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TESTING POSSIBILITIES OF MATERIALS USED IN PERIODONTAL THERAPIES ON LABORATORY RATS

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https://doi.org/10.25241/stomaeduj.2020.8(2).art.2

ABSTRACT

Introduction Periodontitis is an oral inflammatory disease of significant importance, that leads to gingival inflammation, alveolar bone loss and has a high impact on the quality of life and general health. There has been a continuous interest in the scientific world to test new therapies and therapeutic materials for periodontal disease. One of the most critical tools to investigate mechanisms of periodontal pathogenesis and test new therapeutic materials are animal models. In addition, there is a wide range of materials used in periodontal therapy, especially in terms of bone augmentation, so choosing the ideal material is often difficult to achieve.

Methodology In this article, we have evaluated two methods for testing artificially induced periodontal defects - intra-orally and extra-orally - the biological adaptation of the materials used in periodontal and bone regeneration techniques on animal models.

Results We have created two protocols for the extra-oral and intra-oral approaches. By following them we have succesfully managed to create the periodontal defects and to apply the therapeutic materials. We have also made a comparison between the two methods, and the possibilities of materials that can be used.

Conclusion Tests performed on animal models will remain an important asset for evaluating new approaches for the improvement of tissue regeneration therapies. As there are continuous advances in the study of dental materials, we also have to search for new, easy to perform, ethics-friendly methods to evaluate the biological response of these materials.

KEYWORDS

Periodontal Disease; Animal Models; Alveolar Bone Loss; Bone Augmentation.

1. INTRODUCTION

Periodontal disease is known to have a a long history, constantly accompanying the evolution of the human species. New data regarding its prevalence confirm its high value up to 50% around the world, with the highest scores in the older population [1]. It represents one of the major causes of tooth loss which can compromise mastication, esthetics, self-confidence, and quality of life [2].

Microbial dental plaque has been accepted as the primary etiological factor in the occurrence of inflammatory disease. Therefore, the major goal of periodontal therapy was to eliminate the pathological organisms discovered in the dental plaque located on the surface of the tooth [3,4]. Periodontal therapy is complex and it includes: prevention strategies to control the inflammation level and regenerative therapies of all supporting structures and tissues [5]. Root planing leads to clinical improvement by disrupting the subgingival biofilm, which reduces the amount of bacteria, resulting in a delay in the repopulation of pathogenic microorganisms [6,7]

Peer-Reviewed Article
Citation: Mitruț I, Manolea HO, Sălan AI, Neagu I, Stănuși A, Drăghici A. Testing possibilities of materials used in periodontal therapies on laboratory rats. *Stoma Edu J.* 2021;8(2):100-105
Received: April 06, 2020; **Revised:** April 27, 2021; **Accepted:** May 13, 2021; **Published:** May 17, 2021;
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Today many biomaterials are available to use for periodontal regeneration. The vast majority of materials used in periodontal therapies are materials (such as chlorhedine, tetracycline, metronidazole) used for therapeutic purposes in non-surgical procedures. Lately there has been an increasingly frequent use of materials used in reconstructive surgical therapies. Synthetic bone grafts materials are available in particles with different diameters and can offer the benefits of an unlimited amount, without the risk of transmitting the disease and without the need to create an additional surgical area. Polymers are widely used as a barrier material in guided tissue regeneration applications [8]. The interest of the researchers and the support from the companies allow for an enormous amount of clinical and pre-clinical research to be carried out every day. Generally, the repopulation of cells on the root surface after periodontal surgery determines the nature of the attachment that will form. After surgery and removal of biofilm, the root surface of bone defects can be repopulated by epithelial cells, gingival connective tissue cells, bone cells, or periodontal ligament cells [9].

2. AIM

This study is part of a complex in vivo project that aims to test new materials for gingival inflammation and alveolar bone loss on induced periodontal defects. The aim of the current study is to identify and to compare different approaches in the induction of periodontal disease and bone defects.

We have studied, developed and compared two different protocols for inducing periodontal defects on animal models. The two methods consist in two different approaches to the periodontium and alveolar bone – through an intra-oral and extra-oral approach, which will allow the further studies of different materials used for periodontal disease.

This paper aimed to evaluate the different methods of addressing artificially induced periodontal defects through a study performed on laboratory animals.

The study is part of a multidisciplinary project, as it integrates the clinical surgical field, and biomaterials science as well. By developing these protocols, we hope to bring our contribution to the field of dental materials and oral tissues in vivo studies.

3. MATERIALS AND METHODS

The working methodology consisted in a series of steps starting from the creation of periodontal defects in the mandible of laboratory animals, clinical evaluation of the evolution of inflammation in the periodontium, application of materials studied in defects.

3.1. Study Population

We have performed the tests on male Wistar rats. The animals were then kept in separate cages and marked in batches. The cages were well ventilated, with an alternation of 12 hours of light / dark, being maintained at a temperature of about 25^{0}C. The animals were fed with standard fodder combined with granulate, and ad libitum water. Each animal was registered and the data of the experiment were kept in a single register.

3.2. Working methodology

After studying the specialized literature, we selected two types of protocols that we adapted to be used in our project [10,11]. Thus, the testing methods of the materials used in this study were performed through an intraoral and extraoral approach.

The whole operation took place in the animal research facility of the university. The surgical area was equipped correspondingly and located in the same living unit as the animals, so that the stress could be limited as well as the potential danger to the animals health. The surgery room was well disinfected before the operation with disinfectants such as sodium hypochlorite, chlorine dioxide or glutaraldehyde solutions and the animals and instruments were prepared so as to prevent contamination and ensure the success of surgery.

These interventions were performed under general anesthesia with ketamine and narcoxide using Ketamidor 100mg / ml 20 IU (0.2 ml) and Xilazyn Bio 2% 0.3 ml. The injection was made slightly to the right of the white abdominal line.

All experiments were conducted in accordance with local guidelines on the welfare of experimental animals and with the approval of the Ethics Committee of the Research Facility (No. 80/16.04.2019 and No. 101/23.09.2019).

4. RESULTS

We have created both procedures for both intra-oral and extra-oral approaches. Both procedures have managed to induce periodontal defects, and they have proved to be reproduced successfully.

The procedure for the intraoral approach was performed on the lower incisors of the animal models.

As shown in Fig. 1, the depth of the gingival groove measured with the help of a periodontal probe at the level of each tooth in six points MV, V, DV, ML, L, DL.

The procedure for the intraoral approach was performed on the lower incisors of the animal models. As shown in Fig. 1, the depth of the gingival groove measured with the help of a periodontal probe at the level of each tooth in six points MV, V, DV, ML, L, DL.

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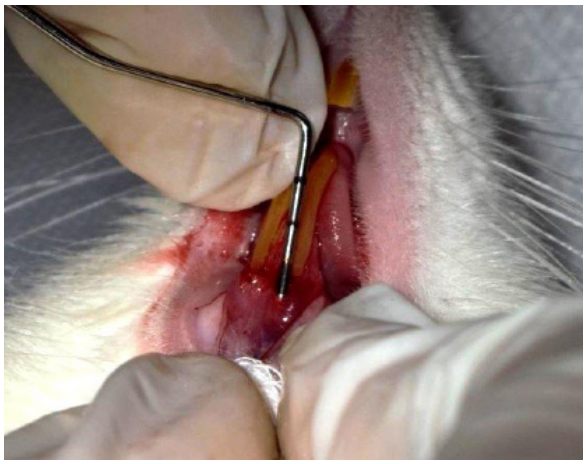

Figure 1. Measuring the depth of the gingival groove using a periodontal probe.

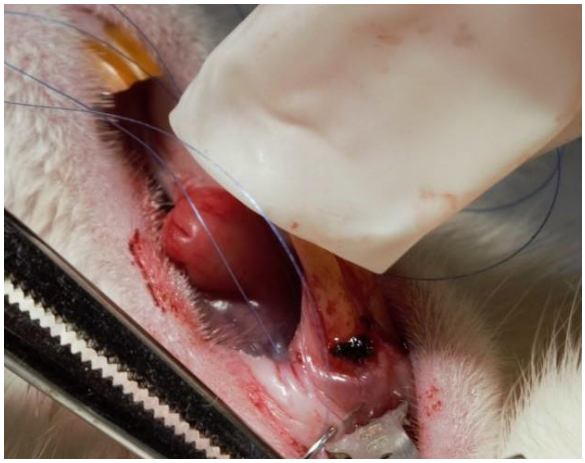

Figure 2. Holding the retraction thread in place by a ligature with a 5/0 sterile non-absorbable silk thread.

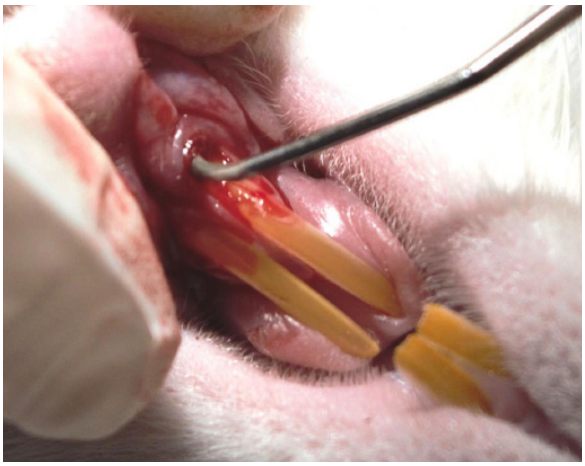

Figure 3. Mechanical scaling and root planning with a Gracey periodontal curette.

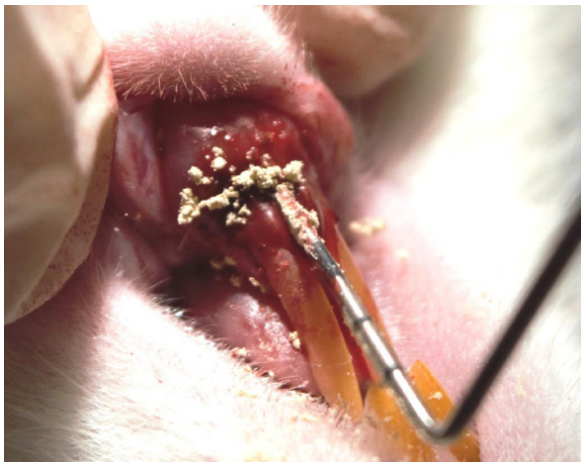

Figure 4. Application of the regeneration material in the periodontal pocket.

After that, we have inserted a piece of a silk thread used for gingival retraction, size 00, into the gingival sulcus, and performed sutures with a 5/0 suture of sterile non-absorbable silk to hold in place the gingival chord. (Fig. 2)

After 7 days, the rats in the study groups were again anesthetized and the sutures and gingival retraction threads were reoved. The periodontal defect obtained in each animal was quantified by noting the depth of the periodontal pocket measured using a periodontal probe at the level of each tooth in six points (MV, V, DV, DL, L, ML), but also the presence and location of inflammation areas. After performing the mechanical scaling and root planning, the materials studied were applied to the corresponding study group by inserting them directly into the periodontal defect from the depth of the periodontal pocket until it was completely filled (Figs. 3,4).

The intraoral approach in order to test the materials used in periodontal therapies had the advantage of an easier application for both surgical and non-surgical materials, but is limited to the incisor group, whose permanent eruption may affect the results obtained.

The method used in this study to create a periodontal space in the vestibular area of the lower incisors and placement of a gingival retraction thread, followed by the suture allowed us to obtain the periodontal defect after one week by maintaining the mechanical irritating factor at this level.

For the extra-oral approach we chose as the initial site the mandibular side and the bone defect to be made on the mandibular molar level. After preparing the animal for surgery, the correct identification of the epithelium and hard tissue should guide the operator for the initial incision. A superficial incision is made for the first time to expose the masseter muscle (Figs. 13, 14) and to have access to the ligament marks that extend in a posterior-anterior direction, approaching the basilar edge of the mandible. An incision can be made below the ligament line at the masseter muscle to expose the mandible.

A distinct ligament usually covers the lateral area at the level of the first molar; it should be dissected to ensure efficient flap take-off and proper surgical access.

After exposing the bone we identified the first molar and the opaquer area of bone in the form of a "tear" that is a characteristic of the vestibular cortex.

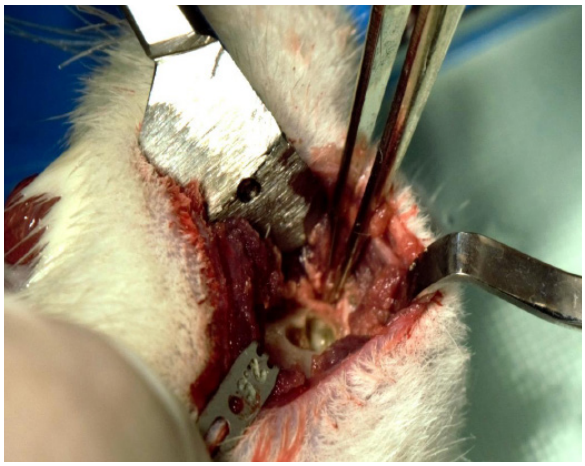

Figure 5. Appearance of the defect created in the mandibular bone.

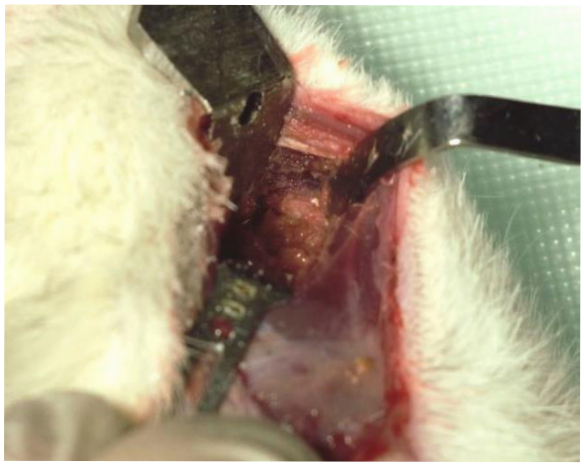

Figure 6. Appearance of the material shortly after its application in the defect created in the mandibular bone.

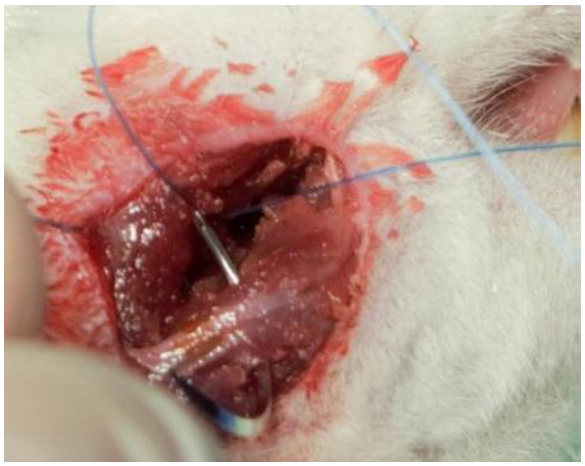

Figure 7. Suture with resorbable threads of the masseter muscle.

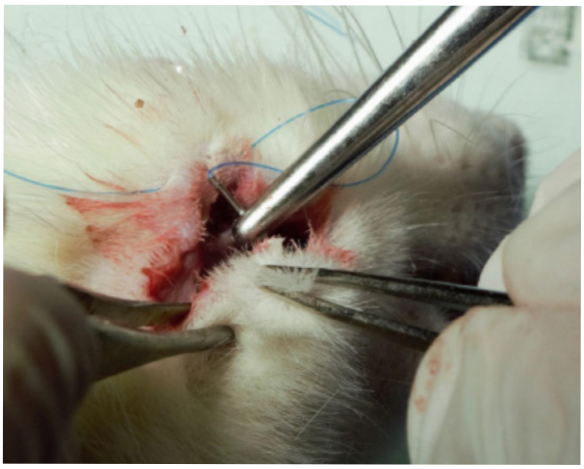

Figure 8. Suture of the skin with non-absorbable threads.

The area of creation of the defect was located at the distal root of the first mandibular molar. Using a surgical 4mm spherical drill, we created a bone defect measuring 8 mm length 4mm wide and 2mm depth. (Fig. 5)
After the test materials were applied to the created defect (Figs. 6,7), the muscle tissue was first repositioned using absorbable sutures.
Once the proper muscle closure and the closure were ensured, the skin was repositioned with non-absorbable sutures. (Fig. 8)

5. DISCUSSION

This study aimed to evaluate the testing methods of materials that can be used in periodontal therapies.
The laboratory rats proved to have similar anatomy of the periodontal tissues with those of humans [12].
Laboratory animals have a significant advantage because they can copy the cellular complexities that occur in humans in vivo and are often more accurate than in vitro studies that takeplace on plastic surfaces with a limited number of cell types present [13].
Animal models are highly susceptible to periodontal disease, as the gingival tissues go through different stages once the plaque occurs.
The gingival tissues become swollen, with pocket formation, accumulation of debris, and ulceration at about 3 months of age. Alveolar bone resorption underneath the gingiva causes the teeth to slide apart and eventually to exfoliate [12].
Alveolar bone is constantly renewed by mode-lling and remodelling mechanisms in response to functional demands, local and systemic factors. Nutritional deficiencies in animals have been shown to affect the periodontal tissues.
One study determined that dietary boron deprivation alters periodontal alveolar bone modelling and remodelling by inhibiting bone formation [14].
Animal models have been frequently used in wound healing studies primarily because of cost considerations.
Wound contraction is considered to be the primary healing method of rats as opposed to re-epithelialisation seen in humans.
Since wound contraction is rapid the overall healing time of rats is substantially reduced, unlike re-epithelialisation which involves the creation of new skin tissue.
The reduced healing time in rodent burn models allows researchers to quickly study the mechanics of wound healing healing [15].

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Studies have proposed many protocols for inducing gingival inflammation leading to periodontal destruction, such as the placement of a retentive silk or ligature in the gingival sulcus of the molars or incisors [10,12,16] or by the injection of lipopolysaccharides or various periodontal pathogens such as Prophyromonas gingivalis, Aggregatibacter actinomycetemcomitans, [12,17, 18].

Both the method of inserting an interdental floss and the repeated intra-gingival injection of bacteria are suitable to analyse the pathogenesis of periodontal disease and therapeutic strategies to modulate the progression of the disease. However, studies of reconstructive therapies require surgically created periodontal defects [11].

The testing methods of the materials used in this study were performed through an intraoral and extra-oral approach. The intraoral approach was achieved by initially creating the periodontal defect by applying a retraction thread maintained in the gingival groove maintained by means of sutures, followed by the mechanical cleaning procedure and the insertion of the tissue regeneration material. The extra-oral surgical approach was performed by creating cavities in the mandible of laboratory animals, followed by the application of tissue regeneration material.

The intraoral approach has the advantage of an easier application for both materials in both surgical and non-surgical procedures, but is limited to the incisor group. The method used in this study to create a periodontal defect on the lower incisors by placing a gingival retraction thread, helped us obtain the periodontal defect after one week. We found that the insertion in the gingival sulcus of a small retraction chord combined with the placement of a ligature acted as a continuous source of irritation to the tissue and led to a more aggressive result.

Complex surgical procedures limit its use, excluding testing of materials used in non-surgical therapies.

The extra-oral approach in order to test the materials used in periodontal surgery has the advantage of easier access to the distal area of the arch, but but complex surgical procedures limit its use, excluding testing of materials used in non-surgical therapies.

5. CONCLUSIONS

Tests performed on laboratory animals will remain one important way to evaluate new approaches that improve modalities tissue regeneration currents. For the materials used in periodontal therapies to be optimally effective, doctors and researchers should have sufficient knowledge of both the ways of applying the materials, as well as their properties and also advantages and disadvantages.

In our study, the intraoral approach allowed us to easily obtain periodontal defects, but which can be maintained only for a short period of time. The extra-oral approach allows access to the lateral area to achieve periodontal defects for a longer time, but is more surgically aggressive.

ACKNOWLEDGMENTS

None.

AUTHOR CONTRIBUTIONS

IM: contributed to creating the concept and structure of the study, in establishing the experimental conditions for the intra-oral protocol, and performed the experimental surgery on laboratory animals. HM: coordinated the experimental surgeries, interpretation of the data, formulated the research conclusions and supervised the drafting of the article. AIS: participated in the drafting of the extra-oral protocol and performed the surgery on laboratory animals with the extra-oral approach. IN: participated in the writing and translation of the paper, contributed to structuring the bibliographical references, and took the photos during the surgeries. AS: participated in researching the bibliographical references, contributed to the introductory part and in the drafting of the abstract. AD: participated in the surgical experiments, writing, editing and translation of the paper.
All authors read and approved the final manuscript

FUNDING

The authors declare that they have not received any funding.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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CV

Dr. Mitruț Ioana has been a PhD student at the Department of Prosthesis Technology and Dental Materials of the University of Medicine and Pharmacy of Craiova since 2018. She is also a Prosthodontics resident at the same university. Her PhD thesis is entitled "Possibilities of using calcium fructoborate preparations in dentistry". Her research interest are testing dental materials and other biomaterials on biological tissues, the applications of boron based compounds in oral health. She has a lot of experience in performing in vivo tests on laboratory animals.

Questions

1. The general goals of periodontal therapy are?

❑a. Regenerative therapies;
❑b. Multiple teeth extraction;
❑c. Prosthetic therapies;
❑d. Aesthetic therapies.

2. Laboatory rats can be used in periodontology research because:

❑a. We can use as many animals as possible for tests;
❑b. They have similar anatomy of the periodontal tissues with those of humans;
❑c. They cannot copy the cellular complexities that occur in humans;
❑d. Even if they are efficient they are less accurate than in vitro tests.

3. The maintenance of the animals consist of:

❑a. Housing them in cages with continuous darkness;
❑b. The surgery facility to be in another building to not disturb the animals in the cages;
❑c. The cages should have an alternation of 12 hours of light / dark;
❑d. The animals should be fed only once a day.

4. The possibilities for creating periodontal defects are?

❑a. Injections of antibiotics;
❑b. Placement of a retentive silk or ligature in the gingival sulcus;
❑c. Extraction of healthy teeth;
❑d. Food deprivation.