UNIVERSITY OF MEDICINE AND PHARMACY
"VICTOR BABEȘ" TIMIȘOARA

FACULTY OF DENTAL MEDICINE
1ST DEPARTMENT DENTAL MEDICINE

GABOR R. ALIN-GABRIEL

PhD Thesis

CONTRIBUTION TO BONE AUGMENTATION METHODS

SUMMARY

Conducători științifici:
PROF. UNIV. DR. COSMIN SINESCU
PROF. UNIV. DR. VIRGIL-FLORIN DUMA

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KEY WORDS: SCAFFOLD, BONE AUGMENTATION, 3D PRINTING, CERAMIC, POLYMER

PHD THESIS SUMMARY

The motivation of choosing the research topic is represented by the need to develop the current state of the technologies for obtaining various bone augmentation materials, and, why not, implanting new ones together with the related material and protocol, in order to be used in the implant-prosthetics restoration field of patients with various bone defects.

The implementation of implantology in dentistry was a threshold in the evolution of dental medicine, being an area where the terms, materials and conceptions are in continuous development. Annually, thousands of dental implants are inserted in order to restore the morpho-functional integrity of the stomatognathic system and to reintegrate patients into society, under optimal conditions. Dental implants have brought significant benefits to dental medicine, thus, by using them in the treatment of patients with various ages, especially the terminal ones, fixed prosthetic restorations with implant support can be performed, the mobile and mobiliz able prostheses becoming more and more rare as a treatment choice. In order to benefit from this type of treatment, the presence of local conditions is required, namely the bone tissue that stabilizes the implant-prosthetic restoration, and this must be quantitatively and qualitatively appropriate.

The importance and topicality of the chosen theme are offered by the primordial conditions for the insertion of dental implants, namely, the presence of an optimal bone offer from a dimensional and structural point of view. Moreover, of major importance in choosing this theme, there is the desire to offer a bone augmentation technique, as an alternative to the established classical techniques.

The chosen topic is of overwhelming international interest, the research groups offering significant importance to the area of bone augmentation, especially to the new materials that can become viable solutions in the treatment of bone augmentations. Also, the digital technologies used in this branch of dental medicine are in the attention of researchers worldwide. This current implies a new starting point in the field of oral implantology, offering another vision and another approach to the cases of edentation, with large bone defects, with the diversification of the possibilities of implant-prosthetic restoration. This current also comes from the problems registered worldwide that bone defects cause prosthetic treatments.

Unfortunately, in most clinical situations, with different classes of edentation, the bone does not correspond and cannot provide primary stability to dental implants, with varying degrees of bone atrophy in the maxillary bones. Atrophy and bone defects have a wide and varied etiology. In order to be able to render the functionality and aesthetics, in such cases, bone augmentation is essential and obligatory in order to perform the implant-prosthetic treatment.

This thesis has been approached interdisciplinarly from several perspectives that include fields such as medical imaging, three-dimensional printing, mechanics, optics, statistics and chemistry.

The thesis is divided into two main parts - 1. General part - with 2 chapters, 2. Specific part - with 4 chapters, and 3. Conclusions and personal contributions. The general part of the thesis describes generalities regarding bone resorption and their classification, as well as the current techniques and materials used in the procedure of improving the bone supply. In addition, general information about three-dimensional printing technology, materials used and various medical applications are described.

For these reasons, the choice of the topic is based on specific research directions that can complement or bring clarifications to previous studies, offering a new vision on possible techniques and technologies useful in the field of bone augmentation in large defects.

Within this research topic, various aspects that may be innovative in the procedures for improving the outstanding bone supply were considered. Thus, a type of scaffold from a ceramic material has been proposed that has been described from a morpho-structural, biomechanical, immunotoxicological point of view.

The general part of the thesis consists of two chapters and it reviews the current knowledge regarding the field addressed, both nationally and internationally. Thus, aspects related to the appearance of bone defects, the therapeutic solutions existing at present, the materials used with various advantages and disadvantages are described. Moreover, the impact of three-dimensional printing on the level of dental medicine is described.

In the first chapter of the general part called „Techniques and materials used in the bone remodeling procedure for dental implant treatment", the author analyzes data about the causes that cause defects in the maxillary bones, which prevents prosthetic treatment based on dental implants, without an additional method that increases bone supply. In addition, there are mentioned the surgical therapeutic solutions currently used, each with the material used, the technique itself and the advantages and disadvantages of each.

The second chapter, entitled „Techniques and technologies for generation of bone remodeling structures", introduces us to an area of current interest, namely new concepts for generating scaffolds using 3D printing (3D), for bone augmentation. It is also described the current 3D printing systems and the materials used by them in applications in the field of dental medicine.

The main scientific objectives of this research are:

1. Imaging evaluation performed with the help of optical microscope and software for measuring the degree of adaptation of scaffolds obtained by 3D printing at the level of a large mandibular bone defect. Comparison of the values obtained within these measurements for

each type of scaffold obtained with the help of different design methods.
2. Evaluation by numerical simulation of the behavior of a 3D printed scaffold of polymeric material and solidarized at the level of a large mandibular defect with the help of dental implants, following the application of forces at the structures mentioned in the study.
3. Morpho-strucutral characterization and evaluation of a type of ceramic scaffold obtained by the method of sponge-based replication, as well as comparing the values obtained with those present in the specialized literature.
4. Evaluation of immunotoxicity and establishing the degree of biocompatibility, in vitro, on cell cultures, of the ceramic samples obtained.

The specific part is divided into four chapters, following the topic chosen in three different research directions, the fourth chapter being a complementary study to the third:

1. Ex vivo experimental study of three-dimensional printing of a mandibular bone defect
2. Study regarding the stabilization of a scaffold with the help of dental implants at the level of a mandibular bone defect. A finite element analysis
3. Study on the method of making a new type of bone remodeling material
4. Cytotoxicity assessment of the proposed ceramic material

In the first chapter of the specific part (3rd of the total), entitled "*Ex vivo* experimental study of three-dimensional printing of a mandibular bone defect", measurements were made at the interface between scaffolds obtained by three-dimensional printing and a large mandibular bone defect.

Using a mandibular bone with a large bone defect, three types of designs of the future three-dimensional polymeric material printed scaffold were made. The designs were realized both with the help of an optical scanner (MAESTRO DENTAL SCANNER MDS400) and with the help of radiological imaging of type CBCT (CRANEX 3DX CBCT) but also with the help of a dedicated software (CATIA V5 - Computer Aided Three Dimensional Interactive Application).

Subsequently, two types of three-dimensional printed polymeric scaffolds were made using the D20 Digital Wax System 3D Printer. They were made of two polymeric materials of different colors to distinguish them from the point of view of the type of design used as a precursor for printing.

After obtaining the samples, they were analyzed in relation to the mandibular bone defect, with the help of the optical microscope technologically realized in the discipline of Propedeutics and Dental Materials, the Faculty of Dentistry, within the U.M.F. "Victor Babes", from Timisoara. Results were

analyzed statistically, the values of the marginal adaptation of the scaffold to the level of the bone defect are on average between 0,200 mm and 0,500 mm.

In the second chapter of the specific part (4th of the total), entitled "Study on the solidarization of a scaffold with the help of dental implants at the level of a mandibular bone defect. A finite element analysis" followed the analysis of the behavior of a 3D printed scaffold made of polymeric material that is solidarized at the level of a mandibular bone defect with one, two and three dental implants. In this study we used an *ex-vivo* mandibular bone with a significant bone defect that prevents surgical treatment with properly inserted dental implants.

By scanning the mandible with MicroScribe 3G (Revaware Systems) and then using a finite element analysis (FEA) with Pro Engineer (PTC) and ANSYS 15 (Ansys Inc.), a numerical simulation is performed within the study and thus evaluated the effects of a force applied to the scaffold, implants and jaw.

This scaffold will be stabilized on the body of the mandible by ordinary dental implants. Given the extent of the defect, one, two or three implants may be used to strengthen this complex.

Considering the above, the purpose of this study is to evaluate, using numerical simulations, the adherence of a scaffold graft of polymeric material to a bone jaw that has a large bone defect and which prevents the clinician from using dental implants as a treatment plan. The second aspect to be considered in this study is the number of implants that are necessary to ensure adequate construction resistance at the usual level of forces acting on the mandible.

Therefore, in this study it was shown that a number of two implants is the best treatment option, offering a good stability of the scaffold, uniformly distributing the forces and improving the resistance at the same time. Also, the cost of treatment is more easily accepted by the patient.

In the third chapter of the specific part (5th of the total), entitled "Study on the method of making a new type of bone remodeling material", the morphological structural characterization of some scaffolds obtained from a type of ceramic material by the method of replication on polyurethane sponge support.

In this study two types of commercial ceramic masses were used to make the samples. The first type of ceramic mass is Ceramco iC Natural Dentine (Dentsply Sirona), which is commonly used for the purpose of manufacturing fixed dental prosthesis. The second product comes from the same company and is called Dentsply Sirona Ceramco iC Natural Enamel.

In parallel, the third type of ceramics with the chemical composition kaolin 47%, feldspar 28.5% and sand 24.5% was studied.

The ceramic scaffolds of all three types of ceramic mass were made by the foam replication method, which involves the production of ceramic foams by coating a polyurethane sponge with a ceramic mass slurry. The result is a solid ceramic structure, with a precise replica of the geometric characteristics

of the polyurethane sponge. The scaffold formed is porous in nature, with high pore interconnectivity. The sponge acts as a template for the porous scaffold. Due to the thermal and chemical stability and the high hardness, the polymeric sponge method has gained wide popularity among all the other processes for the generation of ceramic mass scaffolds. In order to introduce a new material that could have an important medical value, it is firstly necessary to characterize it as accurately as possible from a micro and macro-structural point of view. The means of characterization used in the literature to describe micro and macro-structural a new type of material are the following: X-ray diffraction (XRD) analysis, apparent porosity analysis, scanning electron microscopy (SEM), 3D optical microscopy. I decided to use the same characterization methods provided in the literature and I also added an innovative method to describe the new material proposed, Optical Coherence Tomography (OCT).

At the level of the resulting samples, two distinct regions can be observed. The median region has a relatively uniform distribution of pores with large dimensional scattering. The peripheral region has a smaller porosity, being denser and more compact, simulating the structure of the natural bone. The morphology of the porous structure obtained is illustrated by SEM images, 3D microscopy images and OCT images. The microscopic study was performed on two distinct areas that mimic the natural bone, namely cortical and spongy.

The present study aimed to obtain new types of ceramic, porous masses, with structures and properties similar to the natural bone. It can be concluded that the obtained samples meet the structural morphological characteristics mentioned in the literature as being essential in the surgical procedure of bone remodeling based on scaffolds.

In the last chapter of the specific part (6th of the total), entitled "Evaluation of the proposed ceramic material cytotoxicity", in vitro testing of the ceramic samples obtained in the previous study was carried out on cell culture lines in order to evaluate the degree of toxicity and biocompatibility of the material.

To carry out the experiment, the following reagents were used: culture specific medium - Fibroblast Basal Medium (ATCC PCS-201-030) and Fibroblast Growth Kit - Low Serum (ATCC PCS-201-041) purchased from ATCC, while the others reagents: trypsin - EDTA solution, PBS (phosphate saline), Trypan blue, Alamar blue (resazurin sodium salt) were purchased from Sigma Aldrich (Germany) and Thermo Fisher Scientific (USA). Human primary gingival fibroblasts - HGF were cultured in specific media. Blue Alamar analysis was performed to evaluate the biocompatibility / cytotoxicity of the test compounds on human primary gingival fibroblasts - HGF. The cytotoxicity test protocol complied with the recommendations provided by ISO-10993-5.

The impact of six different types of ceramics (AC, AD, AE, VC, VD and VE) on human primary gingival fibroblasts - the viability of HGF - was evaluated in the present study. The materials were received as solid articles

and solubilized in PBS, an environment that is biocompatible, subjected to ultrasound.

The cell viability evaluation performed using the BlueAlamar test showed that the lowest concentrations tested (50 and 100 μg / ml) for all the compounds tested in this study had no cytotoxic effect, the percentage of viable cells being in the range 95-105%, compared to control cells (unstimulated cells).

The ceramic tested in the present study can be considered biocompatible (according to ISO-10993-5, a compound that induces a viability <70% of control, has a cytotoxic potential), even if their cytotoxic profile was different.

The general conclusions of the doctoral thesis, described at length in the last chapter - "Own conclusions and contributions" were:

- three-dimensional printing method can lead to scaffolding with optimal marginal adaptation
- The use of CBCT in obtaining the design required for three-dimensional scaffold printing is imperative
- a number of two dental implants represents an optimal ratio between treatment costs and functionality, from the point of view of stabilizing the scaffold with the help of implants
- using the method of numerical simulations, that the forces are distributed evenly at the bone and scaffold, if a number of two implants is used and provides good primary stability to it
- The ceramic scaffolds obtained fulfill, according to the specialized literature, all the morphological and structural criteria to be a viable alternative in the bone remodeling procedures.
- The chemical composition of the samples is stable after the burning of the ceramics
- The samples have a unique, dedicated structure, similar to that of the natural bone, the samples having two distinct zones, an external one that resembles the cortex of the natural bone and an internal one, porous, similar to the spongy bone.
- The ceramics used in the previous study were subjected to biocompatibility tests and we concluded that all ceramic types considered in the study are biocompatible according to ISO-10993-5