EVALUATION OF OSTEOGENIC CAPACITY OF THE PERIOSTEUM IN COMBINATION WITH A COLLAGEN MEMBRANE

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ABSTRACT

Objective: The present study evaluated the osteogenic potential of free periosteum graft in combination with collagen membranes. Methods: Twenty white Wistar rats aged 100 days underwent surgery to create a bone defect measuring 2.5-3.0 mm in length in the diaphysis of the fibula. After thirty days, the animals were divided into two groups. Group I received periosteum along with a collagen membrane, while Group II received only a collagen membrane. The animals were X-rayed before the implant surgery and 15 or 30 days post-operation. Results: The results demonstrated that free periosteum graft in combination with collagen membranes was not efficient in the repair of bone defects. Conclusion: We suggest that nonvascularized periosteal grafts do not show potential to form new bone, and that making the implant 30 days after the creation of the bone defect may have interfered negatively in osteogenic process.

Keywords: Bone regeneration. Wistar rats. Transplantation.

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INTRODUCTION

Bone regeneration is an extremely important and extensively studied subject. In the regeneration process, it is very important to study the conditions under which there is bone loss, such as those resulting from traumas, tumors or infectious processes, very common conditions in the human body. Defects of large dimensions do not normally undergo spontaneous repair, due to soft-tissue intervention between the fracture stumps.[1,2]

The therapies of GTR - guided tissue regeneration or GBR - guided bone regeneration were initiated in an attempt to resolve this problem. Due to its properties, GTR has been used extensively as a technique in the treatment of bone defects. Biodegradable membranes are of considerable interest, as they serve to avoid a subsequent surgical stage for membrane removal. The most noteworthy of these membranes are those made of polyglactin 910 (Vicryl)® and the collagen membranes, materials degraded slowly and, apparently, without causing aggressive tissue reactions.[3,4]

Although there are several materials for bone replacement (such as bone of bovine origin or hydroxyapatite), autogenous bone grafts are often used as the material is gathered from the actual patient.

In order to start a fracture repair process, the cell remnants and matrix remnants that were broken should be removed through the action of macrophages. At the same time, periosteum and endosteum respond with intense proliferation, producing a tissue very rich in osteoprogenitor cells that form a necklace around the fracture and that will penetrate between the fractured bone ends. This process evolves in such a way that a bone callus appears after some time, and is finally completely replaced by secondary bone. It is emphasized that all these stages are regulated by biochemical and biophysical factors that should be taken into consideration, such as the presence of chemical substances in the region, released by cells (platelet factors, factors released by bone cells, mediators of inflammation at the site, especially the prostaglandins etc.), bioelectricity in the tissues involved, vascularization of the region, presence or absence of oxygen and other factors.[5,6]

The periosteum consists of multipotential mesodermic cells, with the ability to form all the varieties of connective tissue, presenting great osteogenic potential, but also serving for use to promote

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cartilage formation when in a chondrotrophic environment (with little oxygen).[7-10] Due to these properties, the periosteum has been employed in several forms of graft where there is the need for replacement of skeletal tissues, such as fractures with bone loss, unconsolidated fractures, pseudoarthroses, congenital bone defects and lesions of the articular surfaces.[11]

Although used clinically, the experimental results of the osteochondrogenic capacity of the periosteum are contradictory and conflicting. The contradictions and the different results obtained in the experiments appear to result from a series of factors that may qualitatively and/or its quantitatively influence its osteogenic capacity.

Studies have indicated that periosteal flaps obtained from different bones of the skeleton can produce a larger or smaller quantity of bone when used in the form of grafts and blood supply seems to be one of the preponderant factors in the survival and proliferation of cells from the cambial layer of the periosteum (osteogenic layer) and consequent bone formation.[12,13]

There are three places where periosteal flaps should be obtained: from the iliac fossa, pedicled to the deep circumflex iliac artery; from the distal third of the humerus, pedicled to the deep artery of arm; and from the distal third of the femur, pedicled to the descending genicular artery.[14] Another relevant factor is the decrease of the osteogenic potential of the periosteum with age.[7] The repair of fractures in young individuals is faster and more effective than in adult individuals, since the soft tissues have excellent regeneration capacity and the periosteum can induce the bone neoformation.[15]

Experimental studies on rat fibulas demonstrated that fractures with bone loss, produced between the proximal and medium thirds of the bone, present spontaneous regeneration in defects of non-critical size (2.5 mm or smaller). However defects of significant extension or of critical size (larger than 2.5 mm) do not allow spontaneous reconstitution, requiring the implantation of barrier membranes, or even periosteal fragments, inducing the regeneration process.[16-18]

With a basis on these studies, this paper was aimed at evaluating the osteogenic capacity of periosteal grafts, taken from the anteromedial surface of the distal third of the femur, combined with a collagen membrane. The grafts were implanted in bone defects of considerable extension created in rat fibulas.

MATERIAL AND METHODS

Twenty female white Wistar rats averaging 100 days of age were used in the study. The resection of a 2.5 to 3.0mm fragment of the diaphysis of the right and left fibulas was performed on all the animals at the point where the proximal third joins the medium third. The study was approved by the committee of ethics in research of UNIFAL-MG.

Thirty days after the surgery, the animals were radiographed and divided into two experimental groups each containing 10 animals. In group I, the periosteal graft measuring about 7x5 mm, and taken from the anteromedial face of the distal third of the homolateral femur associated with a fragment of a collagen membrane of the Pro-Tape (Pro-Line) brand measuring 9x6mm was performed on the left hind limb. The animals from group II only underwent implant of the collagen membrane, also in the left hind limb. The right fibula, where the periosteal graft was not performed, served as a control group, to verify whether spontaneous regeneration occurs or not.

The animals were radiographed and euthanized 15 or 30 days after the second surgical procedure, with an overdose of the anesthetic used in the surgeries performed in this procedure.

To radiograph the animals, size 2 (simple pellicle) periapical Kodak Insight dental film was used, with an exposure time of 0.8 seconds and power of 60Kv, through a Radio Esfera (Siemens) model intraoral X-ray unit, revealed in an A/T 2000 Plus (Air Techniques) automatic processor.

The data obtained were analyzed statistically by the Chi-squared test for comparison of proportions at a significance level of 10% (0.10).

SURGICAL TECHNIQUE

For the creation of the bone defects, the animals were anesthetized by means of an intraperitoneal injection of a mixture of ketamine chlorhydrate (Ketalar® - 50 mg/ml) and xylazine hydrochloride (Rompum® - 1g/100ml) in the proportion of 1:1 and in the dose of 0.15 ml/100g of weight. Trichotomy and asepsis with PVPI were performed at the surgical site. A 3cm long incision was made on the lateral side of the animals' legs (Figure 1A), separating the subcutaneous tissues and carefully detaching the muscles from the fibula. (Figure 1B) With the help of cuticle nippers a bone defect was created in the medium third of the fibula, taking care to cause the least trauma as possible. (Figure 1C) The muscles and subcutaneous tissues were repositioned and the skin sutured. All the surgical procedures were carried out under irrigation with a sterile physiological solution.

A 3.5cm long incision was made on the medial side of the animal's thigh for obtainment and transplantation of the periosteal graft. The muscles were separated and the femur exposed. (Figure 2A) Using a no. 15 blade, the periosteum was detached from the femur, taking care to firmly scrape the bone cortical to bring the osteogenic (cambial) layer of the periosteum with the graft. (Figure 2B). It was only at the end of the removal of the graft that the descending genicular artery, responsible for irrigation of the periosteum of this region, was sectioned (Figure 2A) The periosteal graft, measuring 7x5 mm, was kept in a sterile physiological solution until the implantation time. The muscles

Figure 1 – *Creation of the bone defect: (A) Incision on the lateral side of the leg; (B) Fibula exposed; (C) Bone defect.*

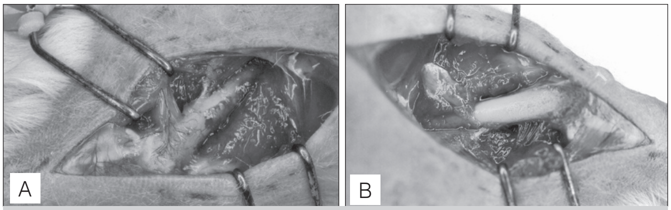

Figure 2 – *Obtainment of the periosteum flap: (A) Periosteum with genicular artery (arrow); (B) Periosteum detached.*

with their fascia and the skin were sutured. Soon afterwards, another incision was made on the lateral side of the animals' left leg; the muscles were separated to once again expose the bone defect of the fibula. The collagen membrane was taken between the fracture stumps, and the periosteal graft was placed on top of it with the osteogenic layer facing the center of the defect. (Figure 3A) The membrane with the periosteum were then rolled over each other and maintained by means of three stitches, one at each one of the ends and one in the central region of the membrane, with 901 (Vicryl) 6.0 polyglactin thread. Hence membrane and periosteal fragment covered the bone defect forming a "tube" at the site. (Figure 3B) The muscles were repositioned and the skin sutured.

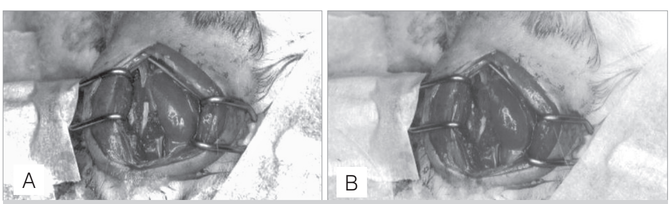

Figure 3 – *Periosteum implant: (A) Membrane positioned; (B) "Tube" of membrane and periosteum.*

RESULTS

The fractures were evaluated radiographically taking into consideration the reconstitution of the left (grafted) and right (non-grafted) fibulas. Those that presented full union between the fracture stumps were considered completely regenerated. (Figures 4A and 4B)

Figure 4 – *Radiographic aspect of a regenerated fibula (A) and of a non-regenerated fibula (B).*

It was observed that both among the animals that received periosteum in combination with the collagen membrane (group I - Table 1), and those that just received the membrane (group II - Table 2), comparing left fibula (grafted) and right fibula (not grafted) in the two groups, the regeneration rate was extremely low, and even lower in the animals from group I. (Table 1)

In group I (collagen membrane in combination with periosteum), of the five animals that were euthanized 15 days after the second surgery, only one presented complete regeneration. The other five, sacrificed at 30 days, did not present regeneration. (Table 3) Of the ten right fibulas analyzed, two were regenerated 30 days after surgery. (Table 1) None of these right fibulas corresponded to the same animal that presented regeneration of the left fibula.

In group II (collagen membrane only) there was complete regeneration in two of the five animals euthanized 15 days after the second surgery. In the five remaining animals, sacrificed at 30 days after surgery, only one appeared regenerated. (Table 3) Of the right fibulas analyzed in this group, three presented complete regeneration and seven did not experience regeneration. (Table 2)

Going by the simple observation of results (Tables 1, 2 and 3), we can see that there are no significant differences, which were verified by the statistical test used.(Tables 1, 2 and 3)

Table 1 – *Numerical result of the animals that received the collagen membrane in association with the periosteum (Group I).*

	Right fibula	Left fibula
With	2 animals	1 animal
Regeneration	20%	10%
Without	8 animals	9 animals
Regeneration	80%	90%
TOTAL	10 animals 100%	10 animals 100%

Table 2 – *Numerical result of the animals that received just collagen membrane (Group II).*

	Right fibula	Left fibula
With	3 animals	3 animals
Regeneration	30%	30%
Without	7 animals	7 animals
Regeneration	70%	70%
TOTAL	10 animals 100%	10 animals 100%

Table 3 – *Numerical result observed in the left fibulas of the animals sacrificed 15 or 30 days after surgery.*

	Group I		Group II	
	15 days	**30 days**	**15 days**	**30 days**
With **Regeneration**	1 animal	0	2 animals	1 animal
	20%	(zero)	40%	20%
Without	4 animals	5 animals	3 animals	4 animals
Regeneration	80%	100%	60%	80%
TOTAL	5 animals 100%	5 animals 100%	5 animals 100%	5 animals 100%

DISCUSSION

Several authors have concluded, through experiments, that the use of the periosteum in several forms of graft is capable of producing the proliferation and differentiation of its cells, with important and indispensable participation in the consolidation of fractures.[5,6,8,11] Even when kept without a blood supply for some time, the periosteum is able to conserve its osteogenic potential.[17]

In the results observed in this study, evaluating the osteogenic potential of the periosteum in combination with a collagen membrane, the free periosteum graft did not interfere in the bone defect repair process, perhaps as it is associated with the collagen membrane, which may have interfered in local revascularization of the graft and contributed toward low rates of regeneration. However, in defects of the fibula with loss of a large quantity of bone substance, periosteal grafts do not resist the pressure of the adjacent muscles and end up interfering mechanically in the regeneration.[17]

In our experiment, the periosteal graft was wrapped in a collagen membrane and implanted in the space between the stumps, in the shape of a "tube". The membrane was used to facilitate the handling of the periosteal graft and to prevent the adjacent muscle tissues from coming into contact with the graft. Use of the membrane may have somehow hindered further contact of the grafted periosteum with the periosteum existing at the extremities of the stumps, making revascularization difficult and interfering in its osteogenic potential. When the free periosteum graft is placed in an environment with little vascularization, it loses its osteogenic potential. This potential can be increased if the graft is put into contact with an intact periosteum.[13]

The use of collagen membranes is efficient in processes that call for the use of guided tissue regeneration and prevents the surrounding tissues from invading the bone defect or delaying or impeding fracture consolidation.[16] Collagen membranes were also used to isolate intramedullary implants performed in rabbit femurs, observing that in the animals where the membrane was applied there was a greater quantity of bone than in the animals that did not receive the membrane.[2]

In our study, we observed that the free periosteum graft was not able to help in the bone defect repair process. It is important to emphasize that all the animals used were young, discarding the "age of animal" variant as a possible justification for the inefficiency of the graft.[7,15]

Through the results obtained, it proved possible to observe that, in group I, one left fibula and two right fibulas appeared regenerated. We stress that these fibulas belonged to different animals. In addition, in group II, we emphasize that one of the 10 animals analyzed presented both fibulas (left and right) regenerated.

The graft implant surgeries were performed 30 days after creation of the bone defect. In some animals it was possible to observe that, instead of having regeneration of the proximal and distal stumps there was their reabsorption, especially in the case of the distal stump. This space of time, between the defect creation surgery and the second surgery, for implantation of the periosteal graft, may have interfered negatively in the osteogenisis process, inhibiting the osteogenic potential of the periosteum.

Studies indicate that there are some sites from where periosteal grafts should be obtained[14], one of which is the distal third of the femur (pedicled to the descending genicular artery); a site that we gave preference to in this study as it provides us with periosteal grafts of considerable size and thickness.

Positive results were obtained with the use of periosteal grafts, yet using re-vascularized grafts.[8,11] Through re-vascularized periosteal implants taken from the femur of rabbits, it was also observed that where vascular anastomosis was performed on the periosteal flap pedicles, there was the bone neoformation, while where the anastomosis was not performed, the graft degenerated and was absorbed. Hence the vascularization of the periosteal graft is essential for osteogenesis to occur.[12]

CONCLUSION

Through the methodology used to evaluate the osteogenic potential of free periosteum grafts in combination with a collagen membrane, we can conclude that:

1.The free periosteum graft was not efficient in the repair of a bone defect created in rat fibulas;

2.Use of the collagen membrane involving the periosteal flap interfered in the osteogenic potential of the periosteum;

3. The free periosteum graft (not vascularized) was not efficient when used late after the fracture, losing its osteogenic potential.

REFERENCES

1. Chakkalakal DA, Strates BS, Mashoof AA, Garvin KL, Novak JR, Fritz ED. Repair of segmental bone defects in the rat: an experimental model of human fracture healing. Bone. 1999;25:321-32.
2. Zhang X, Awad HA, O'Keefe RJ, Guldberg RE, Schwarz EM. A perspective: engineering periosteum for structural bone graft healing. Clin Orthop Relat Res. 2008;(466):1777-87.
3. Aaboe M, Pinholt EM, Hjørting-Hansen E. Healing of experimentally created defects: a review. Br J Oral Maxillofac Surg. 1995;33:312-8.
4. Rossi Junior WC. Influência de uma membrana de colágeno associada a extrato etanólico de própolis na consolidação de fraturas com perda óssea [tese]. Campinas: Universidade Estadual de Campinas; 2002.
5. Ham AW, Cormack DH. Histologia. 8a. ed. Rio de Janeiro: Guanabara Koogan; 1983. p.122-4.
6. Junqueira LC, Carneiro J. Histologia básica. 9a. ed. Rio de Janeiro: Guanabara Koogan;1999. p.122-4,.
7. O'Driscoll SW, Saris DB, Ito Y, Fitzimmons JS. The chondrogenic potential of periosteum decreases with age. J Orthop Res. 2001;19:95-103.
8. Xiao MZ, Huang XY. [An experimental study on the repair of the necrotic tubular bone with pedicled vascular periosteum grafting in rabbits]. Zhonghua Shao Shang Za Zhi. 2003;19:329-31.
9. Malizos KN, Papatheodorou LK. The healing potential of the periosteum molecular aspects. Injury. 2005;36(Suppl 3):S13-9.
10. Augustin G, Antabak A, Davila S. The periosteum. Part 1: Anatomy, histology and molecular biology. Injury. 2007;38:1115-30.
11. Doi K, Sakai K. Vascularized periosteal bone graft from the supracondylar region of the femur. Microsurgery. 1994;15:305-15.
12. Liu JY, Wang D, Cheng HH. Experimental study of the osteogenic capacity of periosteal allografts: a preliminary report. Microsurgery. 1994;15:87-92.
13. Reynders P, Becker JH, Broos P. Osteogenic ability of free periosteal autografts in tibial fractures with severe soft tissue damage: an experimental study. J Orthop Trauma. 1999;13:121-8.
14. Penteado CV, Masquelet AC, Romana MC, Chevrel JP. Periosteal flaps: anatomical bases of sites of elevation. Surg Radiol Anat. 1990;12:3-7.
15. Define D. A aplicação em cirurgia ortopédica do poder osteogenético do periósteo na infância. Rev Bras Ortop. 2002;37:415-23.
16. Caiazza S, Colangelo P, Bedini R, Formisano G, De Angelis G, Barrucci S. Evaluation of guided bone regeneration in rabbit femur using collagen membranes. Implant Dent. 2000;9:219-25.
17. Sant'anna MF. Capacidade osteogênica de enxertos periosteais livres [tese]. Botucatu: Universidade Estadual Paulista; 1997.
18. Steiner GG, Kallet MP, Steiner DM, Roulet DN. The inverted periosteal graft. Compend Contin Educ Dent. 2007;28:154-61.