Revascularization of Autogenous Block Grafts with or Without an e-PTFE Membrane

Andréa C. De Marco, DDS, MS[1]/Maria A. N. Jardini, DDS, PhD, MS[2]/Luiz A. P. A. Lima, DDS, PhD, LD, MS[3]

Purpose: *The aim of this study was to describe the revascularization process of autogenous bone block grafts placed with or without an expanded polytetrafluoroethylene (e-PTFE) membrane.* ***Materials and Methods:*** *Thirty Wistar male rats had their mandibles augmented by either an autogenous bone block graft (group A) or an autogenous bone block graft covered with an e-PTFE membrane (group B). The animals were sacrificed by perfusion at baseline, 3, 7, 14, and 21 days after surgery.* ***Results:*** *After 3 days, the presence of vascular sprouts derived from the recipient bed was observed in group A; more discrete sprouts were also observed in group B. After 7 days, revascularization continued, with vessels derived from both the recipient bed and the surrounding connective tissue in group A but only from the recipient bed in group B. At 14 days, group A showed penetration of vessels at the periphery of the graft; the vessels reached varying distances inside it. In group B, revascularization of the graft occurred mainly near its perforation, its borders, and at the recipient bed–graft interface. After 21 days, graft vascular penetration could be observed throughout the extent of the graft in group A but only approximately halfway through the graft in group B.* ***Discussion:*** *The results emphasized the importance of the vascular network and of the revascularization process of the autogenous bone graft in new bone formation. Early vascular penetration and nutrition of the graft are key factors in its integration with the recipient bed.* ***Conclusions:*** *Revascularization of the bone graft occurred in both A and B. However, vascular sprouts originated only from the recipient bed in group A, while in group B the graft was penetrated by vessels from both the recipient bed and the surrounding connective tissue. The revascularization took place more promptly and was more intense and extensive in group A than in group B for all periods.* INT J ORAL MAXILLOFAC IMPLANTS *2005;20:867–874*

Key words: *animal models, autogenous bone grafting, bone augmentation, e-PTFE membrane, guided tissue regeneration, revascularization*

With the advent of osseointegration, the use of implants has become widespread, and the successful results obtained have made this a reliable long-term treatment.[1–7] Limited bone availability, however, makes it difficult to ideally place implants from a functional and an esthetic point of view, which has led to the development of bone augmentation techniques.

The development of surgical techniques such as guided bone regeneration (GBR) has allowed reconstruction of alveolar bone in a predictable way. GBR involves using a mechanical barrier for protection of the area to be regenerated, thus avoiding the invasion of nonosteogenic tissue cells that proliferate more promptly than bone tissue. Predictable bone augmentation has allowed the placement of endosseous implants in the position that is most suitable, functional, and esthetically pleasing.

The GBR technique has been developed and improved to a point where it has become predictable. Studies demonstrating the advantages, disadvantages, and possibilities of treatment using GBR associated with autogenous grafts have been conducted in humans.[5,8,9] Autogenous bone has been indicated in the literature as the most desirable graft to be used with GBR.

The literature has long emphasized the importance of blood supply to the graft for favorable bone growth and graft substitution. Murray and colleagues[10] conducted a study in dogs which showed

[1]Postgraduate Student, Division of Periodontics, Dental Branch, University of São Paulo, São Paulo, Brazil.
[2]Research Fellow, Division of Periodontics, Dental Branch, University of São Paulo, São Paulo, Brazil.
[3]Associate Professor, Division of Periodontics, Dental Branch, University of São Paulo, São Paulo, Brazil.

Correspondence to: Dr Andréa C. De Marco, Rua dos Atuns 54, apto 134, Jd Aquarius, São José dos Campos, SP, Brazil 12246-320. Fax: +55 12 3923 8024. E-mail: fvcdemarco@directnet.com.br

that preservation of the blood clot and its contact with living tissue is essential in order for bone reconstruction to take place. The same principles were confirmed by Melcher and Dreyer[11] in rats. Successful repair of the graft depends directly on close contact between the graft and the vascularized tissue[12] and on the fixation of the graft to the recipient bed.[13–15]

Describing the biology of bone graft repair, Burchardt[16] emphasized that the recipient bed plays a fundamental role in the graft integration process. Successful grafting depends on a defined sequence of events—*(1)* osteoprogenitor cell proliferation, *(2)* osteoblast differentiation, *(3)* osteoinduction, and *(4)* osteoconduction—and on the biomechanical properties of the graft.

Among the morphologic aspects of autogenous bone graft repair, one of the most relevant is creeping substitution. Burchardt and Enneking[12] described it as a dynamic reconstructive and healing process of the graft, ie, a mechanism in which necrotic bone within the graft is gradually resorbed and replaced by new viable bone. Creeping substitution is only possible because of the presence of adequate nourishment; the delivery of this nourishment is dependent on the revascularization process.

Considering the characteristics and properties of bone grafts, Hammack and associates[17] conducted a study in rats aimed to determine the time intervals needed for blood vessels to penetrate the periosteal region following the placement of autogenous bone grafts. After 2 days, proliferation of bone sprouts could be seen, and by day 10, a more intense and continuous revascularization process had begun to take place. A study carried out by Schmid and coworkers[18] established the significance of angiogenesis or revascularization in GBR. In their experiment, conducted in rabbits, an extensive blood vessel network originated from the calvarian bone and filled the spaces between the filler material used. These observations emphasized the significance of revascularization and its important role in GBR.

A study using a rat model was developed in the authors' laboratories[19] with the aim of analyzing quantitatively and qualitatively the early healing pattern of an autogenous bone block graft. In some cases, the graft was covered with an expanded polytetrafluoroethylene (e-PTFE) membrane. Sixty male Wistar rats had their mandibles augmented using an autogenous bone block graft either with or without a membrane. Animals were sacrificed at baseline, 7, 14, 21, and 45 days. Descriptive histologic and histometric analyses were conducted. The results for the no-membrane group showed bone loss during the observation period. In the membrane group, significant bone tissue gain was seen during the observation period.

In light of the available knowledge, a study of the revascularization of the autogenous bone-block graft with or without an e-PTFE membrane seems necessary to clarify the differences in the repair processes which led to the results obtained in the authors' previous study.[19]

MATERIALS AND METHODS

The present research protocol was approved by the Ethics Committee for Animal Research of the Biomedical Sciences Institute of the University of São Paulo (no. 13/2000).

Thirty Wistar male rats (ranging from 250 to 300 g) were selected. The animals were divided into 2 study groups: group A was to receive only an autogenous bone block graft, while group B was to receive an autogenous bone block graft covered with an e-PTFE membrane (W. L. Gore & Associates, Flagstaff, AZ).

For anesthesia, the animals were given xylazine (Rompum; Bayer, Leverkusen, Germany) and ketamine (Francotar; Virbac do Brasil, Roseira, SP, Brazil) in a 1:1 ratio (0.3 mL per 100 g body weight). After the animals were anesthetized, the skin of the donor and recipient areas was shaved and disinfected with 0.12% chlorhexidine digluconate. The calvarium was the autogenous bone donor area; the angle of the mandible was the recipient site.

To remove the bone graft from the donor area and to drill the holes, a drill was used at 800 rpm, cooled with constant irrigation with saline solution. The bone block was removed from the parietal bone using a trephine bur with a 3.8-mm internal diameter (Nobelpharma, Gothenburg, Sweden). This bone graft was then perforated with a ½-mm carbide bur (KG Sorensen, Barueri, SP, Brazil) used at low speed and cooled with saline solution.

The recipient area was also drilled with a ½-mm carbide bur to allow a green polyester 5-0 suture (Johnson & Johnson/Ethicon, Somerville, NJ) to pass through the mandible and the bone block. This procedure allowed the graft to be positioned in close contact with the bone surface of the mandible and stabilized.

In group B, the suture line was passed through the mandible, through the bone block, and through a 5.5-mm-diameter membrane so that the membrane completely covered the bone block. Cuts were always performed in the occlusive portion of the e-PTFE membranes (GT-10).

The muscle was sutured with 5-0 polyglactine 910 (Vicryl; Johnson & Johnson/ Ethicon). Following that, the skin was sutured using 4-0 black silk (Johnson & Johnson/Ethicon). In the donor area, the skin was

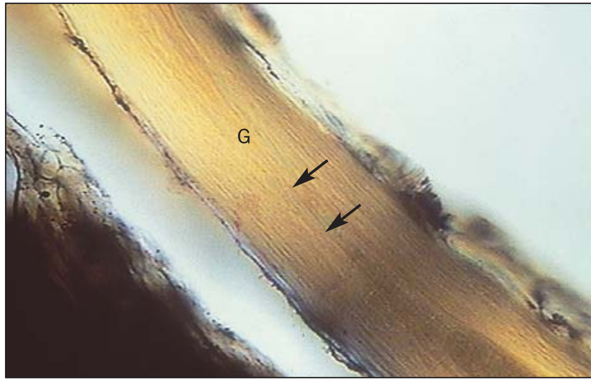

Fig 1a Group A specimen at baseline. A gap may be observed between the graft (G) and the recipient bed as a result of an artifact. The bone graft shows a lamellar structure *(arrows)* (original magnification ×100).

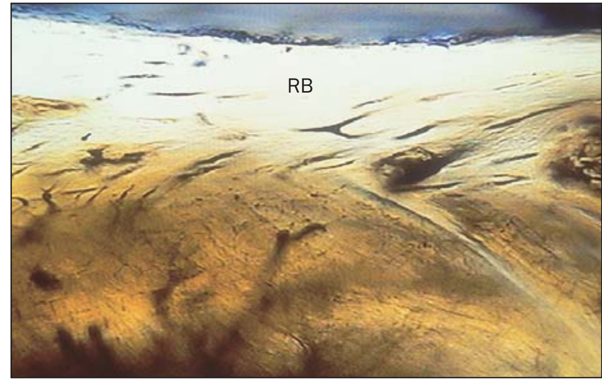

Fig 1b Group B specimen at baseline. Preservation of the vasculature of the recipient bed (RB) is demonstrated (original magnification ×100).

also sutured with 4-0 black silk (Johnson & Johnson/Ethicon). After surgery, the animals received a soft food and water ad libitum.

Sacrifice of the animals in both groups was made at baseline (immediately after surgery) and at 3, 7, 14, and 21 days postsurgery. For each period, 3 animals from each experimental group were sacrificed. At sacrifice, the animals received anesthetic as previously described. A transcardiac perfusion technique was then carried out by first injecting heparin (0.1 mL per animal) to avoid blood coagulation and then injecting a solution of equal parts 4% paraformaldehyde phosphate buffer fixing solution and India ink.

Histologic processing was carried out by immersion in 20% formic acid for approximately 2 to 4 weeks. The acid was replaced weekly. After complete decalcification, a cut was made in the center of the operated area to divide the specimen into 2 parts. One part was sent to routine histologic processing, including staining with hematoxylin-eosin, while cleared specimens were prepared with the other part. Sections 1 mm thick were made by cutting the specimens in a buccolingual direction. Subsequently, the sections were dipped in petri dishes containing methyl salicylate so that the vascular network could be observed.

Both types of sections, those stained with hematoxylin-eosin and the cleared ones, were examined under light microscopy. The most central cuts of each specimen were selected for analysis. The following aspects were evaluated: the recipient bed-graft interface; the main source of the newly formed capillaries in both groups A and B; the penetration of blood vessels into the graft; the features of the recipient bed and those of the graft; and bone resorption and bone forming areas. The microscope was connected to a camera and a computer to capture the images, which were used for the qualitative and descriptive analysis.

RESULTS

No clinical complications were observed following surgery. Thus, 30 animals provided 30 specimens for histologic processing.

Descriptive Histologic Analysis

At baseline, for both groups A and B, the cleared cuts showed dilation of the vasculature throughout the tissues surrounding the graft; however, no carbon black–marked vessels could be seen inside the graft (Fig 1a). In the recipient bed, the larger blood vessels were directed toward the long axis of the mandibular bone (Fig 1b). The interface between the recipient bed and the graft showed a regular bone surface covered with a blood clot.

Revascularization

Group A. By the third day, there was dilation of the vasculature of the tissues surrounding the graft. It was possible to note discrete proliferation of capillary buds from the recipient bed toward the graft. Diffusion of the graft had occurred, as shown by carbon black–marked haversian system canals inside the graft (Fig 2). The bone structure of the graft presented parallel lamellae. By the seventh day, many vascular sprouts could be observed around the graft. In this phase, revascularization developed from the recipient bed and also from the surrounding connective tissue. Vascular penetration could be seen around the graft periphery as well as in its lateral edges, and areas marked by diffusion inside the graft could also be seen (Fig 3). By 14 days after surgery, the graft was connected to the recipient bed by newly formed trabecular bone, and graft revascularization was quite advanced. New capillaries had migrated from the surface of the recipient bed and penetrated into the graft to varying degrees. These

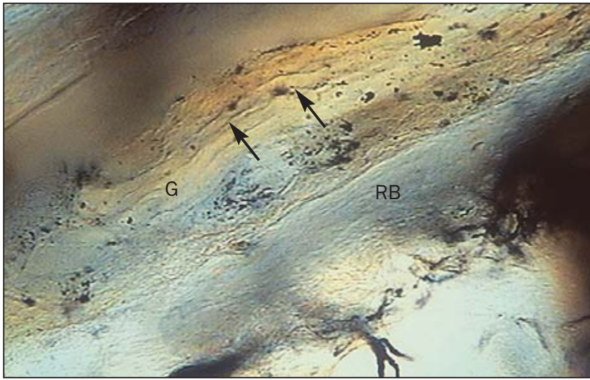

Fig 2 Group A specimen at 3 days. The Haversian system canals of the graft (G) are demarcated by carbon black suspension. The bone graft shows a lamellar structure *(arrows)*.The supraperiosteal plexus that provides nourishment to the internal cortical envelope of the recipient bed (RB) is preserved and shows dilated vessels (original magnification ×100).

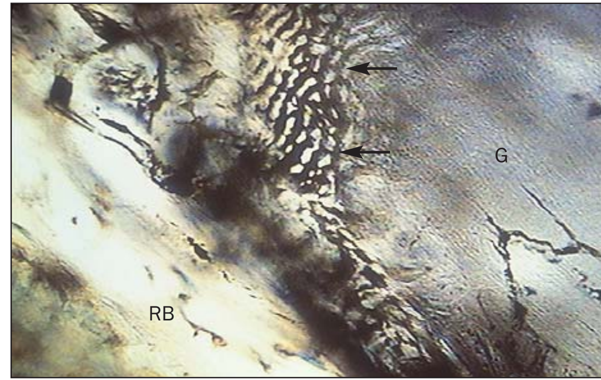

Fig 3 Group A specimen at 7 days. Observe the vascular plexus formed adjacent to the border of the graft (G) and the penetration of its vessels into the graft *(arrows)*. RB = recipient bed (original magnification ×100).

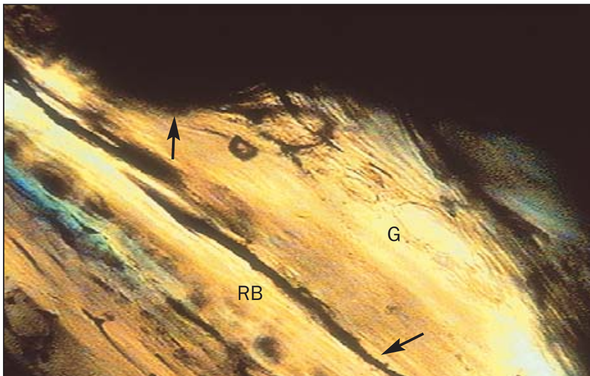

Fig 4 Group A specimen at 14 days. Notice the vessels *(arrows)* penetrating the graft (G) periphery. RB = recipient bed (original magnification ×100).

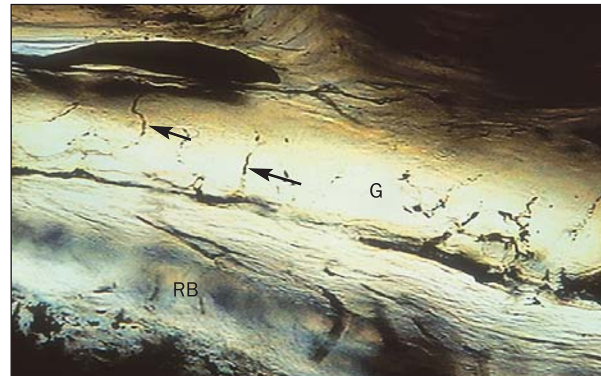

Fig 5 Group A specimen at 21 days. Many capillaries *(arrows)* originating from the recipient bed (RB) and from the surrounding connective tissues penetrated the graft (G) (original magnification ×100).

capillaries, which were formed from previously existing blood vessels, originated from both the surrounding connective tissues and the recipient bed (Fig 4). By day 21 after surgery, the process of revascularization of the graft had progressed further. Many blood vessels had penetrated the graft and extended across the entire graft (Fig 5). Organization of the supraperiosteal plexus over the graft could be observed.

Group B. By 3 days, several vascular sprouts that proliferated toward the graft from the recipient bed could be seen. Revascularization was more intense in the area near the perforation of the recipient bed (Fig 6). No carbon black demarcation was observed in the upper part of the graft, which was in close contact with the membrane surface. After 7 days, vascular sprouts from the surrounding connective tissue did not reach the graft because of the barrier membrane. At this time, the capillaries that migrated in the direction of the graft originated only from blood vessels of the recipient bed. The number of new capillaries formed was greater near the perforation of the recipient bed. Blood vessels from the supraperiosteal plexus of the lingual side of the mandible were dilated, and new capillaries had formed. By 14 days, several blood vessels could be seen inside the graft. The periphery of the graft had been penetrated; some of these vessels reached as far as the middle of the graft. A large number of new blood vessels could be found coming from the recipient bed toward the graft (Fig 7a). Larger blood vessels were observed in the region near the perforation for the fixation of the graft. Vascular penetration in this area originated from the recipient bed and reached almost the entire extension of the graft (Figs 7b and 7c). On the other hand, areas with complete absence of blood vessel penetration could also be seen, despite the fact that vascular sprouts were proliferating toward the graft. Penetration of new capillaries from the recipient bed into the graft could be seen at the lateral borders of the graft (Fig 8). By day 21 after surgery, because of the presence of the membrane,

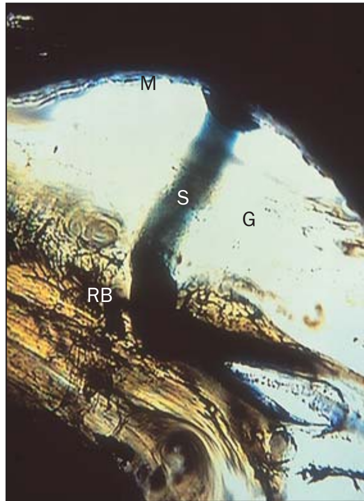

Fig 6 Group B specimen at 3 days. Recipient bed (RB), graft (G), and membrane (M) can be observed along with the suture (S). Vessels of the recipient bed were dilated, and many new capillaries migrated from the recipient bed in the direction of the graft (original magnification ×40).

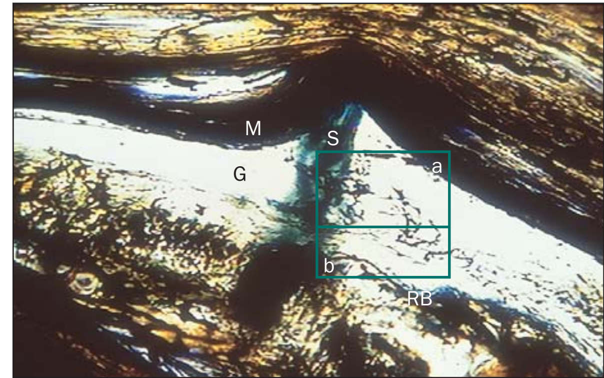

Fig 7a Group B specimen at 14 days. Revascularization of the graft was faster near the perforation of the recipient bed and the graft. G = graft; RB = recipient bed; M = membrane; S = suture. Regions *a* and *b* will be shown at a greater magnification (original magnification ×40).

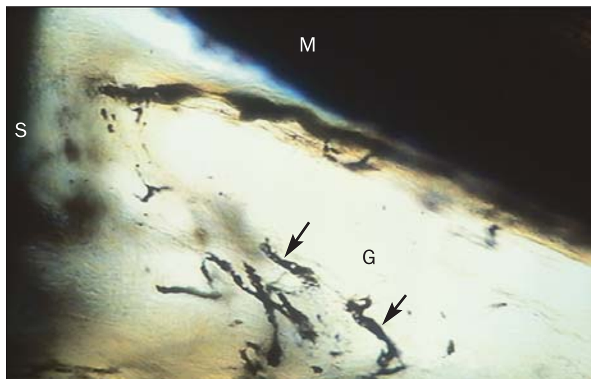

Fig 7b Higher magnification of region *a* from Fig 7a. Vessels *(arrows)* penetrate the width of the graft (G) and reach the surface of the membrane (M). S = suture thread (original magnification ×100).

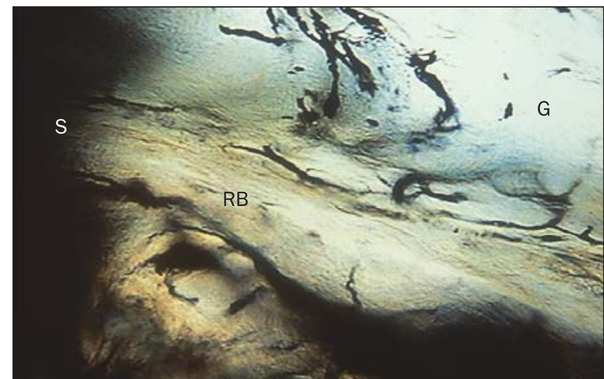

Fig 7c Higher magnification of region *b* from Fig 7a. Vessels originating from the recipient bed (RB) penetrated the graft (G). S = suture thread (original magnification ×100).

newly formed vessels penetrated the periphery of the graft, with the exception of the surface covered by the membrane. Revascularization was more advanced in the regions where the bone had been perforated for fixation of the graft and in the lateral borders of the graft. Near the perforation, a vascular network of vessels of different dimensions could be seen (Figs 9a and 9b).

DISCUSSION

The process of revascularization of autogenous block grafts occurred uneventfully for both groups A and B. However, the origin of the vessels was different. While vascular sprouts originated only from the recipient bed for group B, in group A vessels sprouted from both the recipient bed and the surrounding connective tissue. The revascularization process took place more promptly and was more intense and extensive in A than in B for all experimental periods.

The results for the 3-day specimens from Group A are in accordance with those presented by Hammack and associates[17] for specimens at 2 days. In both cases, marked blood vessels could be seen in the periosteal region and discrete vessels could be seen in the recipient-bed surface facing the graft. At this stage, diffusion of the graft had occurred in A, but was not evident in B. Apparently, in group B the diffusion process reached the lower part of the graft, which faced the recipient bed, but not the upper

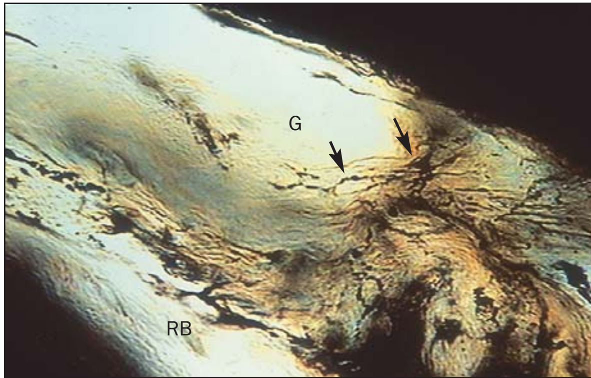

Fig 8 Group B specimen at 14 days. Observe the presence of vessels demarcated inside the graft (G) and the penetration of new capillaries into the graft through its lateral border *(arrows)*. RB = recipient bed (original magnification ×100).

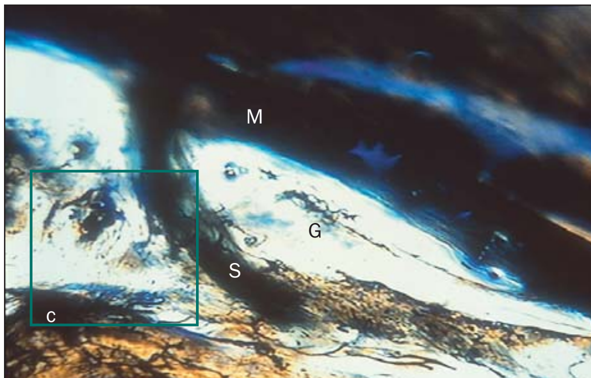

Fig 9a Group B specimen at 21 days. Notice the presence of the membrane (M). Vessels of different dimensions penetrate the graft. RB = recipient bed; G = graft; S = suture thread (original magnification ×40).

Fig 9b Group B specimen at 21 days. Higher magnification of region *c* from Fig 9a. Several small vessels *(arrows)* can be observed inside the graft. S = suture thread (original magnification ×100).

part. This finding may be explained by the fact that in group B, the membrane lay over the upper surface of the graft, separating it from the connective tissue, while the lower part of the graft was in close contact with the newly-formed vascular sprouts that originated from the recipient bed.

Hammack and associates[17] showed that cortical grafts are not penetrated by blood vessels until the sixth day, which agrees with the results of group A described in this study, since by the seventh day, blood vessels from the recipient bed and the surrounding connective tissue were observed penetrating the graft. Nevertheless, it took 14 days in the B group for vascular penetration, which originated from the recipient bed, to be seen. It is interesting to note that although the revascularization process seemed somewhat slower in B than in A, the grafts were completely revascularized in both groups.

By 14 days, group A showed vascular penetration of the graft to varying degrees, while in group B the vessels were still penetrating the periphery of the graft, though some reached as far as the middle of the graft. The finding that revascularization of the graft in group B progressed further near the perforation for fixation of the graft to the recipient bed might indicate that perforation may favor faster healing results.

Twenty-one days after surgery, revascularization of the graft had reached its full extension in group A, and organization of the supraperiosteal plexus over the graft could be seen. In contrast, in the B group, the vessels had fully penetrated the graft only at the perforation of the graft made for fixation purposes. In light of the findings, it is reasonable to conclude that not covering the graft with a membrane favors faster revascularization. However, this could lead to faster resorption of the graft as well.[19] It may be that the vessels originated from the connective tissue, while penetrating and promoting revascularization of the graft, contribute to that resorption.

The importance of revascularization for new bone formation and for substitution of the graft was demonstrated in the work of Nathanson,[20] who observed a strong relationship between revascularization and osteogenesis in and around the grafts. The present study showed that an autogenous bone graft of intramembranous origin, whether covered with a membrane or left uncovered, goes through

revascularization together with remodeling and substitution of the graft, which results in integration of the graft and the recipient bed. This is in accordance with the findings of Alberius and colleagues,[14] who showed that intramembranous onlay grafts integrated better with the recipient bed and that the grafts were better preserved when covered by an e-PTFE membrane. The present results were also in accordance with findings that membrane coverage of the graft better preserves its volume.[19] It is interesting to note that although the process of revascularization of the graft in B was apparently slowed down by the presence of the membrane, the fact that only vessels originating from the recipient bed took part in the revascularization of the graft may favor its preservation.

The 7-day results suggest that woven bone formation is intimately related to the revascularization process. In this phase, Jardini and colleagues[19] (the present authors) showed the interposition of connective tissue between the recipient bed and the graft and the development of newly formed bone trabeculae from the recipient bed. In many cases, bone trabeculae formed bridges between the recipient bed and the graft. This showed that the presence of vascular sprouts from the recipient bed was intimately related to the development of new bone and the consequent formation of newly formed bone bridges uniting the recipient bed and the graft was associated with the penetration of blood vessels in the graft.

The process of substitution of the graft in group B was only clearly observed after 45 days, suggesting that revascularization of the graft developed continuously in that group.[19] In the present study, group A showed faster revascularization, which might have led to faster woven bone formation, but also to early resorption of the graft as in the study by Jardini and coworkers.[19]

To explain the fact that the graft was better preserved in the membrane group than in the no-membrane group, Jardini and associates[19] showed that the membrane group presented more gain in terms of bone area than the no-membrane. This bone volume preservation was attributed to the membrane, which favored more new-bone formation and protected the graft from resorption. It is interesting to note that regardless of these favorable aspects of membrane use, the present results have shown that 21 days is not enough time for complete graft revascularization to take place. However, the observation of a continuous bone gain after 45 days by Jardini and colleagues[19] suggests that this revascularization process will continue.

The results of this study have emphasized the importance of the vascular network and of the revascularization process of the autogenous bone graft in new-bone formation. Early vascular penetration and nutrition of the graft are key factors for its future integration with the recipient bed. Although the membrane seems to retard this process, it does not prevent complete revascularization and substitution of the graft. Furthermore, the membrane barrier is capable of preventing loss of bone graft volume. It seems that faster vascular penetration is not the only important feature for bone graft integration and maintenance of grafted bone. More studies should be carried out to further clarify the mechanisms involved in the early healing process that cause greater bone resorption when the graft is not protected by a membrane and more new bone formation when it is protected by a membrane.

CONCLUSIONS

Revascularization of the autogenous bone graft occurred in both groups. Vessels that penetrated the graft originated only from the recipient bed in Group B, whereas in Group A the graft was penetrated by blood vessels from both the recipient bed and the surrounding connective tissue. The revascularization process took place earlier and was more intense and more extensive in group A than group B in all experimental periods.

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REFERENCES

1. Brånemark P-I, Adell R, Breine U, Hansson BO, Lindström J, Ohlsson A. Intra-osseous anchorage of dental prostheses. I. Experimental studies. Scand J Plast Reconstr Surg 1969;3:81–100.
2. Brånemark P-I, Hansson BO, Adell R, et al. Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. Scand J Plast Reconstr Surg Suppl 1977;16:1–132.
3. Adell R, Lekholm U, Rockler B, Brånemark P-I. A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. Int J Oral Surg 1981;10:387–416.
4. Babbush C, Kent J, Misiek D. Titanium-plasma-sprayed (TPS) screw implants for the reconstruction of the edentulous mandible. J Oral Maxillofac Surg 1986;44:274–282.
5. Buser D, Brägger U, Lang NP, Nyman S. Regeneration and enlargement of jaw bone using guided tissue regeneration. Clin Oral Implants Res 1990;1:22–32.

6. Mericske-Stern R, Schaffner TS, Marti P, Geering AH. Peri-implant mucosal aspects of ITI implants supporting overdentures. A five-year longitudinal study. Clin Oral Implants Res 1994;5:9–18.
7. Leimola-Virtanen R, Peltola J, Oksala E, Helenius H, Happonen RP. ITI titanium plasma-sprayed screw implants in the treatment of edentulous mandibles: A follow-up study of 39 patients. Int J Oral Maxillofac Surg 1995;10:373–378.
8. Buser D, Dula K, Belser UC, Hirt HP, Berthold H. Localized ridge augmentation using guided bone regeneration. I. Surgical procedure in the maxilla. Int J Periodontics Restorative Dent 1993;13:29–45.
9. Buser D, Dula K, Belser UC, Hirt HP, Berthold H. Localized ridge augmentation using guided bone regeneration. II. Surgical procedure in the mandible. Int J Periodontics Restorative Dent 1995;15:10–29.
10. Murray G, Holden R, Roachlau W. Experimental and clinical study of new growth of bone in a cavity. Am J Surg 1957;93:385–387.
11. Melcher AH, Dreyer CJ. Protection of the blood clot in healing circumscribed bone defects. J Bone Joint Surg 1962;44-B: 424–430.
12. Burchardt H, Enneking WF. Transplantation of bone. Surg Clin North Am 1978;58:403–427.
13. Albee FH. Fundamentals in bone transplantation. JAMA 1923;81:1429–1432.
14. Alberius P, Dahlin C, Linde A. Role of osteopromotion in experimental bone grafting to the skull: A study in adult rats using a membrane technique. J Oral Maxillofac Surg 1992;50:829–834.
15. de Carvalho PS, Vasconcellos LW, Pi J. Influence of bed preparation on the incorporation of autogenous bone grafts: A study in dogs. Int J Oral Maxillofac Implants 2000;15:565–570.
16. Burchardt H. The biology of bone graft repair. Clin Orthop 1983;174:28–42.
17. Hammack BL, Enneking WF. Comparative vascularization of autogenous and homogenous-bone transplants. J Bone Joint Surg Am 1960;42-A:811–817.
18. Schmid J, Wallkamm B, Hämmerle CH, Gogolewski S, Lang NP. The significance of angiogenesis in guided bone regeneration. A case report of a rabbit experiment. Clin Oral Implants Res 1997;8:244–248.
19. Jardini MAN, De Marco AC, Lima LA. Early healing pattern of autogenous bone grafts with and without e-PTFE membranes: A histomorphometric study in rats. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005. In press. 2005;100:666–673.
20. Nathanson A. The early vascularization of an autogenous bone inlay into an artificial defect in the rabbit mandibula. Acta Otolaryngol 1978;85:135–148.