The Routine and Specialised Staining for the Histologic Evaluation of Autogenous Mandibular Bone Grafts

An experimental study

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Oral rehabilitation by dental implants is a routine treatment in the common dental practice, and volume reconstruction in cases of advanced alveolar ridge atrophy using bone autografts has become a frequently used therapeutic procedure. The study presents a histological evaluation of autogenous mandibular bone grafts integration in surgically created maxillary bone defects. Seven domestic adult dogs, Canis Familiaris were used in the study. Work methodology was established through maxillary and mandibular morphometry, the donor region being the posterior mandibular body, and the recipient region being the lateral body of the maxilla. In the experimental study, we simulated two bilateral maxillary bone defects, which were augmented with mandibular corticocancellous bone grafts. Biological samples containing the target areas were collected 90-100 days after grafting and the subsequent preparation method of the samples for histological analysis was the standard one.The histological results showed the successful integration and the beneficial effect of corticocancellous autogenous mandibular bone grafts applied in maxillary sites.

Keywords: bone autografts, bone formation and regeneration, histological analysis, integration

The aim of the present study was to histologically evaluate the integration of mandibular corticocancellous autografts applied in maxillary bone defects, on a standardized animal model, determined through study.

The history of autogenous bone grafts dates back to the 19th century; using such grafts for atrophied alveolar edentulous ridges augmentation is the golden standard in implant dentistry today [1].

The use of autogenous mandibular bone grafts is the most frequent alternative for bone volume reconstruction after alveolar ridge resorption [2].

Autogenous bone grafts present a series of great advantages, such as their osteogenic potential, the greater resistance to resorption and horizontal bone atrophy, the possibility to be used in order to correct large bone defects, and the elimination of various immune reactions. They are osteoinductive, osteoconductive and osteogenic [2, 3].

Bone autografts also allow osteogenic cell transfer in the receiving area, which is particularly important for a successful integration [2].

Fresh bone autografts contain surviving cells and osteoinductive proteins, which can stimulate osteogenesis, and represent the best available material, because they are non immunogenic and partially maintain their viability immediately after transplantation [4].

Maxillo-mandibular bone grafts represent a convenient and acceptable source of autogenous bone for alveolar reconstruction, due to the common embryological origin and lower morbidity [5, 6].

Autogenous bone grafts can be obtained from the cortical bone or the cancellous bone but, most of the times, both in implant dentistry and in reconstructive surgery, especially for inlay augmentation grafts, a combination of the two bone types, a corticocancellous mixed graft is used [1, 7].

Mixed, corticocancellous bone grafts, are frequently used in the reconstruction of edentulous alveolar ridges. The cortical layer provides resistance, which is why they can be used to reconstruct the alveolar contour. Corticocancellous bone grafts can be used in order to correct a bone defect up to 5 cm in diameter, as long as an adequate blood supply in the covering soft tissue is ensured. To allow an adequate healing, these mixed grafts must be rigidly fixated to the receiving bone [1, 2, 8].

Using these mixed grafts in cases of advanced alveolar ridge atrophy constitutes a safe and efficient therapeutic approach that provides a favourable bone height and width for implant placement. Their proper fixation reduces the resorption rate and promotes bone formation [9–11].

Special histological techniques followed by classical staining have been previously used in dentistry for the pulpodentinal complex evaluation or for the evaluation of dental implants integration [12, 13].

Experimental part

Material and methods

Seven standard weight (15-20 kg), clinically healthy, adult domestic dogs, *Canis Familiaris*, from the bio base of the Faculty of Veterinary Medicine in Bucharest, were used in the study. The research was organised and accomplished according to current national legislation.

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The work methodology was established after the morphometric analysis of maxillary and mandibular bone structures, originating from another study [14]. Thus, the target donor region was set as the posterior mandibular body, transition area between the mandibular body and the ramus, and the target receiving region as the lateral region of the maxillary body, corresponding to the alveolar area of the bicuspids. In the pre-established experimental model, we performed bilateral maxillary bone defects by drilling at standard speed, and we augmented the defects with a mandibular corticocancellous bone graft. 90-100 days after the surgical intervention, hard tissue fragments were harvested from the grafted sites.

For the histological analysis we used the standard data processing, in accordance with the current national legislation and the medical guidelines for the *Specialty* of *Anatomical Pathology* [13]. The tissue fragments were immersed in 10% buffered formalin immediately after harvesting. 24 hours later, they were decalcified in EDTA for 2-3 days, until a firm-elastic consistency was obtained. Histopathological processing took place via dehydration, clearing and paraffin impregnation, through the automated procedure, according to the work protocol for automated histopathological processing (Leica ASP 200S histology tissue processor). The impregnated fragments were embedded in paraffin blocks with a Thermo Fisher Microm EC 1150 H embedding station, and sectioned to 3µ with a Leica RM 2255 and RM 2265 rotary microtomes. The sections were mounted on plain slides for routine staining (Haematoxylin and Eosin - H&E) or specialised staining (Van Gieson, Trichrome Masson).

Results and discussions

In the periodic (bimonthly) clinical and radiographic check-ups, we noted the favourable evolution and integration of the grafts, excepting one, which on the 30th day radiographic check-up has lost the bone fixation screw, situation we considered a failure. Other noteworthy post-intervention complications did not exist.

Bone regeneration accompanied by remodelling processes with osteoclast activation, along with well represented revascularisation (angiogenesis) was noticeable on the histologically analysed biological products. Capillary vessels were present in the grafts, as well as small areas with local inflammation, presenting normal as well as degraded polymorphonuclear neutrophils, originating from the soft tissue (*lamina propria*). Cell viability, an essential element for successful healing and for the proliferation of bone-forming cells, was proved by the histological analysis which revealed the presence of osteogenic osteoblasts at the level of the grafted areas.

The histological analysis also showed the presence of small areas of osteolysis in the immediate proximity of the areas with osteoblastic hyperplasia and hypertrophy, bordering bone trabeculae and alternating with regions of osseointegration characterised by important osteoblastic hyperplasia on both sides of bone trabeculae.

The newly formed bone tissue also had areas of osteoid deposition with varying degrees of calcification, in a mass of large-cell osteoblastic proliferation, with vesicular nuclei and prominent nucleoli, at the periphery of some mature trabeculae within the pre-existing bone.

From a histological point of view, the results we obtained using block corticocancellous bone autografts were very solid. We shall continue presenting through images 1-9 the results from the histological analysis, along with precise details.

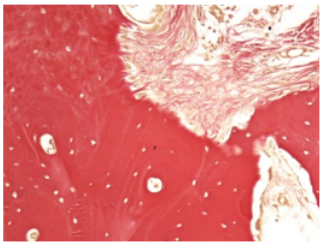

Fig. 1. Grafted area: detail from the areas with osteoblastic hyperplasia and hypertrophy. Van Gieson x 200

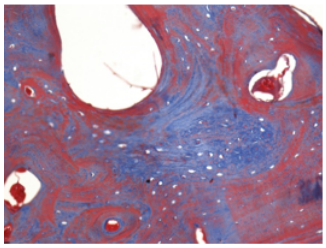

Fig. 2. Grafted area: bone tissue in which numerous lacunae with osteocytes are to be found. Trichrome Masson x 200

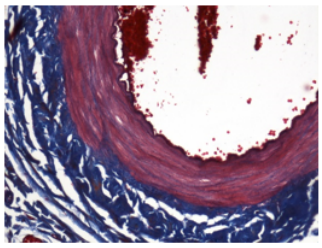

Fig. 3. Small/medium calibre artery vessel in the grafted area. Trichrome Masson x 200

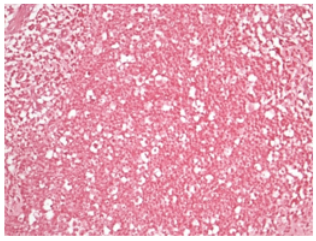

Fig. 4. Area of inflammation with a great number of normal and degraded (pus cells) polymorphonuclear (PMN) neutrophils at the level of the mucosal lamina propria. HE x 200

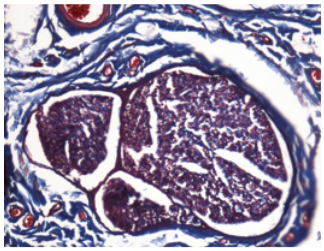

Fig. 5. Nervous fillets in the grafted area. Trichrome Masson x 200

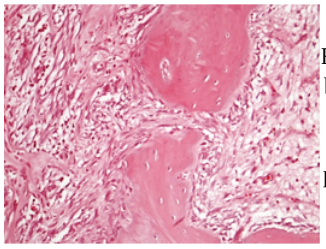

Fig. 6. Grafted area: newly formed bone tissue with varying degrees of calcification and with evident osteoblastic proliferation at the periphery of certain mature bone trabeculae. HE x 200

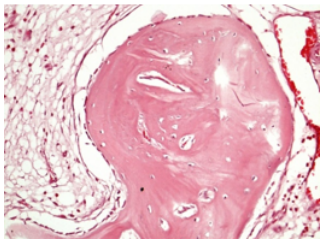

Fig. 7. Grafted area: normal bone trabeculae, bordered by a single row of flattened osteoblasts. Hyperemic state of the bone marrow. HE x 200

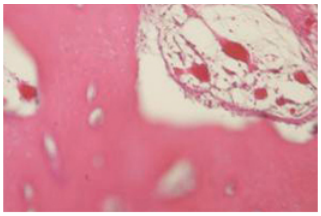

Fig. 8. Grafted area: active bone repairing processes. HE x 100

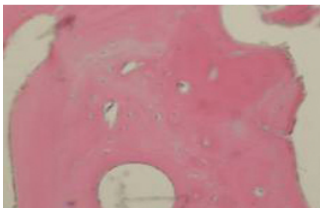

Fig. 9. Grafted area: newly formed bone. HE x 100

Bone healing is a complex biological phenomenon that takes place both during the body's growth and development stages, as well as in certain bone modelling, remodelling and repair processes. The necessary conditions for postsurgical healing are mainly represented by: adequate blood supply, lack of connective tissue at the interface, and primary stability of the grafts [2].

Insufficient sanguine flow in the bone or in the surrounding soft tissue, as well as variations in local vascularisation, can negatively influence healing, resulting in delayed fusions, or non-fusions, between donor and receiving elements [15]. Areas of angiogenesis evidenced by the presence of capillary vessels, alongside areas of reduced local inflammation were found in the grafted sites. This is in accordance with other authors who pointed out that angiogenesis vessels are more numerous in areas where the inflammatory infiltrate is more abundant [16]. Neoangiogenesis involves growth factors and endothelial cell migration and proliferation [17].

During revascularisation phases and multipotent cell differentiation in osteoblasts, the immune system of the recipient is reacting to the antigenicity of the donor [18].

The integration of the bone graft in the receiving area also depends heavily on its adequate revascularisation, as it is independent of the vascular support of the receiving area [19].

Bone regeneration after bone grafting implies a constant, life-long, remodelling process, as other similar studies have proven [20].

The bone regenative processes may be accompanied by the presence of a moderate subepithelial inflammatory infiltrate [21].

Cell viability is influenced by the graft harvesting technique and represents an essential condition for the graft's success, because vital cells will differentiate themselves into bone creating osteoblasts [22].

The success of autogenous bone grafts depends on the survival and proliferation of osteogenic cells, on the pre-existing conditions at the level of the recipient site, on the type of graft, and on the manipulation and modelling of the graft during the procedure of adjusting it to the receiving area. When inductive bone cells appear locally, mesenchymal stem cells are pulled into the grafted area and become capable of inducing new bone formation [15, 23].

The proliferation of bone cells is responsible for tissue regeneration, and osteocyte survival in the grafted areas depends directly on the blood supply and on the vitality of the periosteum [24].

The osteoblast cell line controls the formation and activity of the osteoclasts, the latter being responsible for the initiation and accomplishment of bone resorption in areas requiring remodelling [25, 26].

However, it can be said that for a histological staining to be useful, a high resolution is needed in order to distinguish the qualitative differences of the tissues, as other authors also have shown [27].

After analysing the results, we consider that the use of corticocancellous bone as autogenous block bone graft is a superior alternative, regardless of its embryological origin. The procedure has a high success rate in extensive reconstruction of severely atrophied *alveolar ridges*, finding which is confirmed by other studies [28–30]. The ideal substitute is autologous bone, the gold standard in regeneration [31].

A bone defect formed in various orthopedic and/or trauma pathologies fails to heal and needs bone reconstruction [32].

In order to evaluate the efficacy of various types of autogenous bone grafts, prospective clinical and experimental studies are needed, because the various indications regarding their use require customised solutions [2].

Even though the radiographic examinations can be used in morphometric evaluations regarding the integration of autogenous bone grafts, histological evaluations are far more beneficial. Other authors showed that the grafted areas could also be ultrastructurally analysed through transmission electron microscopy [33].

Because the inability to precisely determine the direction bone formation will take, and since bone modelling and remodelling become evident three months after grafting, future studies should further investigate these aspects.

Conclusions

The histological analysis proved that mandibular autogenous bone grafts have a favourable biologic response and rapidly induce bone formation (regeneration). This process is not completed three months after grafting.

Choice of clinically appropriate grafting material is an important aspect for new-bone formation and perfecting bone regeneration techniques is a permanent challenge in dentistry.

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