SUMMARY

OSTEOINTEGRATION OF A DENTAL IMPLANT IN AN EXPERIMENTAL ANIMAL MODEL OF PERIODONTITIS AND EVALUATION OF THE EFFICIENCY OF ANTI-REJECTION MEDICATION

Ph.D-student: DASCĂLU (ANCUȚA) Diana-Larisa

Scientific coordinator: *Professor, PhD*. CRIVINEANU Maria

KEYWORDS: ***periodontal disease, peri-implantitis, microbiome, bacterial lysate, animal model***

The doctoral thesis entitled „***Osseointegration of a dental implant in an experimental animal model of periodontitis and the evaluation of the efficiency of anti-rejection medication****"*, had as its primary objective the evaluation of the effectiveness of bacterial lysates on periodontal disease and periimplantitis on an experimental rat model. Since both conditions impact the population globally, we set out to reproduce the diseases, in an animal model, using bacterial strains isolated from the oral cavity of human patients, with the aim of contributing to the understanding of the physiopathogenetic mechanisms and contributing to the optimization current therapeutic schemes of periodontitis and peri-implantitis.

The experimental studies in this paper have successively described the way to induce periodontal disease, the development of peri-implant disease, followed by the exposure of the technique for obtaining bacterial lysates and the *in vitro* evaluation of their efficiency. In the last part of the research, we aimed to follow the results obtained following the administration of bacterial lysates on periodontitis and peri-implantitis.

The objectives of the doctoral thesis were multiple and included: induction of periodontitis in an experimental rat model using the method of ligation of maxillary incisor teeth with cotton thread, followed by contamination with bacteria isolated from the human oral microbiome. The second objective addressed the induction of peri-implantitis in the experimental rat model by inserting titanium implants at the site of the maxillary first molar, followed by oral contamination with bacteria selected from the oral flora of patients suffering from periodontal disease and/or peri-implantitis. A third aim of our research was to create an immunostimulatory treatment based on bacterial lysates obtained by ultrasonication of the bacterial strains responsible for periodontitis and peri-implantitis. Last but not least, we verified the influence of bacterial lysates on the inhibition of the activity of pathogenic microorganisms involved in the development of the two oral diseases.

The thesis complies with the current provisions regarding structuring and comprises two parts. In the first part, important data resulting from the bibliographic study are exposed, and the second part is personal research.

Part I presents the latest data on periodontal disease and peri-implantitis in terms of etiology, diagnostic and treatment methods applicable to human and experimental research in animal models. The importance of this part lies in understanding the mechanisms by which diseases are established, in knowing the risk factors, but above all, in understanding the contribution of animals to the progress of science. The bibliographic study is systematized in four chapters, with a number of 52 pages that represent approximately one third of the total information presented.

Chapter I generally named "*Periodontal disease*" provides current data on the etiopathogenesis of periodontal disease, the associated risk factors as well as its influence on systemic health. The immunopathogenic mechanisms triggered by periodontal disease are reported in this chapter, as are animal models used in research on pathogenesis, diagnosis, and tested therapeutics. We have also specified the main methods by which periodontitis can be induced in the animal model, in order to provide a perspective on the selection of the right technique, depending on the intended purpose. Of great use, both for own research and for future studies in the field, were the current methods provided by the specialized literature, which establish the diagnosis of periodontal disease and its therapy, in humans and animals.

Chapter II, like the previous one, is generically called "*Peri-implantitis*" and briefly describes notions regarding the term peri-implantitis, the etiological and epidemiological factors that lead to this condition. The tissues surrounding implants are key elements for the success of interventions of this type and a major challenge in contemporary dentistry. By exposing the immunopathogenesis, the inflammatory mediators as well as the

individual genetic susceptibility for the development of peri-implant disease, the condition of a patient and the result of applied therapies can be evaluated. This chapter focuses on factors affecting implant loss, and comprehensively discusses diagnostic methods applicable to both humans and animals used in implant research. In the latter, the main methods of inducing and evaluating peri-implantitis are addressed because in this field too, animals provide solid support in obtaining new knowledge. In conclusion, current therapeutic protocols including non-surgical and surgical, human and animal techniques are detailed as well as the latest evidence for ensuring oral health-related quality of life in dental implant patients.

Chapter III is called "*Elements in common and differentiating between periodontal disease and peri-implantitis*" and has the role of exposing exactly the information related to the involvement of the oral microbiota and its composition in the development of the two diseases, what exactly we must take into account when a patient it faces the overlap of conditions and especially, what are the similarities and differences between clinical and experimental studies. In the last subchapter, important elements for researchers in the field of implantology that can be taken into account when testing the biocompatibility of an implant or evaluating a new therapeutic protocol using animal models are summarized.

Chapter IV addresses a relatively new topic for dentistry today, namely "*Bacterial lysate as a potential treatment for periodontal disease and peri-implantitis*". The molecular and immunological mechanisms by which bacterial lysates act as well as their use and effect in the therapy of periodontal disease are presented here. Through the multitude of similarities between periodontal disease and peri-implantitis, we wanted to extract all the information related to the therapeutic potential of bacterial lysates and on peri-implantitis, in the literature that describes the immunostimulatory role of lysates on oral diseases, there being no sufficient data to touch the subject of peri-implant disease.

In part II, the research is presented, and structured according to the proposed purpose and objectives. Thus, in the four chapters we described four experimental studies, three of which were carried out on the rat animal model, and one *in vitro*. Each of the sub-chapters respects the order of exposition of the experimental procedure, the conclusions generated, and the recommendations are based on the obtained results. This part represents over 70% of the total volume of the thesis, being reported in 94 pages in which 10 tables and 77 figures are included. All research are made up of sub-chapters touching on the introductory notions, materials and methods, results and related discussions as well as partial conclusions as follows:

Chapter V, "*Induction of periodontitis in an experimental rat model using bacteria isolated from the human oral microbiome*", aimed to evaluate the induction of periodontal disease in a rat model using the bacterial species implicated in the pathology of human periodontitis.

We included in the study 20 male Wistar rats, aged 20 weeks, in which we applied ligatures with gingival retraction wire on the maxillary incisors. 5 days/week, 6 weeks, the ligature and the gingival sac were contaminated, by gavage, with fresh strains of *Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum* and *Streptococcus oralis* at a concentration of 10^9 CFU/ml. During the clinical monitoring period, we followed the expression of specific clinical signs of periodontitis, the evolution of body weight and took samples from the oral cavity for the microbiological identification of the tested bacteria and blood samples for the hematological, biochemical, and immunological examination. We performed radiological analysis of the animals to track bone density and at the end, the rats were euthanized. The sampled periodontal incisors were histopathological analyzed to confirm disease induction.

The characteristic symptomatology of periodontal disease was expressed from the first week of the study and was maintained until the end, with the bacteria being able to be identified at the end of the study. The increase in the number of neutrophils was significant and was associated with local periodontal lesions. These results attest to the fact that the inflammatory process was caused by bacterial invasion and that triggered neutrophil migration. Also, the systemic immunoinflammatory index showed a pronounced inflammatory reaction maintained until the final day of the experiment, as did the activity of proinflammatory cytokines. The biochemical examination helped to complete the diagnosis of periodontitis through increased results of parameters such as ALT and ALKP, which signify the initiation of the bone resorption process, the final stage of the disease. Following the radiological analysis, we obtained the appearance of the bone support, expressed in several degrees of density so that, in each of the examined images, different stages of bone resorption could be observed. Confirmation of periodontal disease induction occurred following the histopathological examination of the incisor and periodontal tissue samples collected at the end of the experiment. Findings such as inflammatory infiltrate, abscesses, necrosis of the alveolar-dental ligament, bone lysis, bacterial infiltrate, or pyogranulomatous inflammation completed the investigative panel for accurately establishing the disease.

Corroborating the data, we can conclude that we induced periodontal disease using bacterial strains isolated from the human oral cavity, at a concentration of 10^9UFC/mL in rats to which we applied ligature wire on the maxillary incisors.

Chapter VI is entitled "*Induction of peri-implantitis in the experimental rat model by inserting titanium implants into the maxillary first molar*" and the aim of this research was to develop peri-implantitis in the rat model

by oral contamination with the same bacteria used for induction of periodontal disease. The study was carried out in three stages: the extraction of the maxillary first molar to reproduce the human dentition, the mounting of the implant and finally, the contamination of the device by gavage with *Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum* and *Streptococcus oralis*, concentration 10^9 CFU/mL, 5 days/week, 6 weeks.

For this experiment we used 20 male Wistar rats weighing 350-400 grams at the start of the study. Dental extraction required approaches and instrumentation similar to human dental technique, and the period required for the regeneration of the dental alveolus was 30 days. This was followed by the implantation of the device on the edentulous site using specific equipment and we also allowed 30 days for integration, a stage preceded by the exposure of the implant to the bacterial action and the follow-up of the clinical evolution. The radiological examination performed after the extraction, after the placement of the implant and after the completion of the period of oral contamination, allowed us to evaluate the bone density, position, or loss of the implant. The hematological examinations carried out during the research showed statistically significant increases for WBC, Hb, RBC, MCH, MCHC and PLT, but especially for the level of neutrophils and lymphocytes, and the systemic immunoinflammatory index completed the picture related to the inflammatory response triggered as a result of the activity of microorganisms' pathogens on oral tissues.

By examining the liver and kidney profile, we hypothesized that peri-implantitis is associated with systemic diseases, and the histopathological examination showed peri-implantitis lesions characterized by a marked inflammatory infiltrate with numerous neutrophils and lymphocytes. The response of the body following the process of bone resorption emerged from the images of bone reorganization complete with abundant fibrous tissue and pronounced inflammatory reaction around the bone sequestrations. Also, the reactivity of the gingival tissue was observed, which was heavily infiltrated with white line cells that defined pyogranulomatous phenomena, the general appearance of the peri-implant border being irregular and hyperemic.

By corroborating all the results, we were able to successfully develop a rat peri-implantitis model using a mixed bacterial infection through the oral gavage technique, a claim supported by the proof of the recovery of the microorganisms used, the end of the study.

Chapter VII is called "*The development of an immunostimulatory treatment based on bacterial lysate by ultrasonication of bacterial strains used to induce periodontitis and peri-implantitis*". Described here are the materials and methods used to obtain bacterial lysates from strains of *Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum*, and *Streptococcus oralis* that induced periodontal and periimplant disease in the rat model. From 24-hour cultures, we obtained the final product, after inactivation and mechanical breaking of the bacterial wall. We checked the effect of the bacterial lysate through an *in vitro* test that involved putting it in contact with live bacteria, in different dilutions. We mimicked the treatment by adding a dose of lysate daily and watched for inhibition of bacterial activity. At a second dose, at least double, the results showed that the lysate neutralizes the microbial agent in the liquid medium. On the solid culture media, we were able to detect the inhibitory effect after the second seeding, that is, when the double dose of the lysate compared to the live bacteria was found in the inoculum to be cultivated.

Through these findings we were encouraged towards *in vivo* testing of bacterial lysates, and in **chapter VIII**, which we entitled "*Evaluation of the effect of antibiotic therapy and treatment with bacterial lysate on periodontitis and peri-implantitis*" we compared the current therapeutic standard with the effectiveness of the product based on of bacterial lysate. He selected for the study 30 adult, male, Wistar rats that we divided according to the induced disease and the applied treatment, resulting in a number of 6 groups: periodontal disease control group and peri-implantitis control group, treated with a solution saline, the groups with periodontitis and peri-implantitis that received antibiotic and anti-inflammatory treatment, and the last category addressed the animals in which we developed periodontal disease, respectively peri-implantitis, where we administered the bacterial lysate. This complex study was carried out over a period of 113 days, during which we analyzed the animals from a clinical, hematological, biochemical, immunological, radiological, microbiological and finally histopathological point of view.

The results obtained showed the reduction of the signs of inflammation of the tissues around the teeth in the case of groups of animals with periodontal disease treated with antibiotics and with lysate. Related to the evolution of peri-implant disease, it was found that the survival rate of implants was higher in the group that received bacterial lysate. The hematological examination revealed a decrease in white blood cells, especially in the animals treated with antibiotics and anti-inflammatory, and the peri-implantitis subjected to the bacterial lysate maintained a high systemic immune-inflammatory index until the end of the study. Biochemically, after the establishment of the therapeutic schemes, we did not observe a reflected influence on the functions of the vital organs. The immunological examination paid more attention to the biological function of IL-6 and analyzed its relation to tissue destruction at the periodontal or peri-implant site compared to IL-1 and TNF-α. IL-6, following the application of the treatments, played a protective role, its level remaining elevated in the treated periodontitis and peri-implantitis groups, demonstrating the similar effectiveness of the two treatments. From the histopathological point of view, the detected lesions confirmed the attenuation of the inflammatory process in the

periodontal tissues and in the peri-implant sac, similarly for periodontal and peri-implant disease, regardless of the applied treatment. Only in the case of peri-implantitis treated with antibiotics, the inflammatory infiltrate remained elevated in the analyzed samples.

By corroborating the final results, we could conclude that the treatment with bacterial lysate had a similar effect to the standard treatment of periodontal disease transposed to peri-implantitis. This treatment option may be valid in the case of periodontitis or peri-implantitis developed as a result of the action of pathogenic bacteria, and a personalized therapy based on bacterial lysate could reduce the unnecessary consumption of antibiotics.

The novelty of the doctoral thesis consists in the possibility of treating both periodontitis and peri-implantitis with bacterial lysates. If the specialized literature mentions some results obtained following the administration of established commercial products based on bacterial lysis, I have never come across a protocol described that uses the bacteria responsible for triggering periodontal and peri-implant diseases, to create a treatment to remedy the conditions. The studies published so far only touch on periodontal disease, and through our studies, we were able to demonstrate the effectiveness of the bacterial lysate including on peri-implantitis. Another novelty element of the work was the systemic immunoinflammatory index applied to both periodontitis and peri-implantitis. Currently, no study has included this parameter in the analysis of the two diseases, although it can be easily calculated based on the data provided by the hematological examination, being a good predictor of oral tissue inflammation. Finally, we pay tribute to the animals whose involvement in research is of great importance for scientific evolution. Our studies, using a rat model, demonstrated the possibility of developing periodontal disease and peri-implantitis by contaminating the oral cavity with a bacterial mix selected from human patients, no other study reporting the induction of conditions with strains of *Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum* and *Streptococcus oralis* inoculated simultaneously.

In **chapter IX**, I included the general conclusions related to the four experimental studies as well as the recommendations aimed at improving the therapeutic protocols of periodontal disease and peri-implantation.

The bibliographic study included 412 references, and in their vast majority they are represented by publications of the last 10 years.

The development of new therapeutic approaches for periodontal diseases and peri-implantitis is today a major concern for the medical world, annually, periodontological and implantological researchers carry out numerous studies that contribute to the updating of current information. The therapeutic success of periodontitis cannot be fully transferred to peri-implantitis and therefore, new protocols are needed to act effectively on both conditions, especially since the incidence and coexistence of the diseases is on an upward trend among human patients.

The studies presented in this doctoral thesis are intended to provide the vast knowledge that characterizes periodontal disease and peri-implantitis. At the same time, the research results contribute to the development of new treatments for the prevention and cure of the two diseases.