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PHD THESIS

MODERN APPROACHES TO TISSUE REMODELING IN DENTAL MEDICINE

A B S T R A C T

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A B S T R A C T

CONTENT

With a strong emphasis on technology, in this digital age of dentistry, I was motivated to find new approaches to the long-term aesthetic outcome of a prosthetic restoration and to deepen my knowledge in finding the best way to design a personalized treatment plan. My professional path, first as a resident in the specialty of oral surgery and later, as a specialist, then as an assistant professor at the Discipline of Prosthodontics, within the Faculty of Dental Medicine of the "Victor Babeș" University of Medicine and Pharmacy in Timişoara, Romania, allowed me to observe and collaborate with other colleagues and students but also to work with patients of different ages and pathologies.

Guided tissue regeneration (GTR) is commonly used in complex oral rehabilitation, in combination with implant insertion and/or bone defect remodeling resulting from periodontal disease. The application of a barrier membrane to exclude contamination by non-osteogenic tissues in the bone regeneration area is a key principle of GTR. Membrane materials possess a number of properties that can be modified. A large number of membranes were introduced for experimental and clinical verification. This determines the need for an update of membrane properties and biological outcomes, as well as a critical evaluation of biological mechanisms governing bone regeneration in membrane-covered defects. Experimental data suggest that various changes in the physicochemical and mechanical properties of membranes can promote bone regeneration.

Optimising membrane materials by systematically addressing both barrier and bioactive properties is an important strategy in this area of research.

Various systemic, general and local factors have a major impact on oral health. Under these distinctive circumstances, periodontal disease is both a dysbiotic condition and an inflammatory response to these triggers.

With the periodontal disease rate rising, it was exciting to explore whether there is a high prevalence among young subjects seeking treatment in the Prosthodontics Clinic, within the Faculty of Dental Medicine of the Victor Babeș University of Medicine and Pharmacy in Timişoara. Thus, the first study aimed directly at **exploring the occurrence of aggressive periodontitis (AgP), which may require guided tissue regeneration interventions**, by screening a conventional group of young patients, based on panoramic X-ray examination, its

association with oral environmental factors and characterizing the prevalence of these patients in the western region of Romania, Europe.

The anatomy of the oro-maxillofacial region is complex, and bone defects caused by trauma, tumors and inflammation in this area are extremely difficult to manage, often resorting to guided tissue regeneration techniques.

The **second research direction** described in this doctoral thesis implies the involvement of three-dimensional (3D) printing technology, which represents a new threshold in the evolution of modern dental medicine. In the area of interest of the present thesis, 3D printing is at its beginning, being still limited both in terms of technology and in terms of materials used for printing, so that it is possible to use them in tissue remodeling in dentistry, respectively at the level of the whole organism.

In the present study, based on a CBCT scan, a digital model of the patient's bone was developed. On this virtual model, a virtual membrane was designed that was printed using a 3D printer. This membrane is very well adapted to the patient's bone. The solid, absorbable membrane is made of a special type of polymer: Resorb X®, a 100% amorphous, non-crystalline, Poly(DL-lactide) (PDLLA). Attempts have been made to develop an easy-to-use, three-dimensional solid membrane capable of protecting the augmented bone from connective tissue and also maintaining space, especially when it comes to vertical augmentation.

A very important characteristic of membranes used in GTR processes is the **degree of permeability**. One of the aspects we wanted to analyze and present in this doctoral thesis is the ability of a material with potential uses in the medical field to be influenced in terms of its permeability when connected to a magnetic flux. Changing the density of this magnetic flux is intended to influence the degree of permeability of the analyzed material.

It has been tried to influence the permeability of membranes by incorporating different nanoparticles, the degree of permeablity of membranes becoming a very important aspect to study, because depending on the properties of the material from which these membranes are made, cells can be directed that will have access to the area where bone regeneration is desired. A hybrid magnetorrheological elastomer (hMRE) was made, which consists of a microfiber structure with two copper electrodes, soaked with a mixture made of a magnetorrheological suspension. The relative dielectric permittivity of hybrid magnetoreological elastomer can be altered in

magnetic field. Thus, relative dielectric permittivities are obtained, up to two orders of magnitude larger, by choosing the value of the magnetic field flux density. The obtained results can be used as a guideline in order to achieve bio-magnetically active membranes usable in guided tissue regeneration.

Furthermore, the cytotoxicity of magnetizable membranes based on cotton microfibers impregnated with honey, propolis, graphene nanoparticles, carbonyl iron powder and/or silver nanoparticles was evaluated. Biocompatibility has been determined by in vitro studies on the human HDFa fibroblast cell line, since they are components of the extracellular matrix and play a role in the synthesis of matrix components, including collagen fibers, thus stimulating conjunctival cell differentiation and tissue regeneration. According to the results obtained in this study, it can be stated that the tested products do not cause any cytotoxicity reaction to the human body, and can become a viable solution for the development of membranes used in guided tissue regeneration.

Regardless of the materials used for tissue regeneration, the bone healing process can always be influenced by the patient's overall health.

The prevalence and severity of periodontitis are further increased in people with diabetes, especially if poorly monitored. Also, the risk and degree of alveolar bone loss are correlated with lack of metabolic control. The changes that occur in bone metabolism are considered to be one of the important long-term complications associated with diabetes. Alveolar bone loss is one of the main outcomes of periodontitis, and diabetes is among the leading risk factors for periodontal disease.

Thus, in the achieved research we also aimed to **evaluate the healing method following guided tissue regeneration procedures in patients with type II diabetes mellitus.**

Regardless of the technique and materials of guided tissue regeneration used, we are currently trying to positively influence the final result through different methods. The **use of lasers in dentistry** can positively influence the treatment by achieving it without anesthetic infiltration, more conservative procedures can be done, resulting less trauma for patients and improving the healing period. Laser modeling of soft tissues has advantages to classical surgical procedures in terms of bleeding and healing. The efficiency over time of this technique lies in reducing the healing period. In the treatment of soft tissues, the laser is a very effective tool that proves its usefulness in various procedures in the dental practice.

The doctoral thesis is structured in two parts: the general part, which presents aspects of the specialized literature with high relevance to the thesis topic and the special part, of personal contributions, which ends with the final conclusions, followed by bibliographic references and annexes.

The **general part** comprises two chapters that present the up to date level of knowledge in the field and frame the research themes chosen in the experimental part of the thesis in the current context of research in the area of guided tissue regeneration.

The first chapter presents an overview regarding guided tissue regeneration and its clinical applications.

The success of targeted tissue regeneration therapy depends on the properties of the membrane used and the biological responses of the hosts. In this sense, many of the future strategies involve membrane modifications to promote appropriate responses (e.g., predictable bone regeneration, appropriate soft tissue reactions, and efficient handling, preventing microbial adhesion and colonization during GBR treatment).

In the **second chapter** are reviewed the materials from which the different types of membranes used in directed tissue regeneration are made of, insisting on their properties, advantages and disadvantages, clinical peculiarities, finally being presented the existing current perspectives.

The special part, personal research, is structured in 6 chapters, presenting the studies performed, which include the working hypothesis, the purpose and objectives pursued, the materials and method used in the research, the results obtained, discussions, conclusions and general conclusions, highlighting personal contributions, plus the bibliography.

In order to carry out the studies described in this doctoral thesis, the approval of the Scientific Research Ethics Commission of UMF "Victor Babeş" Timişoara, Opinion no.07/17.02.2016 (ANNEX 1) was obtained.

Chapter 3 presents a radiographic evaluation study of periodontal disease status, by screening and monitoring a group of patients who requested restorative treatment in the Prosthodontics Clinic of the Faculty of Dentistry, within UMF "Victor

Babes" Timisoara, aged between 18-45 years. The study was conducted to distinguish oral dysbiosis as a possible preclinical factor that can ultimately lead to local conditions which require guided tissue regeneration procedures.

It was found that patients, who requested restorative treatment in the Prosthodontics Clinic of the Faculty of Dentistry, within UMF "Victor Babes" Timişoara for oral rehabilitation, have a high prevalence of periodontal disease. The probability that they will need guided bone regeneration procedures in order to recover the affected teeth for prosthetic purposes is very high.

The purpose of the study presented in **Chapter 4 – Study on Guided Tissue Regeneration Using a 3D Printed Indivual Membrane** was to develop a solid, individualized, three-dimensional, easy-to-use membrane capable of protecting augmented bone from connective cells and also maintaining space, especially when talking about vertical augmentation. Consequently, based on a CBCT scan of the patient's jaw, using the 3D cone beam imaging program of the Soredex Cranex 3D digital device (Soredex, Tuusula, Finland), a digital model of the bone was developed and a virtual model of the future membrane was designed, which was printed using a 3D printer.

The deficient areas were augmented with a mixture of autogenous bone, bovine bone and fragmented PRF. The mixture was inserted into the individualized, three-dimensionally printed membrane, which adapts very well to the area to be augmented.

This study allowed the following conclusions to be drawn:

- The customized 3D printed membrane fulfilled the barrier role very well, effectively preventing connective tissue invagination in the tissue regeneration area.
- In the augmented area, bone begins to form from the natural bone towards the mixture of addition material.
- From a histological point of view, the quality of the neoformation bone is very good.
- The three-dimensional printed membrane offers numerous advantages, reducing the cost and the duration of surgery.

- The risk of perforating the overlay surgical flap is significantly lower.

Chapter 5 describes an **experiment to develop biomagnetically active membranes with potential usage in guided tissue regeneration procedures**, having in mind that a very important feature of GTR membranes is their degree of permeability.

The study represents a low-cost approach to manufacturing a hybrid magnetoreological elastomer (hMRE) with stable electrical properties in magnetic field. The manufacturing process is provided, describing the experimental configuration used to reveal magnetodielectric effects.

It is shown that the dielectric function of the hybrid magnetoreological elastomer is stable over time in the presence of an external magnetic field. We have also shown that its relative dielectric permittivity can be controlled in a magnetic field. Thus, relative dielectric permittivities up to two orders of magnitude higher can be achieved by choosing the value of the magnetic field flux density (e.g. increases from 0 mT to 340 mT). The achieved results can be used as a guideline in order to obtain bio-magnetically active membranes with biomedical applicability, which opens possibilities to new trends in directed tissue regeneration.

The **cytotoxicity study** of magnetizable membranes on healthy human cells, described in **Chapter 6**, was conducted on the Human dermal fibroblasts (HDFa) cell line (Gibco).

The purpose of this study was to evaluate the biocompatibility of biomagnetic membrane impregnation products active on human fibroblasts, given that they are components of the extracellular matrix and have a role in the synthesis of matrix components, including collagen fibers, thus stimulating conjunctival cell differentiation and tissue regeneration.

Basically, the alcoholic extracts of the following products were tested comparatively:

- E1 – extract from the product composed of 75% honey and 25% propolis
- E2 - extract from the product composed of 75% honey and 25% Fe_2O_3 nanoparticles

- E3 - extract from the product composed of 75% honey and 25% graphene nanoplatelets
- E4 - extract from the product composed of 75% honey and 25% silver nanoparticles
- E5 - extract from the product composed of 75% honey and 25% carbonyl iron powder

Microscopic analysis of fibroblasts incubated with the test products showed that the cells did not change their typical fusiform appearance.

It has been demonstrated that biomagnetically active membranes impregnated with apitherapeutic products, graphene nanoplates, carbonyl iron nanoparticles and silver nanoparticles do not exhibit cytotoxicity, allowing cell proliferation, thus being able to serve in the manufacturing of useful devices for biomedical applications.

The choice of membrane composition and/or magnetic induction value allows the value of electric current intensity through the electrical device to be fixed. In this way, it is possible to obtain a magnetic field to facilitate the penetration of useful substances from the membranes into the treatment area, as required in directed tissue regeneration procedures.

Taking into account that type II diabetes mellitus is an increasingly frequent pathological condition among patients, in the study presented in **chapter 7** we **evaluated bone healing and directed tissue regeneration, obtained in patients with type II diabetes**. Consequently, to increase the chances of healing without unforeseen events, we used PRF membranes in two ways: a part of the obtained membranes were shredded and mixed with the bone preparation consisting of autogenous bone and artificial bone, and the other PRF membranes were added over the artificial membrane, in order to facilitate rapid healing. PRF membranes had the role of accelerating the formation of new blood vessels, which would ensure blood supply to the bone regeneration area. The clinical results obtained were more than satisfactory, the patients' recovery being made without unwanted events.

The present study has proved that directed bone regeneration procedures can be successfully performed in patients with type II diabetes, properly monitored, as long as the case is managed responsibly – from anamnesis and objective clinical

examination, diagnosis, therapeutic indication, to compliance with surgical protocol and monitoring of postsurgical healing.

The use of PRF products, in combination with directed tissue regeneration techniques, respectively with GBR membranes, can improve the healing process, with the assimilation of bone addition materials.

In dentistry we are always in a race against time. Healing takes time and this time is sometimes out of reach for us. When referring to soft tissues, this healing process can be influenced by the devices used in their modeling. Laser devices have proven to be a more effective method of soft tissue reorganization compared to classical surgical techniques. In this regard, in **chapter 8** we presented the possibility of **using lasers in soft tissue reorganization, after directed bone regeneration interventions.**

The possibility of using diode lasers in surgical procedures on oral soft tissues is due to the absorption of the laser beam by tissue pigment and hemoglobin, while it is poorly absorbed by hydroxyapatite and water. The study has reconfirmed that laser soft tissue modeling has great advantages in terms of bleeding and healing. Even though cutting soft tissue takes more time with the laser compared to blade involving techniques, this time is regained in the healing process.

When it comes to soft tissues, the laser is a very efficient tool and proves its usefulness in daily procedures in the dental practice.

The **final conclusions**, presented in **chapter 9**, synthesize the essential problems of the topic approached in the thesis, reflecting exactly the results of the research carried out, and respond faithfully to the objectives proposed in the research.

The results of the accomplished studies allow opening future directions and research themes, offering possibilities for further research. The conclusions, the elements of originality and the perspectives drawn from the personal contributions of this doctoral thesis have direct clinical applicability, being for the benefit of the practitioner, but especially of the patient, offering the possibility of improving the quality of the treatments one receives.