Capacity of Guiding Osseous Regeneration: Periosteum Versus Mesenchymal Stem Cells

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Abstract. The study was made on healthy common breed dogs on which, under general anesthesia, bone defects were made. For treatment were used scaffolds with hydroxyapatite-collagen support loaded with mesenchymal stem cells or the bone hole was covered with autologus periosteal flap. Evaluation of the healing process was made by clinical and, x-Ray exam. In guiding osseous regeneration in dog autologous periosteum has similar capacity, even better than autologous mesenchymal stem cells loaded on collagen impregnated with hydroxyapatite scaffolds.

Key words: dog, mesenchymal stem cells, osseous regeneration, periosteal flap.

INTRODUCTION

Tisular engineering catalogues among possible therapeutically methods for the bone defects repair the usage scaffolds implants loaded with stem cells.

The purpose of this study is to compare capacity of dog autologous periosteum and autologous mesenchymal stem cells in guiding osseous regeneration.

MATERIAL AND METHODS

Biologic material: 10 mixed breed weighing approximately 18 kg and 2-3 years old. After clinical examination and baseline laboratory tests all dogs were considered healthy. Before surgery, control X-ray exams of femoral region were carried out to exclude the eventually pathology at the places prepared for orthopedic intervention.

Obtaining the scaffolds loaded with mesenchymal stem cells

Under general anesthesia performed using premedication with acepromazine - ketamine and for maintenance isoflurane in oxygen, from 5 individuals (experimental group B), after the aseptic preparation of the region, bone marrow was aspirated by proximal humeral epiphyseal puncture. The samples were processed to obtain mesenchymal stem cells (MSCs), following a standard protocol and using as support for growing-loading-transplant collagen impregnated with hydroxyapatite scaffolds (Ignä et al., 2008).

Experimental protocol

Under general anesthesia (N-NLA) with inhalatory narcosis (isoflurane), muscle relaxants and controlled ventilation, following a classical approach (Bojrab, 1983), in all ten dogs, in both pelvic legs, large diaphiseal defects were created as 2 cm$^2$ holes (1 cm/2 cm) on the lateral aspect of the femur. The operation was performed under strict aseptic condition.

For experimental group A (n=5), the defect achievement on the right limb was preceded by the elevation of a “U” shape periosteal flap (with a small sharp periosteal
elevator) which served afterwards as cover for the defect site and was fixed by simple interrupted sutures with absorbable material (Fig. 1).

The defects from the left limbs of group B (n=5) were filled with hydroxyapatite scaffolds loaded with autologous MSCs (Fig. 2). In all dogs, osseous defects created in the opposite limb were left empty and were considered control (group C, n=10). Routine wound closure was performed in all cases.

The evaluation of bone defects healing process was made through daily clinical exam for a period of 14 days and at 21 day and by repeated X-rays exam, in craniocaudal and mediolateral view of interested area, immediately after surgery (Fig. 3a, b) and in the postoperative period at 7, 14, 21, and 28 days. Clinical examination includes inspection of the operatory wound, pain sensibility at palpation, general clinical status and presence/absence of lameness. For assessing functional disorders when the animal is walking a five levels scale was used: 0 = normal attitude in station and in walking – without lameness, 1 = in walking difficulties, especially at rapid carriage – fine lameness; 2 = in walking difficulties, intermittent lameness in rapid walking; 3 = evident lameness at every step, pain, 4 = the leg pull out of support in station and in walking, intense pain.
RESULTS AND DISCUSSIONS

At the clinical exam performed in both experimental groups and in control group, during one week after the surgery all the subjects presented 2-3 degree of lameness. In daily exam performed in the next week, in standing position and with walking animal, it comes out that the lameness decreased in intensity, being discreetly and in accordance with the scale used for functional disorders assessing the first level. After 14 days, the functional disorders disappeared completely. In all the subjects the cutaneous incision healed uneventfully. Pain sensibility at the site of incision, lasts in the first postoperative week, and disappeared completely at 14 days in subjects of experimental A and B group. In control group, the region exam by palpation showed an acute pain sensibility in first week which was diminished in intensity and completely disappeared at control made at 21 days.

On radiographic exam, made at 7 days after surgery, it haven’t been observed differences between groups B and C regarding bone density changes in the area of the defect and around it, bone profile remained unaltered in craniocaudal view, the images being similar to those obtained immediate after surgery. On group A, radiological images from craniocaudal view showed at 7 days discreet changes of subperiosteal space with changes of bone defect contour.

Preservation of periosteum is considered critical for limb bones fracture healing (Illizarov, 1989) and radiographic results of the study confirm these assumption. X-ray control made at 14 days on group A, revealed the presence of an inflammatory process that involves the periosteum around the defect, this process being extended even on the opposite site. In craniocaudal view was observed active osteogenesis, newly formed bone filling about 50% of the bone defect. This new bone, immature bone tissue, also modifies the radiodensity of the defect in mediolateral view and has a lower density in comparison with the normal bone tissue surrounding the defect. The mineralization process has progressed at 21 days after surgery so that the new bone fills up totally the defect area and goes beyond that for few millimeters, bone defects covered with periosteum demonstrating slight elevation of their profile, aspects observed in oblique mediolateral view (Fig. 4). Ongoing of the healing process shown by X-ray images was similar at the whole group. The isolation of bone defects with periosteum reduce the involvement of adjacent tissues in the process of bone healing and allow bone marrow, a source of polypotent cells, to grow freely into the defect. Whereas one
third or more of the diaphiseal blood supply comes from the periosteal vasculature (Rhinelander, 1968) in our study the periosteal flap was vascularized. All these elements are responsible for obvious tissue regeneration determined in this study. Our results correspond with those reported by Vögelin (2005) regarding healing of a critical-sized defect in the rat femur who showed that the addition of a vascularized periosteal flap significantly increased bone formation within the boundaries of the defect and prevented heterotopic ossification. The results of morphological, histopathological and scintigraphical studies made on rabbits (Turgut, 2005) showed that periosteal flaps had a much faster and more stable reconstructive capacity of osteogenesis.

![Fig. 4a Group A, subject 1, craniocaudal view at 21 days](image1)
![Fig. 4b Group A, subject 4, neutral mediolateral view at 21 days](image2)

A large number of experimental studies showed that different scaffolds loaded or not with autologous mesenchymal stem cells are effective in promoting bone formation in various animal models such as sheep (Becker, 2006), dogs (Igna et al., 2002, 2008; Malard, 2005), rabbits (Dallari, 2006) and mice (Cancedda, 2003). Hydroxyapatite scaffolds loaded with autologous MSCs used in this study as bone replacement material, provides the same radiological outcomes as our previous studies made in dogs with tibial experimental defects (Dascalu et al., 2007; Igna et al., 2005, 2006, 2008). Radiographic images from the individuals of group B, obtained at 14 days are similar with those from the group A obtained at 21 and 28 days, demonstrating the osteogenesis capacity of scaffolds (Fig. 5). The 28 days control reveals a follow-up of new bone formation which exceeds even the defect area and extends on the opposite site of this. Scaffolds fastening with the absorbable suture material determined a discontinuity of the newly formed bone. At 2 individuals from the group, the newly formed bone tissue proliferated proximal and distal to the defect and has extended on all length of the middle femoral diaphise. Unlike our results, Manjubala et al. (2005) observed that the ossification process of 4-mm holes on the lateral aspect of the femur filled with biphasic calcium phosphate ceramics, started after 4 weeks and the defect was completely filled with new woven bone after 12 weeks. This different result may be explained by the lack of scaffolds loading with MSCs which are considered to be involved in the osseous healing process.

The porous scaffold structure allows controllable ingrowths of new bone tissue into the defect zone and enhancing of this process was achieved by loading the scaffolds’ structure with mesenchymal stem cells. The scaffold used in this study was easy to handle and fix into the defect zone, and finally resulted good performance regarding osseous defect healing.

Results withdrawn from comparative analysis of radiographic images early made in postsurgery period, at 14 and 21 days, demonstrated that bone repair with periosteal flaps
does not lead to a faster rate of healing compared with scaffold loaded with MSCs, but rather to healing in the absence of exuberant bone formation. Moreover, a brief cost-benefit analysis indicates that application of periosteal flap to guided bone regeneration reduces total costs compared with those needed when scaffolds loaded with MSCs are used.

In group C, the X-ray exam performed in accordance with the same protocol reveals to all subjects the absence of osseous healing process at 7 and 14 days and his start at 21 days, put in evidence by the moderate differences of radio opacity and contour of the osseous defect in craniocaudal view in all subjects. On images obtained in booth views, on 60% of subjects it can be observed around the defect an inflammatory periosteal process, which is responsible for identified radiodensity modifications.

Regeneration of bone defects represents a serious challenge in orthopedic surgery and today, promising approaches are available to solve this problem using scaffolds materials, different cell based strategies and the periosteum guiding capacity of osseous regeneration. The use of periosteal flap and hydroxyapatite scaffolds loaded with mesenchymal stem cells offers the surgeon the possibility to enhance osseous defects healing. Results documented in this study regarding the osteoconductive capacity of autologous periosteal flaps represents a promising, easy and real alternative method to improve the results of reconstructive orthopedic surgery. Further investigation must be made to establish the critical defect size in which the healing process conducted by periosteuem remains efficient.

CONCLUSIONS

In osseous guided regeneration on dog, autologous periosteum has similar capacity with autologous mesenchymal stem cells loaded on hydroxyapatite scaffolds, the only difference being the exuberant proliferation of osseous tissue over cortical bone surface in the case of scaffolds.

The healing process evolution on control group proves the reduced capacity of osseous tissue to heal the defect with large substratum loss without a specific treatment.

REFERENCES


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