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Advances in Bone Graft Substitutes in Spinal Fusion

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Bone grafting is essential for reconstruction of spinal defects and a prerequisite to obtaining solid arthrodesis imperative to spinal stability after reconstructive surgery [1]. Spinal fusion is commonly achieved by the adjunctive use of interbody or onlay cortical bone grafts (autograft or allograft). Success depends on factors such as the patient's age, sufficiency of local blood supply, degrees of postoperative movement, and, importantly, the physical and biological characteristics of the graft matrix.

Early attempts at bone grafting date back more than 500 years to the Arab, indigenous Peruvian, and Aztec cultures. In modern times, the first documented case of autogenous bone grafting was reported by Merem in 1810, and the first successful allografting case has been attributed to Macewn in 1881 [2].

Our present knowledge and scientific base for understanding the biology, banking, and widespread clinical applications of bone grafting is largely due to the work of Albee [3], Barth [4], Lexter [5], Phemister [6], and Seen [7] during the late nineteenth and early twentieth centuries. These substantive scientific contributions have made bone grafting techniques common and relatively effective clinical procedures.

There are three biological processes that impact the success or failure of bone graft: osteogenesis, osteoconduction, and osteoinduction [8].

Osteogenesis refers to the process whereby bone forms directly from living cells, such as the stem cells within autogenous bone. Osteoconduction describes the process in which bone grows into and along the surface of a biocompatible structure when placed in direct apposition to host bone through the process of intramembranous bone formation. The ability to osteoconduct is a passive characteristic of bone that allows it to act as a platform on which vascular invasion, resorption, and new bone formation can occur [1]. Osteoinduction is endochondral bone growth stimulated by specific growth factors (morphogens and/or mitogens) on pluripotential cells, such as mesenchymal stromal or stem cells. In particular, bone morphogenic proteins (BMPs), identified through the seminal work of Urist and colleagues [9], has demonstrated the capacity for inducing the differentiation of host perivascular mesenchymal cells into cartilage and bone [10].

In varying degrees, bone grafting source materials and techniques use these mechanisms of bioincorporation. Thus, the ideal bone graft should be capable of these processes and also

be free of immunological antigens and microbial pathogens. There are a variety of bone grafts to choose from, each presenting a unique set of advantages and disadvantages.

Autografts, because they are harvested from host bone stock from one body site for transfer to another location in the same individual, offer the maximum biological potential and histocompatibility. Immunological considerations and disease transmission are obviated through the use of autogenous bone.

The possibilities of meeting the needs of size, shape, and quantity of bone for any given procedure, however, are limited in autografting. The potential for morbidity by harvesting autologous iliac bone graft is ever- present [11] and can be caused by nerve injuries [12,15], vascular injuries [16,17], hernia through the iliac bone donor site [18,19], bowel obstructions [20], and other noteworthy drawbacks. Furthermore, harvesting fibular graft is related to a sense of instability or weakness in the lower extremity [21]. Operating room time is extended, as is the period during which the patient must remain under anesthesia. Any complications arising from these events, especially if compounded by the sequelae of donor site morbidity, may also increase the duration of hospitalization. Moreover, such a procedure often renders the bone donor site unacceptable for a subsequent operation.

Despite these disadvantages, what makes the autograft the gold standard for bone grafting is that it fulfills the three requirements necessary for bioincorporation: it is osteogenic, osteoconductive, and osteoinductive.

Available autologous bone grafts include cancellous, vascularized or nonvascularized cortical, and autologous bone marrow grafts. Bone formation from autologous grafts is believed to occur in two phases. The first phase lasts approximately 4 weeks, during which bone formation is mainly contributed from the cells of the graft. Cells from the host begin to contribute to the process during the second phase [22,23].

Autologous cancellous bone is easily revascularized and rapidly incorporated, leading to a high success rate. It does not provide substantial structural support but is a good space filler. Because only the osteoblasts and endosteal lining cells on the surface of the graft survive the transplant, a cancellous graft acts mainly as an osteoconductive substrate [24–27].

Osteoinductive factors released from the graft may also contribute to bone formation, but this is only a theory based on circumstantial evidence and has not yet been substantiated by scientific documentation [22,28,29]. Cancellous graft achieves strength equivalent to that of a cortical graft after 6–12 months [30].

Autologous cortical grafts include the fibula, ribs, and iliac crest. These grafts can be vascularized or nonvascularized. Autologous cortical grafts are mostly osteoconductive and have little or no osteoinductive properties, but the surviving osteoblasts provide some osteogenic properties [31,32].

Cortical grafts also provide excellent structural support, but nonvascularized cortical grafts become weaker than vascularized ones during the initial 6 weeks after transplantation as a result of resorption and revascularization [31,33]. Vascularized cortical grafts incorporate rapidly, and their remodeling is similar to that of normal bone. They do not undergo resorption and revascularization, thus providing superior strength during the first 6 weeks [31]. However, little difference in strength between vascularized and nonvascularized cortical grafts is evident by 6–12 months [31].

Allografts, usually obtained from cadaveric sources or incidental to operative procedures, offer satisfactory biological potential and eliminate the chance of donor site morbidity. Allografts provide an abundant supply of bone tissue, but their use for spinal fusion has been disappointing, especially for onlay intertransverse bone grafts [34–36]. Their use in scoliosis surgery has been flawed, with poor results in the 1960s [37]. In a recent study in humans, allografts in the form of fresh-frozen human femoral head were found to be at least as effective as autologous bone

in instrumented posterolateral spinal fusion surgery when the results were assessed in terms of clinical outcome [38].

Allografts, including fibular allografts, tricortical ilium allografts, and femoral shaft cortical ''rings,'' have been successfully utilized for anterior interbody fusions [39–41]. Structural femoral ring allografts have proved effective in salvaging failed lumbar fusions with a reported fusion rate of 79–98% [39,42]. Femoral ring allografts with the medullary canal packed with cancellous autograft have been found almost as effective as tricortical iliac autografts (6% vs. 0% pseudarthrosis rate) in anterior fusions in revision surgeries for pseudarthrosis or flatback deformity. Although long-term cortical allograft resorption has been observed in femoral rings packed with cancellous allograft chips that had been used for anterior lumbar intervertebral fusion, the center of the graft usually achieves solid arthrodesis [37].

The use of allografts poses biohazards arising from their potential to act as conduits for disease transmission from donor to recipient and the triggering of immunological reactions. Thus, strict adherence to bone banking methodology and sterilization procedures are essential to proper handling of allografts [43].

Xenograft, or cross-species bone tissue, although in abundant supply, has been found to be a less reliable graft material than autogenous and allogeneic bone. The emergence of such concerns as major histocompatibility difference leading to immune response provocation, the incompatibility of other species' anatomies with human anatomical parts, lessened biological activity, and the need for rigorous, meticulous processing and sterilization of bone derived from nonhuman species have largely reduced the opportunities for effective orthopedic reconstructive use of xenograft bone.

Bone cages have demonstrated great promise for spinal fusion, but they still require a substantial amount of bone graft material, especially for multiple-level fusion. It is therefore likely that the use of bone cages does not diminish the potential for serious complications associated with harvesting autologous iliac bone graft. For these reasons, researchers have directed their attention to the search for suitable substitutes for autograft and allograft bone. The endeavor to transcend the numerous drawbacks associated with natural sources of bone tissue has given rise to the development and manufacture of bone substitutes in various osteoinductive and osteoconductive forms (Table 1) [1,44].

Osteoconductive agents are collagen, tricalcium phosphate ceramics (TCP), hydroxyapatites (Ht), coral-derived biomaterials, mineralized collagen matrix (Healos), some osteoactive polymers, and calcium sulfate (plaster of Paris; POP). Materials with osteoinductive properties contain one or more factors such as bone morphogenetic proteins (BMPs). Whereas numerous growth factors may be involved in new bone formation, including platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), and vascular endothelial growth factor (VEGF), there is evidence that only the BMPs are capable of initiating the entire process of new bone

Table 1 Osteoconductive and Osteoinductive Bone Substitutes

Osteoconductive agents	Materials with osteoinductive properties
Hydroxyapatites (Ht)	Demineralized bone matrix
Coral-derived biomaterials	Bovine osteogenic factors
Tricalcium phosphate ceramics (TCP)	Bone morphogenic proteins 2–8
Mineralized collagen matrix (Healos)	Osteogenin (BMP-3)
Osteoactive polymers	OP-1 (BMP-7)
Calcium sulfate (POP)	OP-2 (BMP-8)

formation [45–49]. BMPs can be found as recombinant proteins produced by genetic engineering or as purified extracts from bone. Demineralized bone matrix is a source of such growth factors but in much lower quantities.

A bone graft substitute material can be used as either a graft extender, a graft enhancer, or a graft substitute. A graft extender is a material that allows the use of less autogenous bone graft with the same end result or one that allows a given amount of autogenous bone to be stretched over a greater area with the same success rate [50]. A bone graft enhancer, when added to autogenous bone graft, increases its successful healing rate. A bone graft substitute is a material that may be used entirely in place of autogenous bone graft to achieve the same or better fusion success rate [50].

There is considerable variation in the type and speed of healing because of biological and biomechanical differences between anterior and posterior columns. The anterior or middle column of the spine is composed primarily of cancellous bone under compression loading, whereas the posterior column consists mostly of cortical bone and is frequently under tension. As a result, the dosage and the ideal bone graft substitute may differ by location, and results of healing for bone graft substitutes or augmentation devices in one region of the spine cannot necessarily be extrapolated for other regions.

For anterior column applications such as intervertebral fusions, biologically compatible materials are most suitable when they provide geometric spaces that invite the ingrowth and osteogenic differentiation of primitive mesenchymal cells. The ''industry'' has exploited this knowledge by providing porous calcium phosphate ceramics [51–53] and orthopedic implants with porous metallic coatings, which are now widely employed in hip and knee replacement surgeries. Suitable ''biological space'' was also found in coelenterate coral skeletons. Once it was discovered how to chemically convert $CaCO_3$ to bone-like hydroxyapatite, this material was marketed as a bone ingrowth system under a number of trademarks (e.g., Interpore 200/400, proOsteon Implant 500). There is now a sizable outcome literature reporting successful use of replamineform coral implants in the canine mandible and tibial plateau [54], in rabbit tibia [55,56], as well as in various human long bones [57]. To date, there are reports that plate-stabilized blocks of coralline material produce new bone within cervical disc spaces [58]. Good results have been reported with implants made of porous hydroxyapatite used to achieve cervical interbody fusion in humans [59]. However, osseous integration, which is usually promoted by hydroxyapatite coating, failed to occur in artificial intervertebral disc in dogs [60]. Studies using HA ceramic spacers to maintain the laminar spread in open door cervical laminoplasty have also reported good clinical results [61].

Although purely osteoconductive substitutes may be suitable in the anterior spine when it is rigidly immobilized, they are less effective in posterolateral spine fusions. In studies assessing posterolateral fusion, hydroxyapatite block alone has not functioned effectively as a complete graft substitute [62]. Osteoinductive substitutes are more likely to be successful as either extenders, enhancers, or substitutes for posterolateral spine fusion [50].

When freeze-dried human bone allograft is demineralized, the allograft is osteoinductive, since it causes bone to form heterotopically [63]. Current use of demineralized, freeze-dried bone allografts is based on this ability [64,65]. Additionally, they provide a space-filling osteoconductive matrix, facilitating bone formation. The osteoinductive ability of demineralized bone is believed to be due to its content of BMPs, other growth factors and cytocines. These factors interact with mesenchymal stem cells or osteogenic precursors in the host tissue [66–68], causing them to differentiate into bone-forming cells. However, the concentration of BMPs in demineralized bone matrix (DBM) is not thought to be sufficient for it to be a complete substitute for autogenous bone graft.

Clinical reports indicate that preparations of this material vary with respect to bone formation [63]. Also, the osteoinductive ability of commercial demineralized, freeze-dried human bone graft, when implanted heterotopically in mouse, varies widely among tissue banks [69].

Differences in procurement and processing methods might play a role, but donor characteristics have the major contribution. Donor age is the most important variable with respect to osteoinductive ability and was inversely correlated with the ability of demineralized, freeze-dried human bone graft to induce bone [63,70]. This age-dependent loss of osteoinductivity is due to a loss of bioactive factors [71]. Only demineralized, freeze-dried bone graft from patients younger than 42 years of age was osteoinductive in a study on humans [63].

The successful isolation and purification of BMPs and the synthesis of recombinant human BMP (rhBMP) was a major step in overcoming the problems with availability of growth factors in demineralized allografts. The BMPs are differentiation factors, causing mesenchymal cells to differentiate into bone-forming cells. In contrast, factors such as PDGF TGF-β and are growth factors, causing cells to divide, thus expanding their numbers. Such growth factors may also be used to enhance bone graft, but combining BMPs with growth factors not only lacks synergistic effect, but the factors may antagonize one another's activity [72,73].

Successful clinical application of rhBMP depends upon the design of appropriate delivery systems. These carriers may not only act as controlled- release delivery systems, maintaining a critical threshold concentration of BMP at the site of implantation for the desired period, but also as osteoconductive materials, serving as a scaffold for the ingrowth of capillaries and osteoprogenitor cells from the recipient host bed [74]. Carriers also contains the BMP at the site of application to prevent extraneous bone. Commonly used carriers include collagen sponges, calcium phosphate ceramics, and degradable synthetic and natural polymers. Ideally, a carrier is a resorbable material with a resorption rate that generally matches the rate of bone formation. If the carrier resorbs before adequate osteodeposition, the result may be misdirected bone formation and pseudarthrosis. If the carrier resorbs too slowly, it might impede bone formation and remodeling [75].

The osteogenic protein-1 (OP-1) is such a combination of human rhBMP-7 in bovine bone-derived type 1 collagen. OP-1 has been demonstrated to be effective as a bone graft substitute when performing posterior lumbar interbody fusion (PLIF) in a sheep model [74]. The amount of bone formation by OP-1 was statistically higher than either autograft or hydroxyapatite in sheep interbody fusion [76]. OP-1 has also demonstrated an ability to induce successful posterolateral spinal fusion in dogs without a need for autogenous bone graft. In humans, OP-1 achieved better results than autograft in posterolateral fusions, in both fusion rates and clinical outcome, when used either alone or in combination with autograft. Preliminary results of similar studies revealed equal or greater bone formation with OP-1 compared with autograft [77]. However, the use of OP-1 did not induce sufficient early structural bone support after intracorporal application on spinal fractures [78].

Hydroxyapatite-tricalcium phosphate has been used as a carrier for rhBMP-2 with good results in a posterolateral spinal fussion model in rhesus monkeys [45]. Tissue engineering using specific scaffold materials to support tissue growth and provide proper osteoinductive agents might make an ideal bone graft substitute in the future. New delivery systems being evaluated include depot delivery systems, viral vector systems, conjugated osteogenic factor delivery systems, and oral small molecule targets [75].

Promising results in recent investigations indicate that gene therapy may have a potential application in spinal fusion. Enhancement of spine fusion by gene transfer in animal models is evidence of the rapid progress that has been made [79]. BMP genes have mainly been used for this purpose. The local production of BMPs using gene therapy may have several advantages over the direct delivery of the recombinant protein. Direct BMP delivery leads to relatively short-term bioavailability. Although it may be adequate for inducing osteogenesis, the physiolog-

ical affects of BMPs may not be maximized using these techniques. The use of BMP gene therapy has the potential to induce long-term, high-level BMP production at sites requiring bone formation. The quality of bone formed using BMP gene therapy may be improved over that achieved with the recombinant protein [80]. Because endochondral bone formation requires angiogenesis within the newly formed tissue, it is possible that the upregulation of VEGF may also improve the efficacy of BMP gene therapy [81].

Percutaneous delivery of the cellular or viral BMP vector, permitting the application of minimally invasive techniques for spinal fusion, may be possible in the future. The incorporation of stereotaxic techniques should make these approaches safe in the clinical setting. However, significant advances need to be made in vector design, gene-regulation techniques, and tissue targeting before human clinical trials can be safely and successfully conducted [81].

Plaster of Paris is an inexpensive and readily available bone grafting material that has proved to be well tolerated by human tissue in nonvertebral settings. POP-filled defects in bone are gradually vascularized and replaced by bone tissue derived from the host [82–86]. To assess the effectiveness and safety of POP in spinal fusion and to compare it to autografts and other graft substances, we conducted three sets of experiments.

In the first set, 20 adult female sheep (30–40 kg body wt) were subjected to L1-L2, L3-L4, and L5-L6 discectomies. The intervertebral discs were excised, and the cartilaginous and bony endplates were cut away to expose the subchondral bone. Each space was then implanted with a 1.0 × 1.5 cm long tubular titanium cage, which had been filled with one of a variety of grafting materials (Table 2) or left empty as a control implant. Implant filling strategy was randomized.

At the time of sacrifice, 4 months postoperatively, host-derived trabecular bone had invested each interbody cage (Fig. 1). All individual segments, including a single interbody graft, were biomechanicaly tested to establish rotational stability and tensile load to failure. The tissues were recovered and sectioned. Sections of the recovered tissues were then microradiographed, and the total area of trabecular bone formed within each cage was quantitated by computer-driven software. Data were expressed in terms of percent trabecular bone volume.

The microradiographic analysis indicated that the different grafts and combinations of tissue types had produced volumes of new bone that were neither significantly different inter alia nor different from the outcome of the empty control implants. All bone present appeared to be of uniform and equal density on microradiographic investigation. Biomechanically, however, the behavior of the control fusion masses was inferior to that of the fusion masses formed under the influence of osteoconductive bony and apatitic substrates. The applied torque of ±2.5 Nm, which was insufficient to break the bony trabeculae, permitted a 1–2° displacement in the experimental groups versus a 3–4° displacement in the trabecular masses formed around the control cages. The POP grafts permitted, quantitatively at least, the smallest angular displacement, but no statistical difference occurred. The ‘‘pull-out’’ tensile test also affirmed that POP

Table 2 Cage-Filling Materials

1. Autogenous iliac crest cancellous bone (Auto)	
2. Frozen allogeneic cancellous iliac crest (Allo)	
3. Plaster of Paris (POP)	
4. Coralline hydroxyapatite (pro-Osteon 500)	
5. Demineralized bone (DBM)	
6. POP + Auto	(admixture 1 : 1)
7. pro-Osteon 500 + Auto	(admixture 1 : 1)
8. DBM + Auto	(admixture 1 : 1)
9. Empty control	

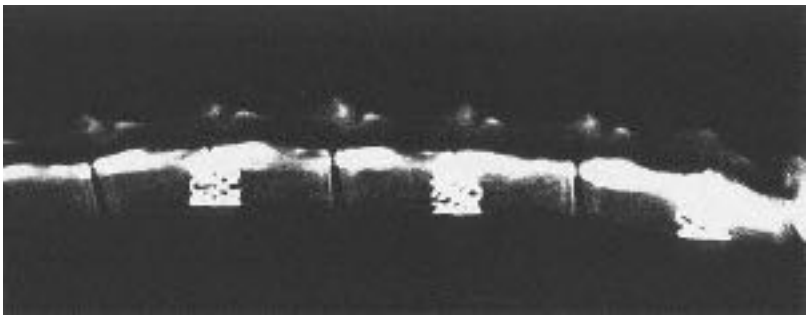

Figure 1 Roentgenograph showing the positioning of titanium cages within excavated disc spaces in the lumbar spine of a sheep 4 months postoperatively. The roentgenograph shows the interbody fusion masses within and around the titanium cages. (From Ref. 89c.)

and most of the other osteoconductive experimental graft types, alone or in combination with autologous bone, performed optimally, and that their fusion masses were biomechanically superior to those formed around the control (empty) cages. The tensile failure load of the sheep demineralized osteoinductive bone was equal to that of the control titanium cages that were implanted empty (Fig. 2). So, despite contrary expectations, the osteoinductive demineralized sheep bone preparations proved the least effective of the different substrates in achieving a solid interbody fusion. The addition of autogenous bone did little to improve DBM performance. The advanced age of the donor animals could have been a factor in its poor performance, since production of bone morphogenetic proteins declines with increasing maturity [87]. Yet it may be that mature sheep are poor BMP responders [70,88].

In that experiment, the small cages in intervertebral disc space provoked an exuberant bone reaction from the host tissues, thus compromising the results of bone graft testing. The mechanisms responsible for the new bone formation that enveloped the titanium-carrier mesh are likely to involve vascular ingrowth from the marrow of the vertebral bodies, with the intercession of the vertebral periosteum and psoas muscle pericytes (osteoprogenitor cells).

In order to prevent reactive exuberant bone formation when testing intervertebral bone cages in sheep, a large bone defect was required.

To address these concerns, a second set of experiments with two series of 15 sheep each was conducted. In a first series, the sheep were subjected to lumbar spine fusion after L4 corpectomy. The body of L4 was osteotomized with preservation of the pedicles and the more posterior components. The subchondral bone at L3 and L5 was removed to prepare a vascular bed into which a bridging titanium (Ti) cage (44 mm $\times$ 15 mm) was inserted to maintain the stability of the lumbar spine. Cages were implanted after they had been filled with either autologous iliac crest bone (five sheep) or POP (five sheep), or were left empty in a third group of five sheep.

At the time of sacrifice, 6 months postoperative, all cages appeared to be fully invested in bone (Fig. 3). Microradiography showed that identical volumes of bone were formed within the autograft and POP cages, but bone within the chambers that had been implanted empty was too little to permit quantitative morphometric evaluation. Furthermore, the quality of bone formed under the influence of POP and autogenous iliac crest graft was equal in terms of stiffness and strength at failure when tested in torsion.

To evaluate the sources of the bone investing the cages, the Ti implants were used in a somewhat different experimental setting, femoral segmental osteotomy, in which tissue geometry

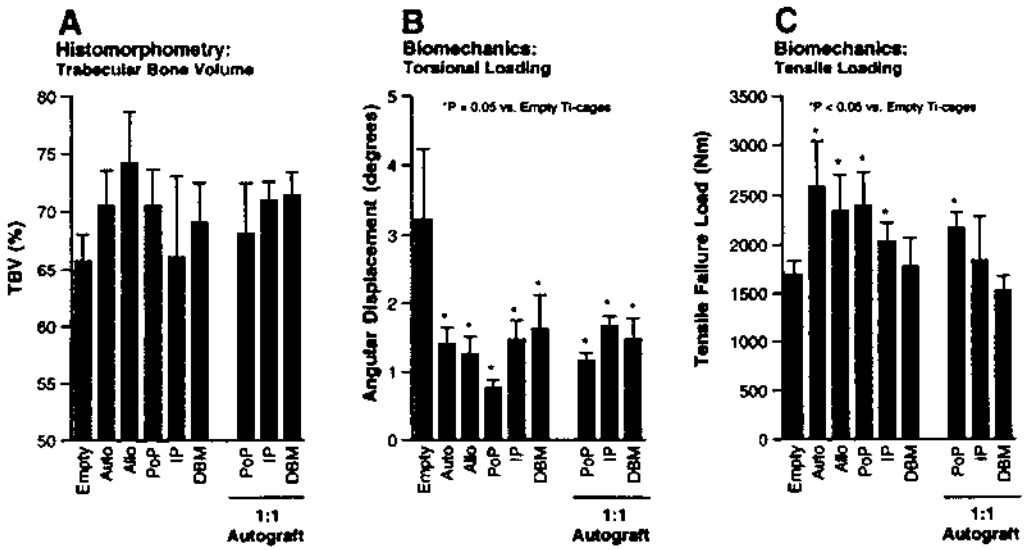

Figure 2 Graphs showing the quantitative histomorphometric and biomechanical evaluations of the bone formed under the influence of osteoconductive and osteoinductive substrates implanted within excavated lumbar spine spaces. (A) Histomorphometry; (B) biomechanics—angular displacements between -2.5 and $+2.5$ N m loads, (C) biomechanics—tensile failure load. Implants of empty titanium cages served as the control group. Data represented by bars marked with an asterisk (*) were statistically different from the empty control data at the $p < 0.05$ level of significance. *Auto*, autograft; *Allo*, frozen allografts; *PoP*, plaster of Paris; *IP*, replamineform coralline substrate; *BDM*, demineralized allogeneic sheep bone. (From Ref. 89c.)

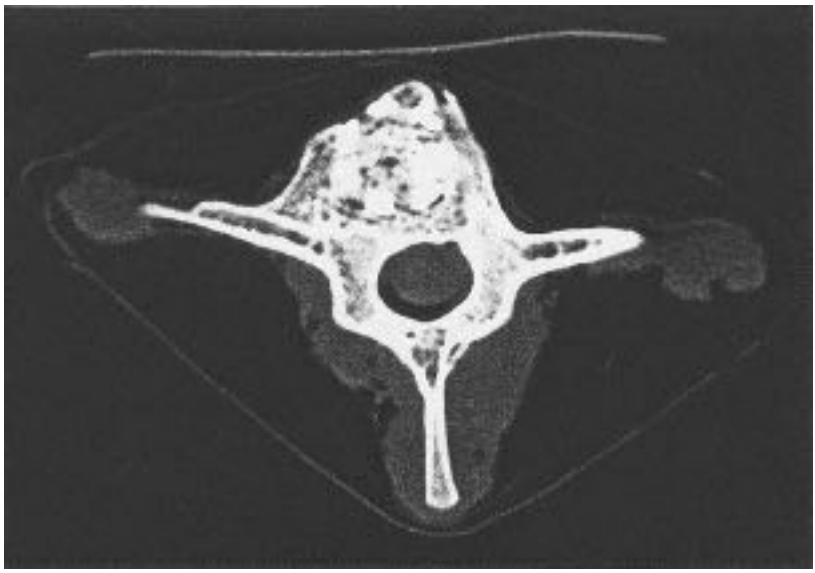

Figure 3 Computed Tomography scan of the L4 replacement titantium cage, showing fusion masses within and around the cage. (From Ref. 89b.)

improves the chance of isolating the contributions of cells made by investing soft tissues from those supplied by the marrow.

An 1 inch (2.5 cm) segmental midshaft defect was created after cortex cut away. The defect was filled with cages and stabilized with a compression plate. The Ti cages were Implanted unfilled (five sheep) or preloaded with either autogenous bone marrow (five sheep) or POP (five sheep).

In order to prevent, or at least retard, the ingrowth of vessels from surrounding tissues, Ti cages were lined with an oversized sheet of Millipore with pore size of 0.45 mm. The protruding ends of the Millipore sleeve were fitted closely over the stumps of the periosteum-free femoral cortex.

At autopsy, 6 months postoperative, the Ti cages had gradually been incorporated into the diaphyseal marrow (Fig. 4). There were no differences in the total volume of bone formed around the cages. Although equally stiff when evaluated in tension, chambers implanted empty remained incompletely filled and were the weakest when tested in torsion. There were no differences in the quantity and mechanical properties of the trabecular bone formed within the chambers by autogenous bone and POP (Fig. 5, Fig. 6).

These studies suggested that POP had an osteoconductivity equal to that of autogenous iliac crest marrow/bone. Both POP autologous bone induced the production of significant new bone with normal histology within and around the Ti cages [89b,c].

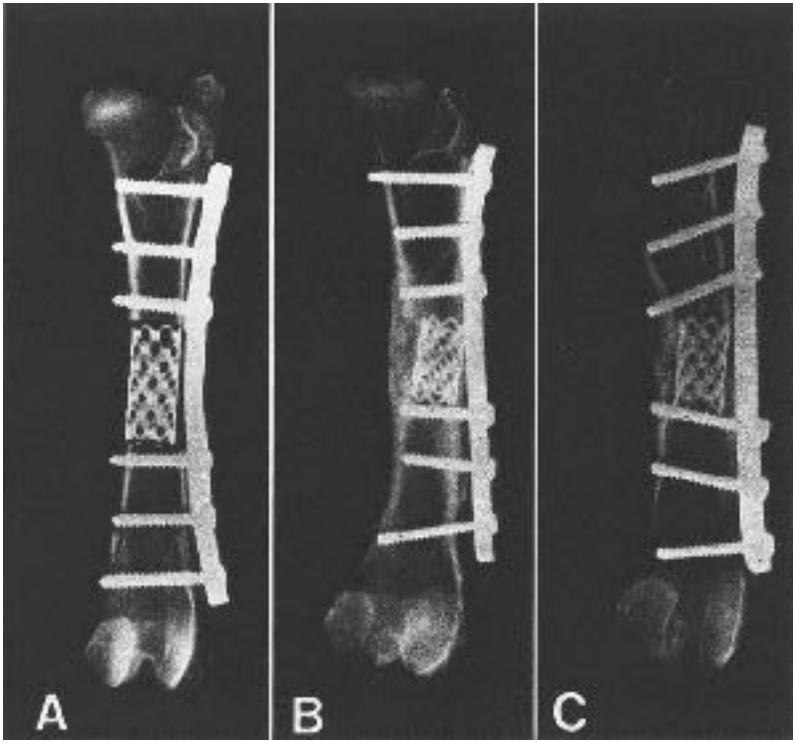

Figure 4 Roentgenographs showing postoperative appearances of a femoral midshaft titanium cage implant in a sheep: immediately after surgery (A), 6 months after iliac crest autograft procedure (B), and 6 months after POP implant (C). All graft sites were stabilized by lateral eight-hole compression plate. (From Ref. 89b.)

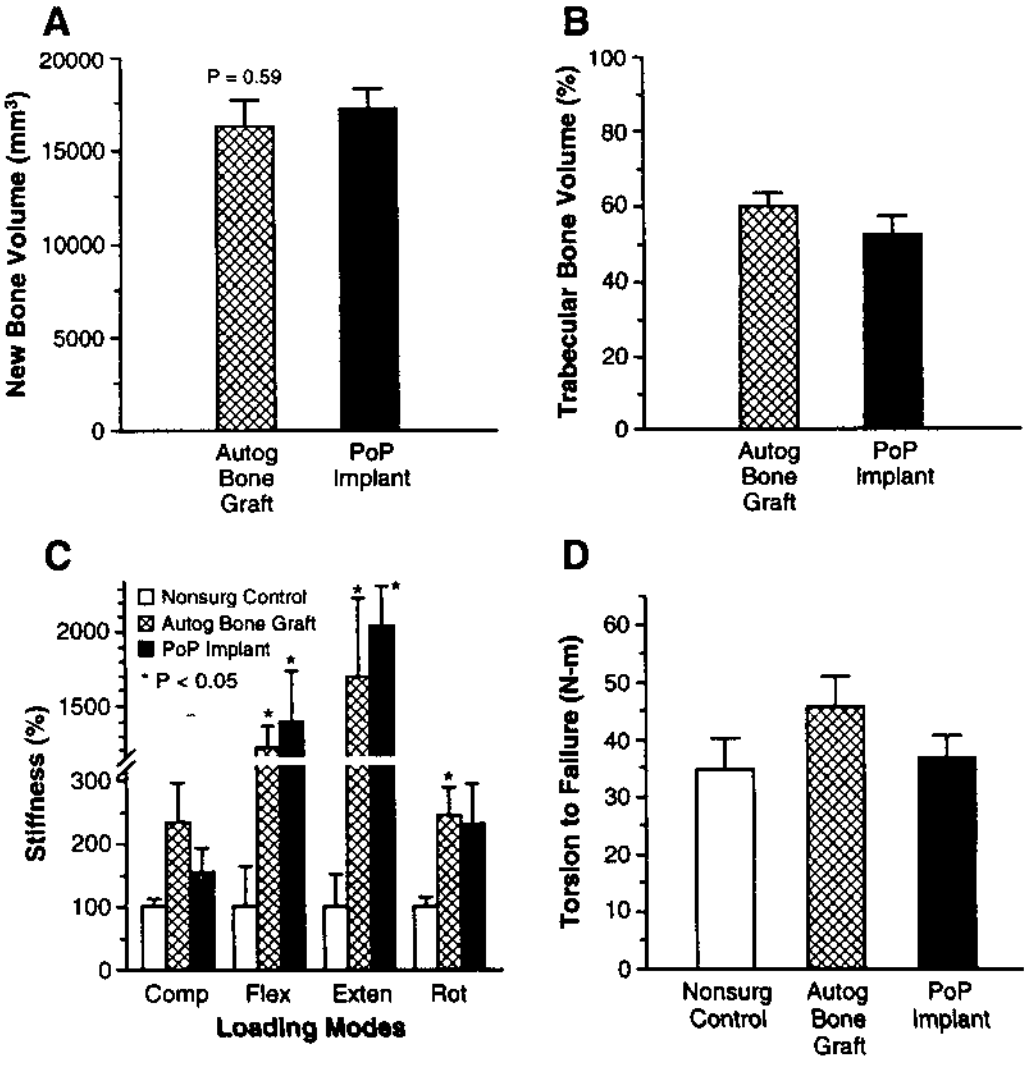

Figure 5 Histomorphometric and biomechanical measures of the stability of the postcorpectomy L4 lumbar interbody fusions in sheep 6 months after surgery. (A) Total volume of bone formed around and within the titanium cage; (B) trabecular bone volumes formed within the Ti cages; (C), flexural rigidity of fusion masses at 15° (Nm degrees); (D), tensile strength of the fusion masses (Nm). (From Ref. 89b.)

As shown by the experiments with femoral segmental osteotomy, we can conclude that bony core within the Ti cage largely derived from medullary osteoprogenitor elements, while bone that invested the Ti cages externally was the product of surrounding tissue cells. The millipore liner delimited the new bone that formed the central core within the Ti mesh from that which invested the cage externally. Significant displacement of the liner occurred only with the empty implants, whose crimping was caused by the more rapid formation of bone from the investing soft tissues. In those implants, the trabecular bone volumes attributable to a marrow stromal source were the lowest (Fig. 7). The original conformation of the Ti Millipore contact was well maintained, as showed by microradiography, in situations wherein osteoprogenitor

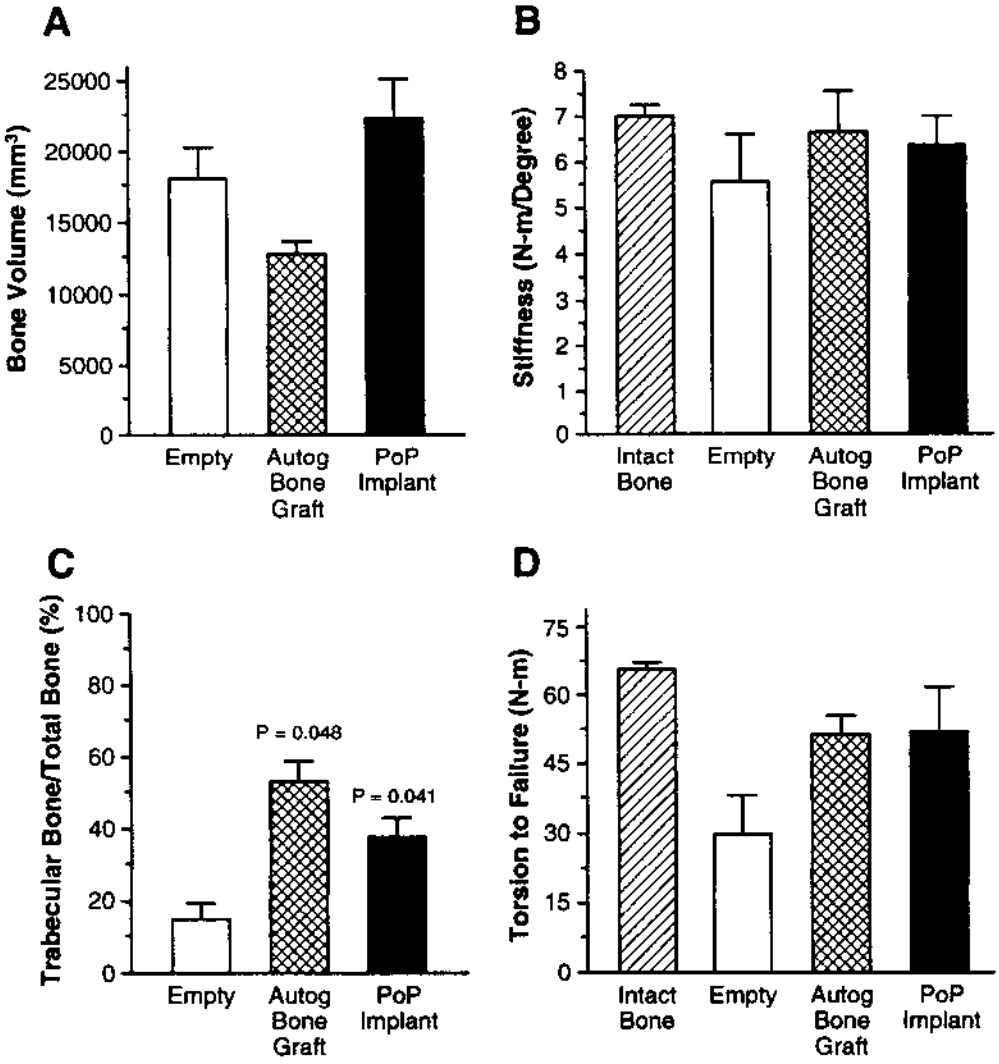

Figure 6 Histomorphometric and biomechanical measures of the stability of the midshaft femoral titanium cage implants. (A) Total volume of bone formed; (B) stiffness; (C) trabecular bone volumes formed within the Ti cages; (D) tensile strength of the fusion masses (Nm). (From Ref. 89b.)

cells were drawn from all sources. Accordingly, it seemed likely that the bony core had been derived largely from medullary osteoprogenitor elements, the stromal cells [89], whereas the bone that invested the Ti cage externally had been the product of periosteal osteoblasts and osteoprogenitor cells derived from muscle connective tissue elements [90].

The late results of plaster of Paris when used as a bone filler, to heal osseous defects, have been investigated. The early osteogenic effect on healing at the molecular level, however are not clear.

To study how implants of POP affect the time course during the first 3 months, we conducted a third set of experiments using a sheep lumbar vertebral defect model in 20 adult

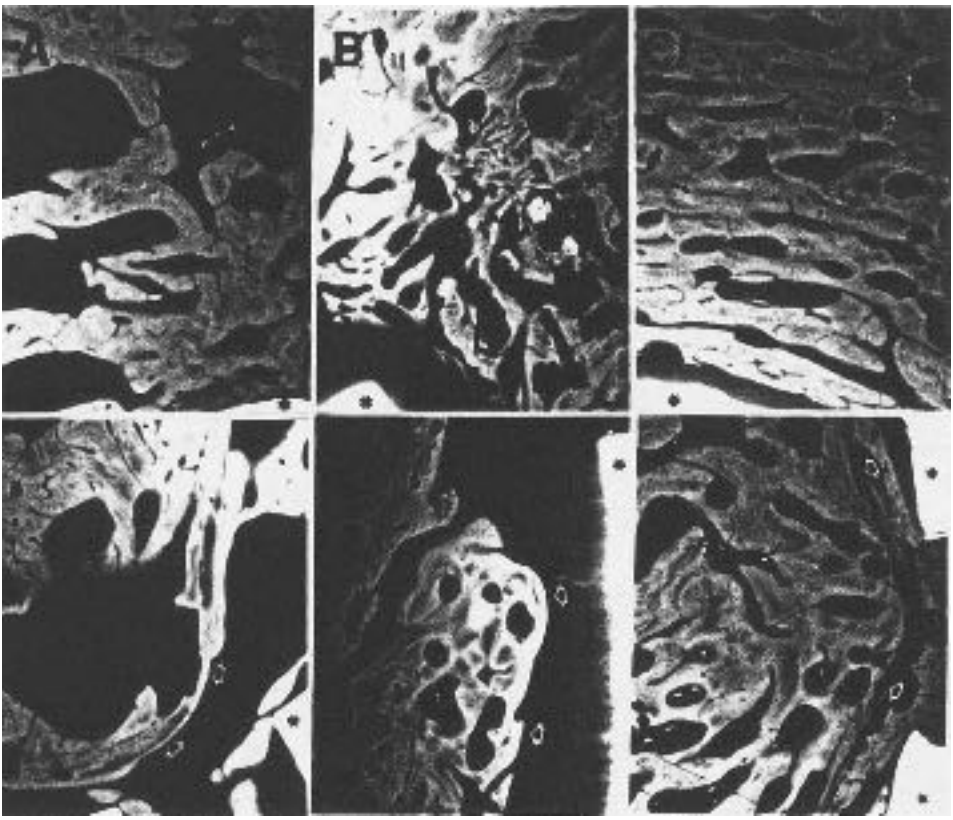

Figure 7 Microradiographs showing the relative amount and structure of the bone formed external to (*top*) and within (*bottom*) the titanium cage midshaft femoral implants in sheep 6 months after surgery. (A) Control/empty; (B) autogenous bone/marrow; and (C) POP. (From Ref. 89b.)

female sheep. In 15 sheep, defects 5.0 mm in diameter and >10 mm in depth were created in a ventral-dorsal direction, equally spaced from L1 to L5 (one hole per vertebral body) with a microscopic ring saw. In five sheep, the defects were 10 mm in diameter and >10 mm in depth, leaving a hole volume of ~740 mm^3. The 5 mm defects were packed with either POP or autogenous cancellous bone and marrow cored from the defects. The same procedure was followed in sheep bearing 10 mm defects, but a certain number of defects were left unfilled as controls.

The animals that received 5 mm defects were sacrificed at intervals of 1, 2, and 3 months postoperatively, while animals bearing 10 mm defects were sacrificed at 3 months.

The volume of the new bone filling the defect spaces was determined using 3D reconstructions of transverse images of the vertebrae. Implants of autogenous bone and POP afforded an equal stimulus to repair. In those cases ~96% of the original bone mass was restored after 3 months, while the defect left empty contained only half as much new bone (Fig. 8).

Histological sections were also analyzed to determine the percentage of the defect space occupied by mineralized bone and osteoid, the percentage of mineralized surface invested in osteoid, and the percentage of available trabecular surface covered by osteoblasts as well as the percentage of bone surface that had been eroded as index to remodeling. Tissue and cellular

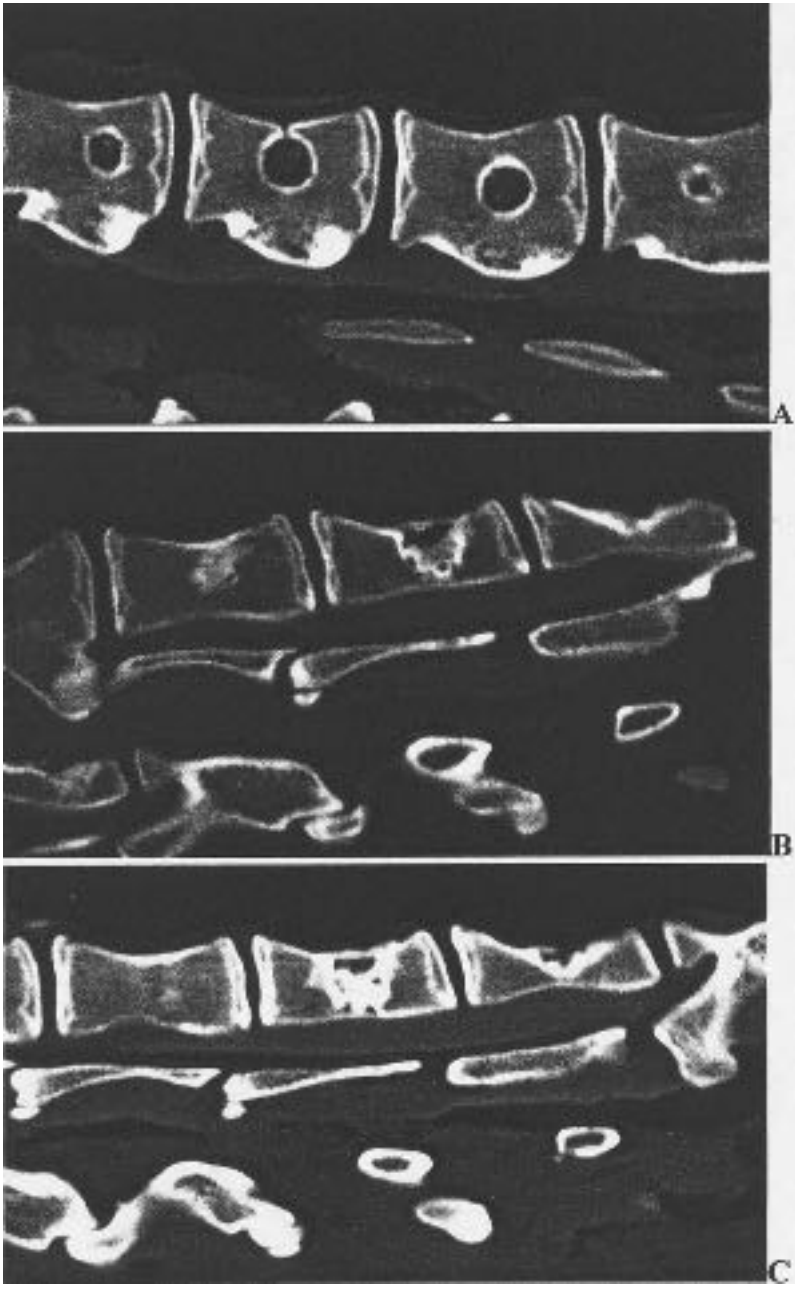

Figure 8 Computed tomography of lumbar spines with 10 mm defects at three months. (A) Defects initially left empty remained poorly filled. The implants of (B) autogenous bone and (C) POP showed a similar pattern of healing.

Table 3 Effects of Autogenous Bone Marrow Grafts and POP Implants on the Repair of Lumbar Yertebral Defects in Sheep

Defect size (mm)	Postop group	Treatment	*N*	Trabecular bone density (%)	Osteoblastic surface (% total)	Erroded bone surface (% total)
Baseline			1	30.07	3.27 (1)	22.27
5.0	1 month	Autograft	3	12.55 ± 3.20	16.62 ± 4.00	14.66 ± 3.81
		POP	3	20.67 ± 6.66	24.97 ± 4.51	20.64 ± 2.58
5.0	2 months	Autograft	3	29.91 ± 1.87	00.73 ± 0.63	08.03 ± 3.27
		POP	5	26.91 ± 2.75	05.81 ± 1.93	12.38 ± 2.31
5.0	3 months	Autograft	4	45.16 ± 7.32	1.71 ± 0.81	9.34 ± 2.49
		POP	6	39.70 ± 5.89	2.37 ± 1.26	7.85 ± 2.06
10.0	3 months	Empty	12	29.24 ± 3.86	16.31 ± 2.8	
		Autograft	4	46.47 ± 7.55	13.8 ± 4.09	
		POP	8	42.61 ± 6.30	12.93 ± 3.21	

Data expressed as the mean ± standard error of the mean (SEM).

profiles for the small 5.0 mm defects (Table 3) show that no matter nature of the graft, the trabecular bone volumes progressively increased with time from an average of ~17% at 1 month to ~42% at 3 months. However, POP improved repair processes at 2 months postop, increasing the remodeling as indicated by a 5-fold increase in fraction of trabecular bone surface involved in osteoblastic activity and 1.5-fold increase in bone surface involved in resorption (eroded bone surface).

The larger (10 mm) defects showed a similar pattern of healing. The implants of POP and autogenous bone were equally effective in promoting a 2-fold ingrowth of new bone with respect to defects left empty (Table 3). At 3 months the fractional cellular components involved in remodeling (osteoblasts and osteoclasts) and the levels of the osteoblasts activity (osteoid surface and bone mineralization rate) were similar (Table 4).

The principal finding from our studies was that POP has an osteoconductivity equal to that of autogenous iliac crest graft when used for filling bone cavities. This corroborated the findings of Peltier showing that the most important property of POP as a ''filler'' is its apparent natural rate of absorption—one that was equal to the rate at which new bone can grow into the

Table 4 Computer Analysis of Hematoxylin-Eosin Stained Slides Showing the Effects of Autograft and POP on Repair of Defects

Defect size (mm)	Postop group	Treatment	Osteoid surface (%)	No. osteoblasts/ unit trabecular perimeter	Bone minelalization rate (μm/day)	No. osteoclaststst/ unit trabecular perimeter
10.0	3 months	Control	35.31 + 6.63	11.40 ± 2.11	0.375 ± 0.034	0.53 ± 0.14
		Autograft	31.83 + 8.05	9.42 ± 2.90	0.415 ± 0.091	1.25 ± 0.87
		POP	29.04 + 7.31	9.22 ± 2.31	0.377 ± 0.021	0.99 ± 0.54

Data expressed as the mean + standard error of the mean. Bone mineralization rate was measured after labeling with tetracycline on the 20th and again on the 10th day prior to sacrifice.

defect [85]. This permits POP to provide structural support and prevent fibrous tissue ingrowth, while facilitating creeping substitution.

For promoting intertransverse posterior spinal fusion, POP should be used in combination with other grafts as graft enhancement material [91,92]. When used in combination with bone procured from the decompression sites, the results were equivalent to that of autogenous iliac bone crest bone for lumbar fusion [93]. POP is also a suitable vehicle through which osteoinductive materials may express optimal osteoactivity [94]. POP may also serve as an ideal carrier for osteoinductive agents as BMPs. Combination of POP with bovine osteogenic factors [95] or fibroblast growth factor (FGF) [96] can induce and increase the rate of bone formation.

Plaster of Paris can also be used as an effective carrier for local delivery of antibiotics. Antibiotic-loaded cylindrical pellets prepared from bone graft or demineralized bone matrix elute 70% and 45% of their antibiotic load by 24 hours, and negligible amounts are detected at 1 week. Plaster of Paris releases 17% of its load by 24 hours, with trace amounts detected at 3 weeks, while polymethylmethacyilate elutes 7% at 24 hours, with trace amounts detected for as long as 14 days [97]. Coating plaster of Paris pellets with a poly(lactide-co-glycolide) polymer decreases the burst effect of the elusion occurring on the first day and extends efficient release to more than 5 weeks [98].

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