Osseointegration Evaluation of Two Socket Preservation Materials in Small Diameter Bone Cavities

An *in vivo* lab rats study

FLORIN OBADAN[1], MONICA CRAITOIU[1]*, HORIA MANOLEA[1], LAURENTIU MOGOANTA[1], EUGEN OSIAC[2], RADU RICA[1], ALEX IOAN SALAN[1], KAMEL EARAR[2]*

[1]University of Medicine and Pharmacy of Craiova, 2 Petru Rares Str., 20349, Craiova, Romania

[2]Dunarea de Jos University of Galati, Medicine and Pharmacy Faculty, Department of Dentistry, 47 Domneasca Str., 800008, Galati, Romania

The present study aims to evaluate the osseointegration in small diameter rat bone cavities of a collagen-based material and a synthetic bone graft by using a protocol consisting of three evaluation methods: direct macroscopic examination, optical coherence tomography (OCT) and a histological study. For this study we made three study groups, each of them consisting of twelve laboratory Whistar rats, one for each studied material and one control group. For each study group, six laboratory rats were sacrificed after two months, and the other six after four months in order to evaluate the bone wound healing.The total amount of augmentation was significantly greater in the augmented groups than in the control group. Macroscopic examination of the evolution of augmented bone wound healing with the collagen-based material offered spectacular results especially in the cavities prepared in the calvaria, while in the bone wounds augmented with OsseramNano we always noticed the presence of synthetic material residual particles. The OCT evaluation highlighted the degree of filling of the defect through the lack of refractivity of the collagen-based material, while the higher refractive index of the synthetic bone graft material allowed some spectacular observations. On the histological samples from the first study group, filled with the collagen-based material, we generally have observed the filling of the experimental bone defects with repairing connective tissue with various bone extensions from the surrounding bone tissue The histological assessment of the synthetic bone graft augmented cavities showed firstly the presence of synthetic material residual particles surrounded by a newly formed connective tissue in early stages or a young bone tissue with many osteoblasts in the advanced stages of osseointegration.

Keywords:bone augmentation, collagen, synthetic bone graft, optical coherence tomography, histology.

A lot of surgical techniques and materials have been used over time for bone replacement in the human body.When choosing a bone graft material the surgeon should consider its ultimate effect on healing patterns in and around the alveolar bone at the endpoint of the procedure [1].

For early biomaterials, it was required to have a combination of physicochemical properties, suitable to replace human body tissues and to be biologically inert. Since then a long way has been brought to the third generation of biomaterials, whose role is to be both resorbable and bioactive, that is, to be able to elicit a controlled action in physiological conditions [2].

Bone substitutes used in oral and maxillofacial surgery could be categorized according to their biologic origin and source as autologous bone graft when obtained from the same individual receiving the graft; homologous bone graft, or allograft, when harvested from an individual other than the one receiving the graft; animal-derived heterologous bone graft, or xenograft, when derived from a species other than human; and alloplastic graft, made of bone substitute of synthetic origin [3].

Alloplastic synthetic biomaterials were developed to overcome the disadvantages of autografts and are fabricated in various forms with varying physicochemical properties [4].The most routinely used alloplastic materials are hydroxyapatite, tricalcium phosphates and bioactive glasses. Calcium phosphate biomaterials are of great interest to be used as bone replacement graft materials as they have a similar composition to bone mineral, are osseoconductive, form bone apatite like material or carbonated hydroxyapatite and form a very strong bone-calcium phosphate biomaterial [5]. Also over the last few years, tricalcium phosphates has been used and extensively investigated as a bone substitute. Its crystallographic form β-TCP exhibits good biocompatibility and osseo-conductivity and is used commonly as a partially resorbable filler allowing replacement with newly formed bone [6].

Topical application of type-I collagen sponges in the extraction sockets could be also a useful method due to its effectiveness in hemostasis, wound stabilization, and promotion of healing [7].

The present study aimed to evaluate the osseo-integration in small diameter rat bone cavities of two socket preservationmaterials *Alveoprotect* and *Ossceram Nano*(Bredent, Selden, Germany). For the assessment at several levels of these socket preservation materials we decided to use a protocol consisting of three evaluation methods to provide a comprehensive image about the behaviour of the used materials [8].

Experimental part

Material and methods

For this study we made three study groups, each of them consisting of twelve laboratory Whistar rats. On the calvaria (fig.1a) and maxilla (fig.1b) of thoseanimals 4-mm

*email: mcraitoiu@yahoo.com; erar_dr.kamel@yahoo.com

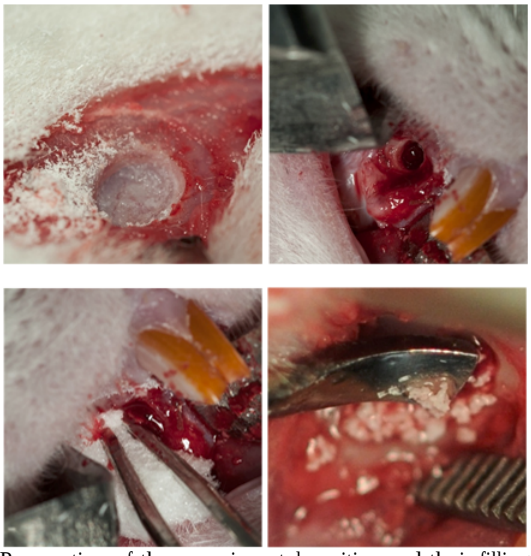

Fig.1 Preparation of the experimental cavities and their filling with the studied socket preservation materials (from left to right) 1a. Calvaria cavity 1b.Maxillary cavity 1c.Insertion of the Alveoprotect material 1d.Insertion of the Ossceram Nano material

diameter experimental cavities were carried out. Surgical procedures were performed under general anesthesia using Ketamidor 100 mg/mL 20 UI (0.2 mL) and Xilazyn Bio 2% 0.3 mL within the laboratory animal facility of the University of Medicine and Pharmacy from Craiova and the study protocol was approved by the Ethics Committee of our university (no.101/ 12.12.2014).

For the first study group the cavities were augmented with the collagen fleece material *Alveoprotect* (Bredent Medical, Senden, Germany) (fig.1c). This collagen-based material is used to prevent the post-extractional bone loss, to stabilize the dental alveolar boneand to facilitate an implant insertion at a later time. For the second study group we used for the augmentation the synthetic bone graft Ossceram nano (Bredent Medical, Senden, Germany) (fig.1d). This material is a two-phase calcium phosphate ceramic consisting of 60% hydroxyapatite (HA) and 40 % ß-tricalcium phosphate (ß-TCP). The third group was the control group to which the experimental cavities were left unaugmented the healing being achieved without any external intervention. After augmentation the wound was closed and sutured with 5.0 nonresorbable thread.

Laboratory animals were kept under observation and fed according to the standard diet. For each study group, six laboratory rats were sacrificed after two months, and the other six after four months in order to evaluate the bone wound healing. The euthanasia of laboratory animals was performed according to the current standards by administration of an overdose of anesthesia. Samples were obtained from the maxilla and calvaria bone, which had been cut to adequate sizes using a handpiece to cover both the areas of bone healing and adjacent normal bone. The obtained samples were fixed in 10% formalin solution and subjected to three examination methods.

The first method was a direct macroscopic examination performed immediately after rats euthanasia during the samples preparation.

For evaluating the surface and subsurface of the new-formed bone tissue we used OpticalCoherence Tomography (OCT) which is an imaging technique characterized by high spatial resolution and non-invasive detection. We have used for our measurements an SS-OCT device provided by THORLABS (OCS1300SS; Munich, Germany). The laser source is a swept laser (55 kHz) working on a central wavelength of 1325 nm (average power ≈12 mW). The system allows 2D and 3D scans. Axial resolution is about 12 μm and lateral resolution is about 15 μm. Optical power on the sample is 5 mW.

Finally, the obtained samples were submitted to the classical phases necessary to the histological study. Bone samples were fixed in 10% buffered formaldehyde for two weeks and then decalcified in an EDTA solution, which was refreshed at regular intervals. Decalcification was considered complete when the samples reached a rubber consistent. Samples were then dehydrated in increasing degrees of alcohol (50, 75, and 100%), cleaned with xylene and then embedded in paraffin. The paraffin embedded samples were serially sliced in cross sections with 5 microns thickness and mounted on glass slides. The sections were deparaffinised, hydrated and stained with hematoxylin and eosin and Masson trichrome colorations. The sections were examined with an Olympus CX 20 microscope attached to a camera and a computer.

Results and discussions

A *macroscopicexamination* was performed immediately after rat's euthanasia during the samples preparation.

Macroscopic examination of the evolution of augmented bone wound healing with Alveoprotect offered spectacular results especially in the cavities prepared in the calvaria. Thus, two months after inserting the material into cavities from the calvaria, they were already occupied by a bone tissue, even if it was less dense than neighbouring structures (fig.2a). After four months, the healing bone had a dense, homogeneous look, making it difficult to clinical differentiate from the native adjacent bone tissue (fig.2b).In the maxilla case, the Alveoprotect integration speed appears to have been lower than in the calvaria. Thus, after two months from the material insertion, we could see the emergence of a dense fibrous connective tissue at the level of the created defect (fig.2c). The samples appearance at 4 months has highlighted bone bridges in the cavity created in the jaw bone giving the appearance of a young bone tissue that probably requires more time for a full maturation in this anatomical region (fig.2d).

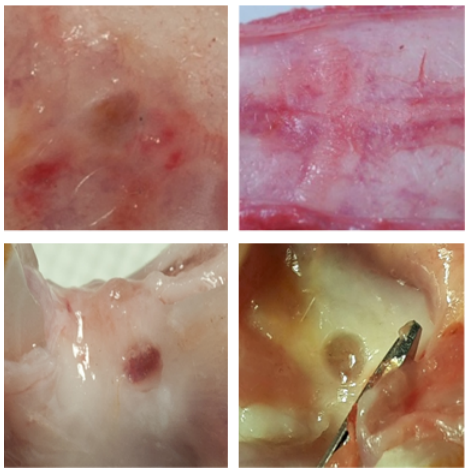

Fig. 2.The macroscopic appearance of the samples filled with the Alveoprotectmaterial(from left to right). 2a.Calvaria sample obtained at two months after the material insertion. 2b Calvaria sample obtained at four months after the material insertion. 2c. Maxillary sample obtained at two months after the material insertion. 2d. Maxillary sample obtained at four months after the material insertion

Macroscopic examination of the evolution of healing bone wounds augmented with OsseramNano always highlighted the presence of synthetic material residual particles. They were highly present in the augmented cavities at just two months after their insertion, occupying

almost entirely the bone defect volume, both in the calvaria and maxilla. Four months after, their biological integration could be much better clinically appreciated, especially in

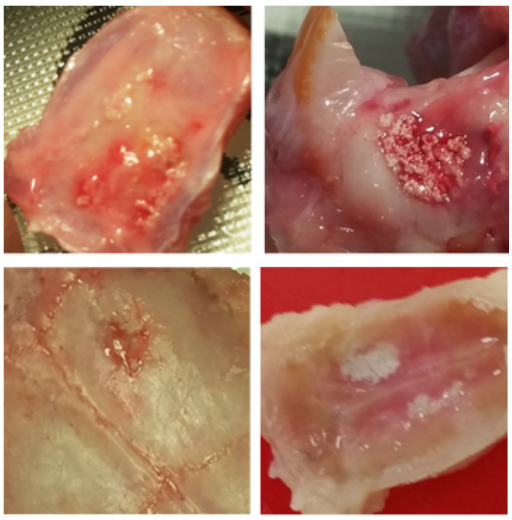

Fig. 3.The macroscopic appearance of the samples filled with the Ossceram Nanomaterial (from left to right). 3a.Calvaria sample obtained at two months after the material insertion. 3b Maxillary sample obtained at two months after the material insertion. 3c. Calvaria sample obtained at four months after the material insertion, view from the sample interior face.3d. Calvaria sample obtained at two months after the material insertion, after sample demineralization, view from the sample interior face

the calvaria samples. Thus, even it still could be clinically noticed, the bone defect area appeared to be more uniform and better anchored to the adjacent bone tissue (fig.3).

The OCT study allowed us to evaluate the surface and subsurface of the new-formed bone tissue in the experimental cavities.

Thus, for the collagen-based material Alveoprotect, the OCT evaluation highlighted the degree of filling of the defect through the lack of refractivity of this socket preservation material.The cavities created in the calvaria filled with this material had a homogeneous OCT aspect, with some isolated gaps in samples obtained at two months after the material insertion, but with a denser look for samples obtained at four months after insertion. In the maxilla samples, this imaging evaluation allowed us to highlight the filling progress of the created defects, with the presence of bone bridges two months after the material insertion defining a plurality of various sizes cavities and a more homogeneous filling four months after the material insertion (fig. 4a-d).

The surface and subsurface evaluation by optical coherence tomography of the new bone formed within the experimental cavities filled with Ossceram Nano synthetic material, made from hydroxyapatite and tricalcium phosphate, allowed some spectacular observations due to the higher refractive index of the synthetic material than the adjacent bone structures one. Thus, OCT examination enabled us to obtain images with a better view of the persistence of residual material particles and their arrangement on the surface and subsurface of the analysed augmented bone defects(fig. 4e, f).

On the histological samples from the first study group, filled with the Alveoprotect material, we generally have observed the filling of the experimental bone defects with repairing connective tissue with various bone extensions from the surrounding bone tissue, but we noticed the

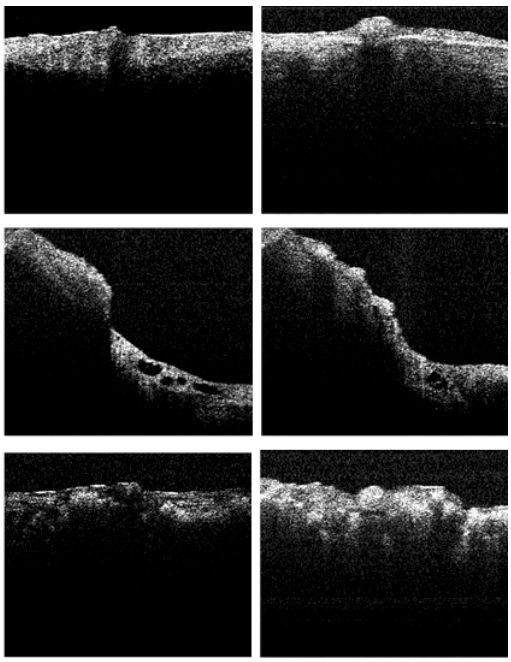

Fig.4.OCT aspects of the bone samples, highlighting the healing defects covered with the socket preservation materials (from left to right). 4a.Calvaria defect covered with the Alveoprotect material at two months after material insertion 4b.Calvaria defect covered with the Alveoprotect material at four months after material insertion 4c. Maxillary defect covered with the Alveoprotect material at two months after material insertion 4d. Maxillary defect covered with the Alveoprotect material at four months after material insertion 4e. Calvaria defect covered with the OssceramNano material at two months after material insertion 4f. Calvaria defect covered with the OssceramNano material at four months after material insertion

apparition of ossification centres also inside the repairing connective tissue away from the osteoid front coming from the bony defect edges.The collagen arrangement of the Alveoprotect material also provided a specific pattern for the colonization of the created defect by the connective tissue and then by the bony tissue. The intensity of the inflammatory reparative processes was observed in the examined defects by assessing the lymphocyte infiltration and also by the presence of foreign body reaction, manifested by the apparition of multinucleated giant cells in a granulomatous inflammatory response type.

Bone forming processes specific structures have been observed, with numerous blood vessels, young bone with numerous cells and the existence of a mineralization front with osteoblasts into a rich in cells fibrous connective tissue (fig. 5 a-d).

The histological assessment of the Ossceramnano augmented cavities showed firstly the presence of synthetic material residual particles surrounded by a newly formed connective tissue in early stages or a young bone tissue with many osteoblasts in the advanced stages of osseointegration. On all the studied preparations there were no areas of necrosis or encapsulation and rejection of the applied material. The synthetic material resorption degree was related to the particles size, and time elapsed from their insertion. In advanced stages we have met incorporated particulate matter into the new formed bone (fig.5 e,f).

We choose in our study to insert the tested materials in two anatomical region of the laboratory animals: calvaria and maxilla.The calvaria region is frequently used in animal model studies for evaluating osteogenesis induced by biomaterials as it has similar embryological origin and

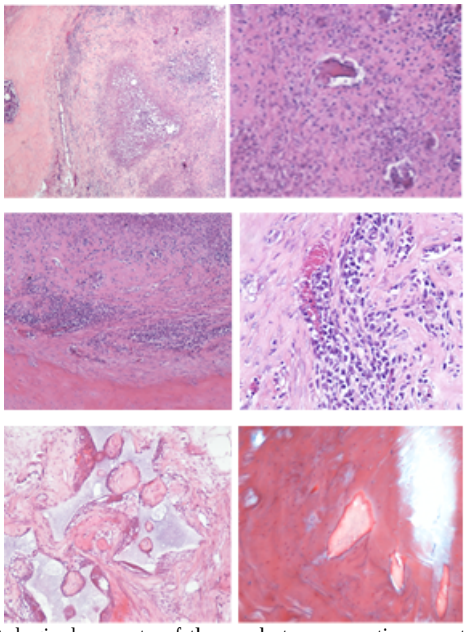

Fig.5 Histological aspects of the socket preservations materials tissular integration in the created defects.5a.Overview of Alveoprotect augmented bone defect two months after inserting the material. Col H&E. 10x 5b.Apparition of ossification centers also inside the repairing connective tissue away from the osteoid front coming from the bony defect edges in a Alveoprotect augmented bone defect Col H&E. 20x 5c. Highlighting the mineralization front of the Alveoprotect augmented bone defect occupied by a fibrous connective tissue with collagen fibers arranged parallel to the osteoid front. Col H&E. 20x. 5d. High caliber blood vessel located on the periphery of the bone defect filled with red blood cells surrounded by a tissue rich in cells. Col H&E. 20x. 5e. Overview highlighting the Ossceram Nano synthetic material particles surrounded by newly formed connective tissue two months after their application. H & E. 20x5f.Large Ossceram Nano synthetic material particles surrounded by young bone tissue four months after their application. H & E. 20x

morphology to the maxilla and has limited anatomic area of mechanical stress and relative stability of the neighbouring structures [9-11]. Moreover, rat calvaria is one of the most commonly used pre-clinical settings for the testing of bone graft biomaterials, because it is relatively cheap and offers favourable anatomical access and surgical handling [12].

The edentulous posterior maxillary region presents in implantology a more difficult situation than any other region of the maxillary. The absence of teeth often triggers a progressive reduction of the alveolar process volume, a reduction that aims particularly at the bony vertical dimension, namely the region between the top of the alveolar ridge and the floor of the maxillary sinus, called the sub-sinus Misch vertical dimension. The presence of the maxillary sinus, combined with the reduction of height of available bone in the terminal maxillary region poses serious problems for implant insertion [13].

The main alternative for the reconstruction of bone defects is the sinus augmentation method with alloplastic materials which proves to be reliable and clinically applicable, with similar results to those obtained by augmentation with autologous material. Unlike in augmentation with autologous material, postoperative risks and possible postoperative complications are minimal, while the functionality of the grafted area is similar [14]. Otherwise, in a 2014 systematic review and meta-analysis bone substitute materials are described as a reasonable alternative to autologous bone and implant survival seemed to be independent of the biomaterial used in maxillary sinus floor augmentation and alveolar ridge augmentation [15].

In our study we choose to sacrifice half of the laboratory animals sacrificed after two months, and the other half after four months in order to evaluate the bone wound healing evolution. A 2010 study suggested that a period of eight weeks may be appropriate to assess new bone tissue and the resorption of the graft material, but longer periods are needed for late healing, such as bone incorporation, resorption of materials, bone remodelling, or the amount of bone regeneration [16].

Autogenous bone is still considered the gold standard for most applications; it becomes vascularized and osseointegrates with surrounding bone, thus minimizing the risk of infection, dislodgement, or break-down. Limitations include added operative time for graft harvest, donor site morbidity, graft resorption, molding challenges, and limited availability [17]. Alloplastic materials are synthetic bone substitutes that act as a biologic filler. They are osseoconductive bone substitutes, do not require a donor site, are available in unlimited quantities, and do not pose a risk of disease transmission [18].

In our study the collagen material Alveoprotect offered spectacular results especially in the cavities prepared in the calvaria. Thus, two months after inserting the material into cavities from the calvaria, they were already occupied by a bone tissue, while at four months, the healing bone it was difficult to clinical differentiate from the native adjacent bone tissue. In the maxilla case, the Alveoprotect integration speed appears to have been lower than in the calvaria.

In 2017 we already stated from our results that a collagen material did not change the natural histological pattern of the regeneration process,but offered a support for the bone wound healing which enhanced the bone formation speed and it can be used in the guided bone healing process to prevent the bone loss in areas with small bone defects [19]. Also, Cioban C found in 2015 that the preservation using the collagen matrix alone allowed the development of more mature and continuous external bone structures four weeks after extraction. The use of the collagen matrix seems to be an interesting ridge preservation option but only after obtaining further information on its barrier function. The use of a bovine bone substitute seemed to delay hard tissue development after tooth extraction [20].

In the category of bioactive materials performance, calcium phosphate and bioactive glass based materials have attracted a significant attention being widely used in bone tissue engineering. In particular, calcium phosphates are more traditional for bone graft substitution, since their composition is close to the mineral part of the bone tissue [2].

In our study examination of the evolution of healing bone wounds augmented with OsseramNano always highlighted the presence of synthetic material residual particles. They were highly present in the augmented cavities at just two months after their insertion, occupying almost entirely the bone defect volume, both in the calvaria and maxilla, while four months after, their biological integration could be much better appreciated, withincorporated particulate matter into the new formed bone.

In a 2015 study Onisor-Gligor Fl. et al. found that subantral augmentation with autologous bone leads to a higher degree of osseointegration of the dental implants placed in this material compared to those placed in alloplastic material, but without a statistically significant

difference. However, alloplastic grafts have a lower rate of resorption compared to autologous grafts [21].

Cioban C. et al. in 2013 state that the use of a bovine xenograft with a bilayer pure collagen matrix was associated with an increased osseous deposition and a less osteoclastic activity in the post-extraction socket in comparison with the use of a double membrane layer [22].

The quality of bone grafting was already evaluated by OCT in other studies [23] and validated by using micro-CT. For the collagen-based material Alveoprotect, our OCT evaluation highlighted the degree of filling of the defect more through the lack of refractivity of this socket preservation material, but for the new bone formed within the experimental cavities filled with Ossceram Nano synthetic material the higher refractive index of the synthetic material than the adjacent bone structures one allowed some spectacular observations.

Our study confirmed the observations of other authors as we also always highlighted the presence of synthetic material residual particles [24, 25]. Connective tissue content decreased with the use of the bone substitutes, but considerable residual hydroxyapatite and xenograft particles (15% to 36%) remain at a mean of 5.6 months after socket augmentation procedures. Whether these changes in bone quality will influence implant success and peri-implant tissue stability remains unknown [26]. However, a 2014 study showed that low-level laser therapy may be effective in the healing of bone defects, especially when associated with a filling material as it accelerates the resorption of the graft material particles [27,28].

The role of hydroxyapatite and collagen was highlighted also in a 2014 study which showed that hydroxyapatite and collagen combination-coated dental implants display greater new bone formation and bone-to-implant contact in the peri-implant area than the same combination plus bone morphogenetic protein-2-coated implants, hydroxyapatite only coated implants, and uncoated implants [29,30].

Conclusions

The collagen-based material offered spectacular results especially in the cavities prepared in the calvaria leading to the formation of a young bone tissue even at 2 months after insertion, while at four months, the healing bone it was difficult to clinical differentiate from the native adjacent bone tissue. The collagen arrangement of the material also provided a specific pattern for the colonization of the created defect by the connective tissue and then by the bony tissue.

The synthetic bone graft material always left residual particles in the evolution of healing bone wounds highly present at just two months after their insertion and to a lesser extent at four months.The higher refractive index of the synthetic material than the adjacent bone structures one allowed some spectacular observations using optical coherence tomography. Thesynthetic material residual particles were surrounded by a newly formed connective tissue in early stages or a young bone tissue with many osteoblasts in the advanced stages of osseointegration with fully incorporated particles into the new formed bone with a tight contact in the late stages.

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Manuscript received: 21.02.2018