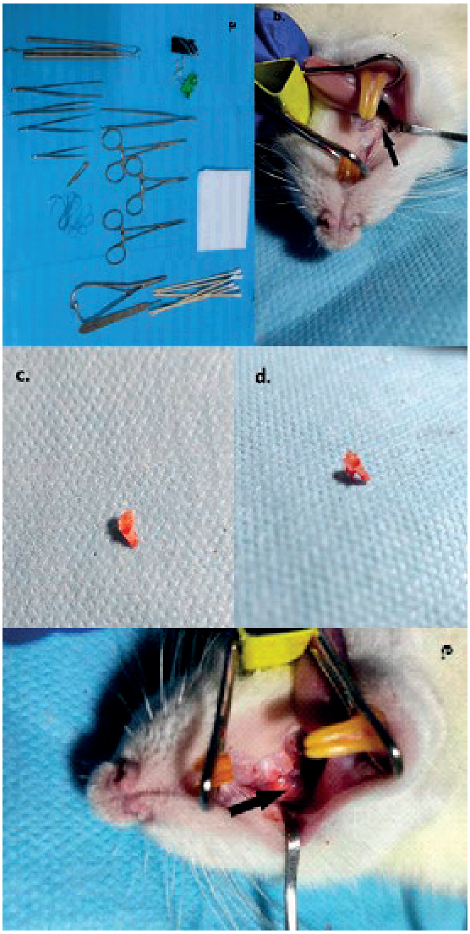


Figure 1: Dental extraction procedure (a - surgical instruments, b - maxillary left first molar, c, d - extracted teeth, e - tooth socket after extraction)

2. The implants mounting

After the 4 weeks necessary for the regeneration of the dental alveolus, the rats were anesthetized again using the same protocol as in the case of extraction and positioned in the same decubitus position. On the site of the extracted molar, the gingiva was sectioned with a scalpel blade, no. 15, followed by its detachment from the bone. After exposing the bone support, a 1.5 mm deep cavity was created with the help of a 1.2 mm diameter drill into which a 1.5 mm long and 1 mm diameter titanium implant was screwed (Figure 2). The gingiva was sutured over the implant with a 6/0 non-resorbable monofilament thread (Dafilon, Romania). After another 4 weeks, necessary for osteointegration, the radiological examination was performed.

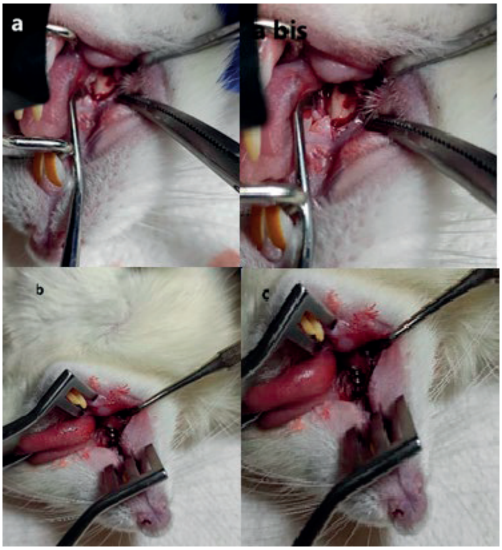


Figure 2: Experimental implantation operation in rats in (a, a bis - exposure of the bone support, b, c - mounting of the implant)

The animals were monitored daily by a veterinarian. On day 0, the animals were weighed, and blood was collected from the retroorbital sinus for hematological evaluation (complete hemoleucograms) after extraction. The monitoring of the weight of the animals was carried out every 2 weeks, and the hematological exams was repeated after the installation of the implants to evaluate the health status and also to follow the systemic immunoinflammatory index (SII). SII is frequently used in human medicine to predict several diseases, including bone inflammation, even in the absence of other specific signs. It is calculated based on the results obtained from complete blood counts by applying the formula (NEU×PLT)/LYM (NEU - neutrophil counts, PLT - platelet counts and LYM - lymphocyte counts). The radiological examination was performed to verify the regeneration of the bone support after extraction but also to evaluate the integration of the implants.

## RESULTS AND DISCUSSIONS

### Statistical analysis

Analyzes were performed using Prism 9 for Windows software (GraphPad LLC, USA). To compare the data, the One-way ANOVA function was used, and a value of $p < 0.05$ was considered statistically significant.

Clinically, the animals had a favorable evolution, but the post-extraction recovery, in the first 2 days, showed an alteration of the general state, represented by apathy, but as time went by and with the installation of analgesia after the institution of post-operative treatment, the rats returned to a good condition.
Body weight in the case of all animals registered a significant decrease ($p<0.05$) in the first 14 days post-extractive, following that until the day of mounting the implants this loss is recovered (Figure 3). Also, compared to day 0 and until day 74, weight increases were visible after each procedure applied to the animals, less pronounced after the installation of the implants, a sign that the animals tolerated these devices better.

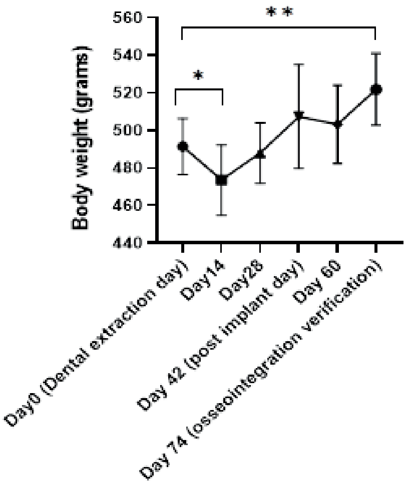


Figure 3: Evolution of body weight post-extraction-post implant

Following the SII analysis, surprising results were obtained, in the sense that it was significantly higher ($p = 0.0006$) after the installation of the implants, compared to the results obtained after extraction (Figure 4).

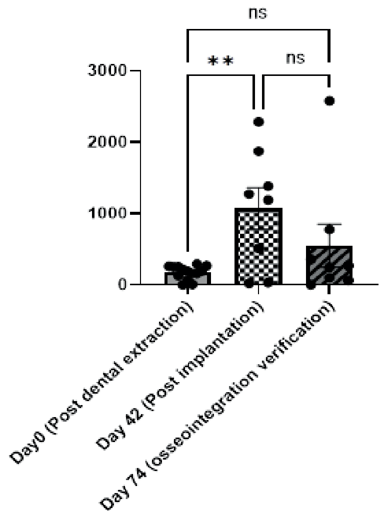


Figure 4: The difference between SII on the day of extraction, the day of implant mounting and the day of osteointegration verification

The radiological examination performed one month after the extraction showed an uniform bone support, the regenerative phenomena settling within the physiological limits (Figure 5).

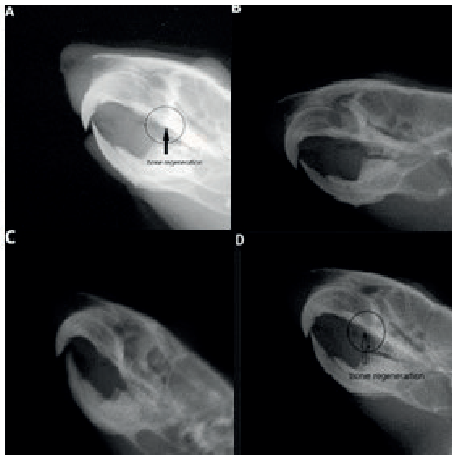


Figure 5: Bone bed appearance after extraction

At 74 days, when the osteointegration of the implants was checked, an optimal bone density could be observed around the implants (Figure 6), but out of the total of 15 mounted implants, 5 were lost, in these animals, cavity refilling was observed bones, shows other specific signs of device rejection.

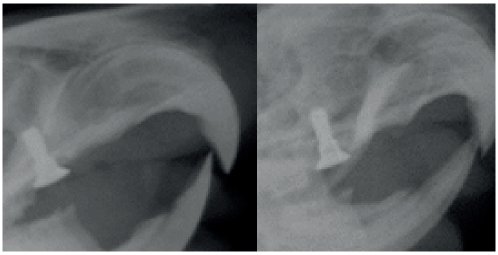

Figure 6: Appearance of dental implants (day 74)

The stability of an implant can be assessed both invasively and non-invasively.
Invasive methods include the pull/push test (Swami, 2016; Blazsek, 2009; Brunski, 2000), the disassembly test (Carvalho, 2010) or histological analysis (Bernhardt, 2012; Bissinger, 2017). These methods cannot be applied in clinical practice, therefore it is necessary to refine the non-invasive methods (Davies, 2007; Rodrigo, 2010) which refer to post implant radiological analysis (Atsumi, 2007), resonance frequency analysis (Huwiler, 2007) or clinical evaluation. For preclinical tests, the combination of both non-invasive and invasive methods could provide the best result, providing a safe basis for clinical applications.
Animal models seem to be the ideal solution to develop better devices for medical applications (Spicer, 2012; Van Griensven, 2015) because they offer the possibility of verifying osteointegration in a living organism. The medical world is still looking for the best animal model and testing method to increase the reliability of experiments (Hartung, 2010; Renaud, 2015), so that they are reproducible and reliable (Schmitz, 1986). The ISO/TS_22911:2016 guide provides indications for the preclinical evaluation of implants from a morphological, radiographic and histopathological point of view (ISO/TS_22911:2016, 2016)
Osteointegration refers to the direct contact between an implant and living bone tissue (Branemark, 1983). Moreover, the term also refers to the process of formation of this direct fixation which has a high dependence on the previous surgical procedure and preoperative circumstances (Trisi, 2009). Therefore, the implant-bone interface represents the area of major interest for researchers in the dental or orthopedic field. Through this study, we sought to create an animal model for testing dental implants that would approach the bone microstructure of the human jaw.
By extracting the maxillary left molar, we aimed to achieve the edentulous space of the human patient who needs an implant. Moreover, because this need for dental implants is more common in elderly people, the age of the rats was chosen accordingly, so that after 20 weeks, they are considered old. Aging influences numerous cellular processes, including immune responses, which may impact the outcome of bone injury healing, whether accidental or induced (Clark, 2017). Research's predominant use of young, healthy animals in preclinical models does not typically reflect the advanced age and potential comorbidities, such as impaired vascular function and reduced angiogenic responses, present in human patients (Stegen, 2015).
The systemic immuno-inflammatory index (SII) is a novel inflammation marker that is highly predictive of tumor prognosis and immune response status (Shui, 2021, Ji, 2020). Clear associations between IBS and inflammatory conditions have been observed. (Hamad, 2021), being also correlated with the loss of bone density (Du, 2021), in the case of our study, a much higher SII was observed in the condition of the loss of bone tissue following the creation of the implantation cavities but also of the secondary inflammatory reaction. The human equivalent for the bone healing process, in the case of rats, is 4-8 weeks (Hatt, 2022). Unlike histological and immunohistochemical analyses, which require animal euthanasia, radiographic imaging can be used to longitudinally assess bone healing in the same animal over time, which is an attractive means of reduction. the use of animals. New bone regeneration quantified from radiographic imaging is mostly expressed as bone volume/total volume (BV/TV), bone mineral density, new bone formation, or units. In this study, the X-ray analysis for evaluating bone

support regeneration post extraction or for evaluating the integration of implants was the ideal choice that allowed keeping the animals alive, thus making possible their transition to new stages of study. However, histology remains the main method of analysis and is used in all the studies presented. Histology is a powerful tool to assess native tissue infiltration within the construct, making it one of the most important outcome assessments. This is closely followed by CT/iCT, immunohistochemistry and radiography (Tcacencu, 2018).

## CONCLUSIONS

Rats have proven to be suitable animal models for the study of dental implants. The implantation technique required additional attention, the working field being a limited one, the size of the implants being an adapted one. The body's response to the infamous post-implantation processes was an obvious one, but it was remitted through usual therapeutic protocols. The radiographic analysis completed the clinical picture so that through the technique approached on the chosen model, physio pathological conditions related to the implant, devices and innovative therapies can be tested.

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