Eur Spine J (2001) 10 : S110–S113
DOI 10.1007/s005860100273

ORIGINAL ARTICLE

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Influence of bone environment on ceramic osteointegration in spinal fusion: comparison of bone-poor and bone-rich sites

Received: 30 January 2001
Accepted: 15 February 2001
Published online: 13 April 2001

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Abstract Quantitative experimental data showed differences in bone quality and ceramic incorporation between bone-rich and bone-poor implantation sites. Bone in-growth was significantly lower for ceramic implanted at a lumbar intertransverse than a laminar site. Bone-marrow enrichment of the lumbar intertransverse site (regarded as bone-poor) greatly facilitated ceramic osteointegration. The vertebral interbody site, despite theoretical richness in osteogenic precursor cells, might be bone-poor at the time of grafting as compared to the reference iliac crest site. These data have important clinical implications concerning the potential benefit of enriching both bone-poor and bone-rich sites.

Keywords Bone substitutes · Spinal fusion · Calcium phosphate ceramics · Osteogenic precursor cells

Introduction

The biological processes of ceramic osteointegration depend partly on the quality of host bone surrounding bone graft material [6]. The ideal recipient site should be conducive to osteoconduction, osteoinduction and osteogenesis [6]. In practice, these general conditions require either extensive contact or a small space between local host bone and bone substitute in the presence of viable osteogenic precursor cells. These conditions relate to surgical preparation of the recipient site, i.e. the quality of local decortication, the technique of bone substitute positioning and the intrinsic state of the implantation site (quantity of host bone surface available, local richness in osteoprogenitor cells, etc.).

Thus, the potential for bone growth into a bone graft substitute may differ depending on whether the material is on the lamina in contact with host bone or positioned laterally close to the transverse process and surrounded mainly by muscle tissue. Moreover, the addition of progenitor cells from a bone-marrow puncture to graft materials, i.e. enrichment of the graft area with osteogenic cells, may improve the outcome of bone substitute osteointegration [2, 8].

In cases of spinal fusion, the clinical validation of these basic concepts is not very apparent. The present study, based on experimental data from the literature and personal work, attempted to quantify the differences between bone-rich and bone-poor implantation sites in terms of bone quality and ceramic incorporation. The main considerations were the differences between laminar and intertransverse sites at the lumbar level, the benefit of enriching the poor intertransverse site with bone-marrow aspirate, and the richness in osteoprogenitor stem cells at the rich vertebral interbody site as compared to the reference iliac crest site.

Laminar versus intertransverse process site

The posterolateral lumbar fusion site can be divided into two areas with respect to the local biological and mechan-

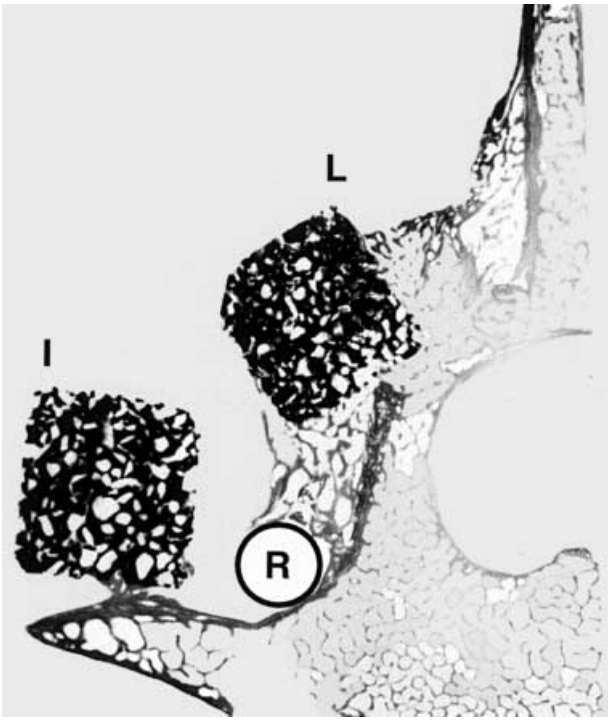

Fig. 1 Juxtaposed microradiographs of ceramic blocks at the laminar (*L*) and intertransverse (*I*) process sites. (*R* indicates location of the rod)

ical environment: a laminar area associated with the facet joint and an area between and including the transverse processes. This distinction is clinically relevant, since the intertransverse site constitutes the poorer bone environment and is often used alone as a surgical site, given the frequency of bilateral laminectomy combined with facetectomy.

In a previous study [4], we evaluated bone growth into calcium phosphate ceramic in a canine model with respect to localization on the lamina or transverse process sites of the lumbar spine. This model represented a clinically relevant setting for the use of pedicular instrumentation and the achievement of posterolateral arthrodesis. The ceramic used was a macroporous biphasic calcium phosphate (40% β-tricalcium and 60% hydroxyapatite) in parallelepipedic block form implanted into both laminar and intertransverse sites, from L2 to L4, on eight dogs. The animals were sacrificed 9 months after surgery.

In the present study, the local environment was taken into account in characterizing each site, i.e. in determining whether bone contact occurred for each side of the square section of the block. Bone in-growth was determined as a function of the local environment by comparing the laminar and intertransverse sites as well as each block side in contact or not with bone within each site.

Analysis within each site confirmed the influence on bone in-growth of close contact between macroporous osteoconductive material and host bone. Ceramic block sides in direct or close contact with the bone of lamina or intertransverse processes exhibited the greatest amount of new bone formation (Fig. 1). These results indicate that a suitable surgical technique requires that ceramic blocks be in contact with host bone.

The comparison of laminar and intertransverse sites showed a significant difference in bone formation. The 9% lower bone growth at the intertransverse site represented nearly one-third of the total amount of bone growth into ceramic at the laminar site (17% vs 26%). This difference, which may have potential clinical relevance, suggests that bone substitutes for posterolateral arthrodesis should be evaluated separately for each site, since good results for the laminar site are not necessarily valid for the intertransverse site. Finally, the intertransverse site appears to be bone-poor, and could benefit from enrichment with osteogenic precursor cells obtained, for example, from an iliac crest puncture.

Addition of bone marrow to a bone-poor intertransverse process site

The osteogenic potential of marrow-derived cells has been largely demonstrated [2, 9]. However, only a few experimental studies have considered the application of bone marrow to the spine as a useful adjunct to enhance rates of bone formation and fusion [1, 3, 5].

In a rabbit posterolateral fusion model grafted with autogenous iliac crest bone, Curylo et al. [3] quantified the effect of bone-marrow enhancement of spinal arthrodesis. The measured mean bone volume determined from computed tomography images was almost 30% greater when bone marrow was added to autogenous iliac crest bone. The fusion rate was achieved in 61% of cases versus 25% in the control group.

Lindholm et al. [5], who added bone marrow obtained by aspiration from the femoral medullar cavity to demineralized bone matrix in a posterior thoracic spinal fusion model in rabbits, found that transplantation of both materials provided more rapid fusion than that of demineralized bone matrix alone.

The association of ceramics with bone marrow has shown osteogenic potential in in vivo situations, e.g. in a rat model in which a combination of bone-marrow cells and calcium phosphate ceramic allowed bone formation in extraosseous sites and also potentiated bone in-growth in osseous sites [8]. It has also been shown in vitro that human marrow-derived cells cultured on macroporous calcium phosphate ceramic express and conserve their osteoblastic phenotype, and that these osteogenic cells are able to form new bone matrix in a ceramic in vitro [10]. Macroporous calcium phosphate ceramics are a main vector associating a mineral volume beneficial to mineralization with a wide specific surface allowing attachment of a large number of cells.

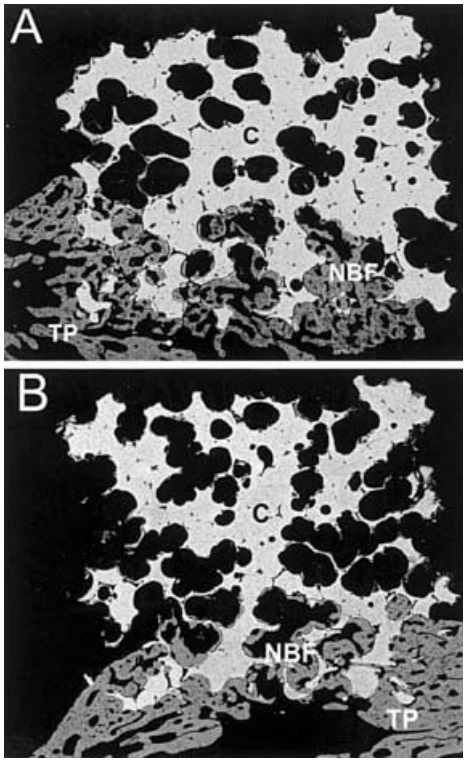

Fig. 2 Scanning electron photomicrographs: transverse sections of calcium phosphate ceramic blocks (*C*) loaded with autologous bone-marrow aspiration (*A*) or grafted alone (*B*) on transverse processes (*TP*). Bone-marrow loading obviously increased new bone formation (*NBF*)

However, Boden et al. [1] found that the use of a ceramic corallin hydroxyapatite with bone marrow did not provide an acceptable bone graft substitute (no solid fusions) in a posterolateral lumbar arthrodesis rabbit model.

In the present study involving a biphasic calcium phosphate ceramic (40% β-tricalcium and 60% hydroxyapatite), the enhancement of bone formation was quantified after addition of bone marrow in an original rabbit intertransverse process lumbar fusion model. Three graft materials were compared: iliac bone grafts (controls) and ceramics, associated or not with a standardized bone-marrow puncture. The originality of this method consisted in performing two independent intertransverse grafts by two independent lateral approaches and stabilizing the spine with posterior instrumentation to minimize movements that might cause non-union. With the transverse process, the graft materials were also secured by a suture.

Ceramic parallelepipedic blocks were randomly loaded with bone-marrow cells. After 6 weeks, the fusion rate was assessed by a manual test and determined for each interface between transverse processes and graft materials. Bone formation was compared on three sections for each specimen using quantitative histomorphometry performed in the coronal plane at the level of the two decorticated transverse processes and in the median area. As compared to ceramic block alone, the rate of new bone formation into ceramic loaded with bone marrow was significantly increased (50%) when the three sections were considered for each block (Fig. 2). The manual palpation test, when applied to each transverse process/graft material interface, showed that fusion was achieved in 91.6% (22/24) of the intertransverse sites in the control group and 92.5% (37/40) in the ceramic group, with no significant difference between blocks loaded or not with bone marrow. However, vertebral fusion was not achieved for any grafts with ceramic alone, as all the blocks were fractured in the median area.

Although these results do not indicate that biphasic calcium phosphate ceramic associated with bone marrow is a completely suitable graft substitute for lumbar fusion, they confirm that the addition of bone marrow to ceramic can greatly facilitate bone formation at a bone-poor (e.g. intertransverse) fusion site.

Richness in osteoprogenitor cells at the vertebral interbody site as compared to the reference iliac crest site

The vertebral interbody site surrounded mainly by spongy bone was presumably bone-rich. The potential richness of this site in osteogenic precursor cells suggests that ceramic material for implantation would not require addition of a bone-marrow puncture from the reference iliac crest site.

The number of alkaline phosphatase-positive colony-forming units was used to estimate the amount of osteoblast progenitor cells contained in bone-marrow aspirates [9] obtained from vertebral interbody and iliac crest sites in the same patient during 25 anterior spinal surgery cases for scoliosis treatment. A syringe containing heparin was used to collect 2–3 ml of sample from the medullary cavity of the iliac crest and the cancellous bone of the vertebral body reached through the interbody space after removal of the disk and subchondral bone. The bone-marrow samples were placed in tissue-culture medium, and the number of alkaline phosphatase-positive colony-forming units was counted after 9 days of culture, using appropriate staining.

No correlation was found between richness in osteoprogenitor bone-marrow cells at the iliac crest and vertebral interbody sites. In most cases, a large difference was observed between the two sites within the same patient. In some cases, the vertebral interbody site was poorer in osteoprogenitor bone-marrow cells.

The number of osteoprogenitor cells from a puncture may vary for different reasons. In histological terms, the number of nucleated cells may increase if local bone marrow is highly cellular and loosely connected, allowing bone-marrow cells in marrow space to flow into the aspiration needle [7]. Apparently, no histological studies have

documented differences in cellularity and cancellous bone architecture between iliac crest and vertebral body sites. However, it has been reported that the concentration of bone-marrow-derived cells obtained by puncture depends on the rate of dilution with local blood [7]. The rate at which dilution occurs probably depends at least on the density of the local vessels within cancellous bone. Muschler et al. [7] noted that variation in the dilution rate is due primarily to puncture volume, since contamination by peripheral blood becomes greater as aspiration volume increases. In our study, the dilution effect was partly minimized, as approximately the same volume was collected from both sites.

The differences between the two sites in the same patient could have been due to another cause of blood contamination. As the puncture within the vertebral interbody site was made in spongy bone below the decorticated osseous surface, bleeding may have modified the cell content of the surrounding spongy bone.

In terms of grafting conditions, our results are probably more indicative of cell richness at the interbody site than in the vertebral body itself. Moreover, the vertebral interbody site might be bone-poor at the time of grafting. Therefore, from a clinical point of view, it would seem advisable to perform an iliac crest puncture even when ceramic is implanted in an interbody site, in order to provide a potentially abundant and readily available source of osteoprogenitor cells.

Conclusion

The benefit of adding a bone-marrow puncture from the iliac crest to ceramic is two fold. First, it serves in all cases as a binding medium between ceramic granules, making them easier to handle and place in the graft site. More specifically, it maximises the number of viable osteogenic precursor cells at both bone-rich and bone-poor sites, especially since a site theoretically rich in bone cells could be poor at the time of implantation because of local bleeding.

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