Osteopontin and Bone Sialoprotein Distribution at the Bone Graft Recipient Site

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Background: The area in close proximity to a bone graft is subject to marked remodeling activity, which may dramatically affect graft survival.

Objective: To specifically analyze the effects at the recipient bed–onlay graft interface.

Design: In 22 adult Lewis rats, bicortical grafts were positioned below the temporal muscle and subperiosteally over the parietal bone. The recipient bone was left intact or ground to remove the external cortical layer, thereby exposing the graft to the osteopontin cells of the bone marrow. The rats were killed after 4 or 20 weeks. The outcome was assessed by routine histologic examination and immunohistochemical labeling for 2 bone matrix proteins, osteopontin and bone sialoprotein, which are involved in bone resorption and formation, respectively.

Results: Placement of the grafts submuscularly or grinding of the outer cortical layer of the host bed increased recipient site resorption. Resorptive activity (labeling) was concentrated to a subzone below the surface of the recipient bone; neither the graft surface nor the interface soft tissues were labeled.

Conclusion: The successive loss of skeletal contour after bone grafting, in many cases, may largely result from recipient site failure rather than graft size reduction.


AUTOGENEIC BONE grafts are used in reconstructive craniofacial procedures to facilitate union between skeletal segments and to create improved contour and shape. In many respects, their predictability in terms of incorporation and volumetric persistence is still unclear. Multiple investigators have tried to find means to improve graft survival and operative success. Several factors have been identified that are considered to influence the long-term outcome, such as the embryonic origin of the graft,1,2 its extent of revascularization,1,3,5 its architectural features,1,3 and fixation.5,8

One factor of fundamental importance to this issue is the recipient site–graft interface and its acceptance of the new graft. Surprisingly, despite the numerous articles published in this field, this issue has frequently been neglected. Theoretically, an increased tissue pressure resulting from the introduction of a more or less voluminous graft may change the normal recipient bone remodeling response to a more aggressive bone resorption and bone loss. Large grafts in a nonresilient tissue environment would then promote bone resorption and negatively influence the ultimate and hoped-for result.

This investigation aimed to study the reactions at the recipient site after various bone grafting procedures. This was accomplished by descriptively analyzing the distribution of 2 noncollagenous phosphoproteins, osteopontin and bone sialoprotein (BSP), which are believed to be involved in the regulation of bone formation.

RESULTS

All animals tolerated the operative procedure without complications. All wounds healed uneventfully. Macroscopically, the bone grafts seemed incorporated into the recipient bone without evidence of infection or graft dislodgment.

HISTOLOGIC FINDINGS

At 4 weeks, integration of subperiosteal grafts to the recipient bed was most pronounced peripherally adjacent to the peri-

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MATERIALS AND METHODS

ANIMALS AND ANESTHESIA

Twenty-two male isogeneic adult Lewis rats, with a mean (SD) weight of 359 (34) g, were used. Four additional animals of identical size were used to obtain donor tissue. Sedation, anesthesia, and animal care were provided as described previously.9-13 The animals were killed 4 or 20 weeks after grafting. Institutional guidelines regarding animal experimentation were followed. All research protocols were approved by the Animal Ethics Committee at Lund University, Lund, Sweden.

SURGICAL PROCEDURE

The surgical protocol has been described previously.9,10 Briefly, identical-size bone blocks, 4 mm in length, from the femur and tibia (without their periosteum), were harvested from donors using a low-speed trephine mounted in a dental drill. During drilling, the surgical field was continuously irrigated with sterile saline to reduce thermal damage. A paramedian skin incision was made to expose the cranial vault. On one side, a fascial incision parallel to the temporal line was made, and a tight pocket was produced under the temporal muscle. On the contralateral side, a subperiosteal pocket was created. At some sites (Table), the recipient bed was gently ground under irrigation to expose the marrow of the recipient bed to an area corresponding to the size of the graft. All animals received 2 bicortical bone grafts, except for 2 animals that only received 1 graft (the contralateral side received unicortical grafts for a pilot study). The experimental design is presented in the Table.

HISTOLOGIC EXAMINATION

At autopsy, the bone grafts and the recipient bed were carefully excised en bloc without stripping away the soft tissues, immediately frozen in isopentane, and stored at −70°C. Six-micrometer sections were prepared using a cryostat. The sections were incubated with rabbit antibodies against proteins prepared from rat bone matrix, ie, BSP14 and osteopontin.15 Immunolabeling was performed with antiserum (diluted 1:50-1:200) from immunized rabbits.16 The sections were stained with hematoxylin-eosin, safranin O, and van Gieson stain. Also, a control specimen without antibody labeling was prepared. The specificity of the immunolabeling was checked as described by Hulth et al.18 All histologic examinations were performed by an investigator who was not informed about which specimens belonged to which group. The analysis focused on the recipient site, but the interface region and the graft contact area were also included. Detailed studies in identical circumstances on the specific maintenance of various onlay bone graft regimens have been reported elsewhere.9,13

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IMMUNOHISTOCHEMICAL FINDINGS

Osteopontin

Labeling was fairly uniform between groups. Submuscular grafts disclosed slightly more intensive labeling. In general, osteocytes were distinctly labeled. After 4 weeks, the soft tissue in the interface between the graft and host bed was almost nonlabeled, while the newly formed trabeculae and the undersurface of the graft revealed distinct labeling (Figure 1). After 20 weeks, the labeling pattern was identical to that described above, but less intensive (Figure 3). The recipient bone showed a nonlabeled outer zone, which demonstrated a lamellar structure but was dissimilar in appearance from the remaining external cortical layer. Below that, a zone of more intensive labeling was detected, its bone structure being more immature, with large osteum but was incomplete along the remainder of the graft-host interface. Areas of localized bone resorption were distributed over the host bed, but the graft’s surface facing the interface was mainly intact and seemed relatively nonreactive. Ground areas were fairly intact superficially, with new bone trabeculae reaching toward the interface region, while more resorptive activity, appearing like vacuoles, was found deeper in the recipient bone. Graft integration was markedly more pronounced in this group. Submuscular grafts were more integrated than the subperiosteal ones, but otherwise disclosed the same histologic pattern. The ground areas below submuscular grafts showed excellent bony union to the graft.
vascular channels. Most inferiorly (closest to the brain), the lamellar bone was vaguely labeled. In some specimens, small triangular tongues, with their base at the interface and demonstrating more labeling peripherally, were directed endocranially.

**Bone Sialoprotein**

Labeling generally showed low intensity (Figure 2 and **Figure 4**). The findings were almost identical regardless of group, except that submuscular groups disclosed slightly more labeling. The BSP was localized below the external zone of the recipient site, with activity concentrated in the central part and less activity more peripherally (Figure 4). No labeling was observed at the host bed’s surface (Figure 2). All osteocytes were distinctly labeled. Labeling was less pronounced after 20 weeks.

**COMMENT**

Onlay bone grafts are widely used in the restoration and augmentation of the craniofacial skeleton. However, an initial satisfactory result is sometimes followed by a less than optimal long-term outcome because of graft resorption or recipient site remodeling causing loss of contour and volume. Consequently, prognostic assessment of graft success is difficult, and various techniques and experimental protocols have been tested to improve the procedure; yet, the problem remains partly unsolved. The present study focused on the bone remodeling activities of the graft–host bed interface, and with the use of immunolabeling to localize 2 proteins believed to be important markers for various aspects of osseous response, a detailed analysis of the problem was expected.

In previous reports on onlay integration, sparse comments on the status of the recipient bed have been provided. For example, Goldstein et al noted intensive bone remodeling after 15 days in the recipient nasal dorsum of rabbits after placement of grafts from the zygomatic arch, as well as multiple areas of bone formation in both graft and host bed after 40 days. Ermis and Poole observed, after 16 weeks, resorption of the underlying mandible in most rabbits receiving bicortical iliac grafts. Simi-
larly, Phillips and Rahn\textsuperscript{5} described resorptive areas in the underlying mandible for nonfixed (but not fixed) rib and skull grafts in sheep after 20 weeks. Chen et al\textsuperscript{3} observed vigorous osteoclastic activity in the recipient surface 10 days after subperiosteal placement of unicortical iliac and calvarial rigidly fixed grafts to the rabbit snout. Lin et al\textsuperscript{5} postulated that once a graft becomes adherent enough to the recipient bone to resist outside mechanical forces acting on it, the type of fixation used would make little difference. This conclusion was based on the findings that fixed or nonfixed iliac unicortical and full-thickness calvarial grafts transplanted to the snout or femur in rabbits resulted in no significant difference in residual graft volume for neither host bed. Finally, Gosain et al\textsuperscript{11} observed conversion from a cortical to a trabecular bone structure in vascularized bone transfers and their recipient zygomas in rabbits after 1 year. Consequently, information is rhapsodic, incomplete, and contradictory, and further data are necessary to achieve a full understanding of this important issue.

The major constituent of bone matrix is hydroxyapatite, and the most abundant organic constituent is type I collagen. The extracellular matrix contains several non-collagenous proteins that appear to serve important functions in the regulation of mineralization, collagen fiber growth, and cell-matrix interactions.\textsuperscript{12} Osteopontin and BSP are the major phosphorylated proteins of mammalian bone, and both are produced by the osteoblasts. These proteins function in the initiation of mineralization, bind tightly to hydroxyapatite, and possess cell attachment activity via an RGD (arginin-glycine-aspartic acid) amino acid sequence.\textsuperscript{15} However, they are different in many respects.

Osteopontin is abundantly present in both membranous and enchondral bone, primarily in osteoblasts and osteocytes, but also in osteoprogenitor cells.\textsuperscript{22} It is less acidic than BSP and is present not only in bone but also in other tissues, such as the kidney, placenta, and some parts of the central nervous system.\textsuperscript{23} The protein presently is believed to have a dual function. First, it has been shown to recruit both osteoclast precursor cells and osteoclasts and to bind them to the mineralized matrix of bone.\textsuperscript{24,25} Second, the protein is enriched at the mineralization front in enchondral bone\textsuperscript{26} and is supposed to regulate the mineralization process by inhibiting calcification and crystal growth.\textsuperscript{27}

Bone sialoprotein is present only in bone and dentin.\textsuperscript{28} It is apparently restricted to osteoblasts in areas of initial bone formation.\textsuperscript{29,31} In vitro, it promotes nucleation and crystal growth\textsuperscript{32} and hence appears to be a marker of new bone formation. After the osteoblasts have been embedded in the mineralized matrix of enchondral bones as osteocytes, labeling is markedly reduced.\textsuperscript{33} Interestingly, the osteocytes of the membranous bones studied (the parietal and temporal bones) were distinctly labeled, even in the adult animals used.

In accord with the findings of our previous studies,\textsuperscript{9,10} in which immunolabeling of osteopontin and BSP were localized mostly to a bicortical graft’s interior during the investigation periods, in the present study we found labeling in general to be weak in the areas investigated. The recipient site, however, disclosed quite dramatic anatomical changes. Generally, it was markedly and quite rapidly reduced in height, while the contact surface of the graft appeared relatively nonreactive. Mostly, the outer surface (zone) of the host bed was intact and nonlabeled, although rich remodeling activity and bone protein labeling were observed in a subzone paralleling the surface. Seemingly, the resorptive activity is concentrated some distance away from the actual interface region. Also, in ground specimens, the endocranial surface was quite undisturbed and kept its lamellar structure. The resorptive activity therefore seems to be quite strictly localized. Both osteopontin and BSP labeling clearly supported this conclusion and highlighted the subzone activity. It is possible that the triangular bone formations observed in some specimens might participate in such a localized manner of transferring the resorative signals to the bone tissue interior.

The graft’s contact surface to the interface showed marked local remodeling and resorptive activity. This remodeling was more pronounced after submuscular placement of the graft, a finding that was apparent for both intervals. Maturation was slow for the bicortical grafts irrespective of placement. Surprisingly, full bone maturation was not observed in any group, but after 20 weeks reduced labeling was observed for both proteins investigated. This indicates that maturation of the recipient bone with decreased bone turnover was approaching.

The spectrum of successful graft incorporation ranged from the subperiosteally placed graft, which obtained improved integration after the recipient bed was ground, to the submuscular graft, with further improvement after grinding. This pattern was applicable for both intervals tested. Also, the early dramatic gain in bone production obtained after grinding was only temporary, as this bone was resorbed later. The reason for the divergent response between the submuscular- and subperiosteal-placed grafts is unclear. The influence of graft fixation was probably negligible; both placements clinically seemed quite stable during operation, and all grafts were fixed to the host bed at the end of the study.

The periosteum is a vascular membrane that consists of a fibrous layer and a cellular cambium layer. It gets less osteogenic with age.\textsuperscript{33,34} Interestingly, its osteogenic potential seems to be dependent on the type of bone (membranous or enchondral) being covered.\textsuperscript{35,36} Speculatively, the passive nature of the periosteum does not produce very much tension or pressure on an onlay graft. In contrast, a more or less continuously moving muscle must exert an intermittent stress, which should negatively affect graft size. However, to what extent this muscle activity will affect the integration per se is not known. LaTrenta et al\textsuperscript{7} emphasized the importance of 2 factors, apart from the physiologic stress placed on the graft, that affect the extent of bone graft resorption (and integration): the vascularity of the host bed and the recipient-to-host bone contact. The recipient-to-host bone contact was presumably identical with both graft positionings, while the extent of revascularization may have been different. As theorized by Ermis and Poole,\textsuperscript{38} the increase
in vascularity noted for submuscularly placed grafts may promote rapid and optimal graft incorporation. The graft resorption induced by stress implies that relatively larger grafts should be used when they are being placed below muscle, rather than subperiosteally positioned, to acquire the desired graft volume.

Different views prevail concerning the importance of revascularization for survival and volumetric maintenance of free bone graft may hamper its resorption, while the richly vascularized calvarial bone, constituting the recipient site, is more vulnerable to pressure and local stress. This conclusion agrees with Albrektsson’s observation that neither osteogenesis nor resorption occurs before a bone is revascularized. Consequently, recipient site failure rather than graft volumetric changes may be a logical explanation in many cases of loss of skeletal contour after bone transplantation. Furthermore, the positive impact on graft integration that was observed after grinding secondarily facilitated graft revitalization and, in turn, resorption, which induces doubts as to its long-term benefits.

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REFERENCES


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