Autograft versus allograft with or without demineralized bone matrix in posterolateral lumbar fusion in rabbits

Laboratory investigation

**JULIO URRUTIA, M.D., NICOLAS THUMM, M.D., DANIEL APABLAZA, M.D., FELIPE PIZARRO, M.D., ALEJANDRO ZYLBERBERG, M.D., AND FELIPE QUEZADA, M.D.**

Department of Orthopaedic Surgery, Pontificia Universidad Catolica de Chile, Santiago, Chile

Object. Posterolateral spinal fusions are performed to treat different spinal disorders. Autograft continues to be the gold standard; it is, however, associated with donor site morbidity and limited sources. Allograft has been used, but has been reported to result in lower fusion rates. Demineralized bone matrix (DBM) has also been used and reportedly increases the fusion rate in a variety of critical defect models. Different forms of DBM are available, not all have been independently studied. To evaluate the effect of a xenogenic DBM added to allograft on the fusion rate of posterolateral lumbar spine arthrodesis the authors designed an experimental study comparing posterolateral fusion rate using autograft, allograft, and allograft plus a xenogenic DBM in a validated animal model.

Methods. A bilateral, 1-level (L4–5) intertransverse process fusion was performed in 45 male New Zealand rabbits. Iliac crest bone graft was harvested bilaterally from each rabbit. The rabbits were randomly assigned to 3 groups: Group I, Autograft, 15 rabbits; Group II, Allograft, 15 rabbits; and Group III, Allograft plus DBM in a paste form (Dynagraft). The animals were killed 8 weeks after surgery. Fusion was assessed radiographically and by manual palpation by 2 independent observers. The results were analyzed using the Fisher exact test and chi-square test.

Results. The fusion rate was 46.6% (7 of 15 rabbits) in the autograft group, 33.3% (5 of 15 rabbits) in the allograft group, and 33.3% (5 of 15 rabbits) in the allograft plus DBM group (p > 0.05).

Conclusions. Autograft produced a higher fusion rate than allograft in this spinal fusion rabbit model, but the difference was not statistically significant. Allograft plus xenogenic DBM showed the same fusion rate as allograft alone. (DOI: 10.3171/SPI/2008/9/7/084)

**KEY WORDS** • allograft • autograft • bone graft • demineralized bone matrix • rabbit model • spine fusion

**POSTEROLATERAL** spinal fusion is used in the treatment of different spinal disorders, including deformities, infections, traumatic injuries, tumors, and degenerative conditions. This procedure has been associated with significant non-union rates; however, associated with donor site morbidity and limited sources. Allograft has been used, but has been reported to result in lower fusion rates. Several alternatives to autograft have been used. Allograft has been used for spinal fusion as an autograft extender or substitute. It is highly osteoconductive, less osteoinductive, and does not contribute osteogenic cells. It entails the potential risks of disease transmission and immune response and is associated with lower fusion rates than autograft when used posteriorly for spinal arthrodesis. Allografts, however, avoid the complications associated with autograft harvesting and their supply is potentially unlimited. Allograft is usually used as fresh-frozen, freeze-dried, or lyophilized bone. Whereas fresh-frozen bone allograft is potentially associated with a higher immune response, freeze-dried allograft (dried at −60°C) has the biomechanical properties of fresh-frozen allograft with less immunogenic response, but it has unreliable viability (20–70%). Lyophilized allograft has a low antigenic response and maintains the biomechanical strength and some osteoinductive properties of fresh-frozen allograft.

**Abbreviations used in this paper:** ANOVA = analysis of variance; BMP = bone morphogenetic protein; DBM = demineralized bone matrix.
Osteoinductive materials such as bone marrow and growth factors have also been studied, however their real benefit has not been clearly defined.6,15

A large group of osteoinductive molecules, able to stimulate ectopic bone formation, have been described and studied in animals.28 Among them, BMPs have been, perhaps, the most widely assessed in spinal fusions.19,20,23,27 Their clinical use in human patients has been reported with good results,9,11–14 but they are expensive.

Urist6 originally described the osteoinductive capacity of DBM. It has been considered a useful substitute for bone autograft. It provides variable osteoconductive properties, and no osteogenic cells; its success has been attributed to the osteoinductive capacity provided by the BMP content.6,15

Demineralized bone matrix has been used as a graft extender, or substitute, and to increase the fusion rate of spinal arthrodesis.9,26,29 Autograft volume can be supplemented with DBM to achieve good fusion rates.28,30 In general, DBM is prepared by decalcification of cortical bone (acid extraction of bone), exposing the extracellular matrix, which contains small amounts of osteoinductive growth factors, including BMPs. Demineralized bone matrix is less immunogenic than mineralized bone allograft.22 Different formulations have been developed;28,30 they can have variable osteoinductive capacity depending on the steps of processing, sterilization, and final formulation (including the carrier). Demineralized bone matrix may represent an attractive alternative when graft expander is needed. The potential advantages include lower immunogenicity than mineralized allograft bone and the exposure of extracellular matrix and osteoinductive proteins by the decalcification of cortical bone.

The purpose of this study was to evaluate the effect of a xenogenic DBM (human DBM, Dynagraft, IsoTis) in addition to allograft in an established posterolateral intertransverse process spinal fusion rabbit model.17,39 compared with allograft alone and compared with autograft. We did not include a group in which autograft was used in addition to DBM, as we wanted to determine whether the addition of a xenogenic DBM to the allograft could overcome the lower fusion rates expected with allograft compared with autograft.

Methods

Control and Experimental Groups

Bilateral, intertransverse process fusion procedures were performed at the L4–5 level in 45 male New Zealand rabbits. Corticocancellous bone graft was harvested from both iliac crests in each rabbit (1 cc from each side). Rabbits were randomly assigned to 3 different groups of 15 animals each: Group 1 (positive control) rabbits received autograft (1 cc per side). Group 2 (negative control) rabbits received allograft (1 cc per side). In this group, surgery was performed in 2 correlative rabbits at the same time; fresh bone was obtained from the iliac crests of one, then immediately prepared and put fresh into the other. Group 3 (study group, allograft plus DBM) rabbits received 1 cc allograft plus 1 cc DBM in paste (Dynagraft) in a 1:1 proportion on each side. Fresh allograft was also obtained as described above.

Institutional review board approval for animal research was obtained for this study.

Surgical Procedure. Each rabbit was sedated with 0.2 mg/kg of acepromazine hydrochloride and 15 mg/kg of ketamine hydrochloride administered intramuscularly. General anesthesia was induced with an endovenous solution consisting of 20 mg/kg of ketamine hydrochloride plus 5 mg/kg of xylazine. Postoperatively, enrofloxacin (5 mg/kg) was administered for 5 days for infection prophylaxis.

Bilateral posterolateral (intertransverse) spinal fusion was performed at L4–5. A standard posterior midline incision was performed through the subcutaneous tissues. Through bilateral, independent, intermuscular approaches, the transverse processes of L4 and L-5 were exposed and decorticated with a high-speed circular bur. Bone graft was obtained through the same skin incision, but through a different fascial approach. Either autologous corticocancellous iliac crest bone graft or allogeneic iliac crest bone graft alone or mixed with DBM was applied between the decorticated intertransverse processes according to the randomization protocol. A similar amount of bone graft (1 cc per side) was used in every rabbit in Groups 1 and 2, but the Group 3 animals received a total of 2 cc of graft material in each side as they received 1 cc of allograft and 1 cc of DBM. The increased amount of total graft used in Group 3 (DBM plus allograft) did not create any difficulty when placing the graft in the intertransverse bed.

The fascia and skin were closed with absorbable 1-0 and 3-0 sutures, respectively.

Postoperative analgesia was obtained with tramadol hydrochloride 4 mg/Kg (Tramal, Grunenthal, GmbH) administered subcutaneously every 12 hours for 5 days and then every 24 hours for 3 more days.

Analysis of Fusion

The animals were humanely killed 8 weeks after surgery. The whole lumbar spine was dissected and soft tissues were removed to allow complete visualization of L4–5 (Fig. 1). The presence of fusion was analyzed by means of 2 different methods by an investigator other than the one who harvested the spine.

A manual palpation test, as described by Boden and colleagues,8 was applied by 2 independent observers blinded
A dorsoventral digital radiograph of the dissected lumbar spine was also obtained immediately after specimens were dissected. A solid fusion mass was felt to be present if there was a continuous bone bridge seen on the radiograph (Fig. 2). In order for a specimen to be considered fused, both manual testing and radiographs had to show evidence of solid fusion.

**Statistical Analysis**

Differences in body weight between the 3 groups were evaluated by ANOVA with application of the Bonferroni correction. The differences in fusion rate between the 3 groups was analyzed using the Pearson chi-square test. A probability value < 0.05 was considered statistically significant.

The sample size estimation was based on data from a study by West et al. We assumed that the allograft plus xenogenic DBM group would show a 50% greater fusion rate than the fusion rate that would be expected for allograft alone. With a significance level of 5% and a power of 80%, the smallest appropriate sample size for each group was 15 animals.

**Results**

The rabbits in the 3 groups had similar body weights (p = 0.59, ANOVA, Table 1).

Seven (46.6%) out of 15 specimens from Group 1 (the positive control group, treated with autograft) were considered fused; solid fusion was present in 5 (33.3%) of 15 specimens from Group 2 (the negative control group, treated with allograft) and 5 (33.3%) of 15 from Group 3 (the study group, treated with allograft plus DBM). The differences between groups were not statistically significant (p = 0.69, Pearson chi-square test, Fig. 3).

No macroscopic inflammatory reaction was observed in the Group 3 specimens (allograft plus DBM group) that could be attributed to the presence of the xenogenic graft material used.

**Discussion**

We report the effect of different graft alternatives in spinal fusion using a rabbit model. Our results showed a 46.6% rate of consolidation using autograft and 33.3% using allograft with or without xenogenic DBM in a paste form (Dynagraft). Our results with autograft show a fusion rate comparable to several other reports using this same model. We also obtained a higher fusion rate with autograft compared to fresh allograft, which is concordant with previous reports. Our data also show that xenogenic DBM does not improve the fusion rates of allograft in posterolateral arthrodesis.

In our study we tried to overcome the lower fusion rates of allograft in posterolateral fusion by combining allograft with DBM in a rabbit model. Demineralized bone matrix has been considered a useful substitute and/or enhancer for bone autograft as it has osteoinductive capacity, due to its BMP content. Previous studies have shown that autograft volume can be supplemented with DBM gel to achieve good fusion rates.

Although we used human DBM in a rabbit spinal fusion model, we believe that the xenogenic nature of Dynagraft does not explain its lack of effect. The fact that we did not find any macroscopic inflammatory reaction in the rabbits that received human DBM suggests that there was no significant immunogenic reaction to this xenograft. In addition, BMPs, which explain the osteoinductive properties of DBM, are not species specific. Although it has been reported that xenogenic DBM produces less bone formation in normal animals than in athymic ones, more recent publications have shown that xenograft can be an appropriate alternative in immunocompetent animals, both in a rabbit.

![Fig. 2. Radiographs showing a solid fusion mass (A) and non-union (B).](image-url)
spinal fusion model\textsuperscript{23}\textsuperscript{26} and in a non-human primate model.\textsuperscript{26} Human studies using xenograft for spinal fusion are scarce and show contradictory results; some reports discourage its use,\textsuperscript{41} while others conclude that xenograft seems to be a valid alternative.\textsuperscript{25,27}

Different commercially available DBM products have different osteoinductive properties when tested in spinal fusion models. Wang et al.\textsuperscript{50} compared 3 different commercially available DBM products (Osteofil, Medtronic Sofamor Danek; Grafton, Osteotech, Inc.; Dynagraft) in a rat spinal fusion model; they reported a lower fusion rate for Dynagraft. The same authors also demonstrated different fusion rates between Grafton putty, DBX putty (Synthes), and Allomatrix putty (Wright Medical Technology, Inc.),\textsuperscript{32} as well as among other DBM presentations.\textsuperscript{24} It has also been shown that different lots of the same DBM product may have different BMP content;\textsuperscript{5} we used DBM from different lots to avoid the lack of effect of a single lot with a low osteoinductive capacity. Nonetheless, the low fusion rates observed with Dynagraft in other studies may be an explanation for our results.

Only one human study has compared autograft to allograft plus DBM. An et al.\textsuperscript{5} conducted a nonrandomized, prospective clinical study of cervical spinal fusion and found that the autograft group had lower rates of graft collapse and non-union, but that the differences between groups were not statistically significant, similar to our results.

Several studies have demonstrated a higher fusion rate with autograft than with allograft alone,\textsuperscript{1,47,50} a finding that is concordant with our results. Although processed allografts are more commonly used in humans, we decided to use fresh, not processed allograft, intending to produce a more challenging environment for spinal fusion in the negative control group (allograft alone). Thus, we could better determine whether the addition of DBM was able to increase the fusion rate.

The amount of bone graft is considered an important factor influencing the fusion rate in any spinal arthrodesis. Our model was designed to include the same amount of bone graft in the 3 groups, which necessarily led to a higher total volume injected in Group 3; thus the results could not be attributed to a lower amount of bone used.

We should also remark that we used 1 cc of bone graft per side, which was the maximal amount that we could safely harvest from each iliac crest in every animal under study. Although this is a lower amount of graft than what is usually considered the standard for this rabbit model,\textsuperscript{12} and the quantity of bone graft is considered an important factor influencing the fusion rate in any spinal arthrodesis, this amount of bone graft allowed a fusion rate comparable to the result of several studies in which this same model was used.\textsuperscript{16,17,40,43}

Several limitations must be considered when analyzing our results. We used fresh, not processed allograft, which may limit the applicability of our results to human spinal fusion; future studies should evaluate less immunogenic allograft alternatives combined with DBM. In addition, other studies should also evaluate whether the use of allogenic DBM is able to increase the fusion rate when added to allograft.

**Conclusions**

In this rabbit model of lumbar spinal arthrodesis, autograft showed higher fusion rates than allograft or allograft plus xenogenic DBM, but the differences did not reach sta-
tical significance. Adding xenogenic DBM in a paste form (Dynagraft) to allograft did not change the fusion rate achieved with allograft alone. Future experimental studies should address whether adding other forms of DBM to allograft can overcome the less favorable fusion rates of allograft compared with autograft.

Disclaimer

None of the authors of this paper have any financial relationship with or any other conflict of interest involving manufacturers of any of the products mentioned in this paper.

References


J. Urrutia et al.
Autograft versus allograft in a rabbit model of lumbar fusion


Address correspondence to: Julio Urrutia, M.D., Marcoleta 352, Santiago, Chile. email: jurrutia@med.puc.cl.