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REVIEW ARTICLE

# An update on bone substitutes for spinal fusion

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**Abstract** With the current advances in spinal surgery, an understanding of the precise biological mechanism of each bone substitute is necessary for inducing successful spinal fusion. In this review, the categories of bone substitutes include allografts, ceramics, demineralized bone matrix, osteoinductive factors, autogenous platelet concentrate, mesenchymal stem cells, and gene therapy. Further, clinical studies have been evaluated by their levels of evidence in order to elucidate the precise effect of the bone substitute employed and to establish clinical guidance. This article will review both clinical studies based on evidence and basic research in current advances in order to avoid as far as possible any chances of failure in the future and to understand cellular biology in novel technologies.

**Keywords** Bone substitutes · Spinal fusion · Demineralized bone matrix · Bone morphogenic protein · Gene therapy

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## Introduction

Spinal fusion is one of the frequently employed procedures for treating various morbidities such as deformity, trauma, and degenerative disc disease with instability. Over the past century, this technique has been enhanced by the use of autogenous bone grafting. However, a significant rate of pseudoarthrosis has been reported in the literature, ranging from 5 to 43% [24]. Moreover, host risk factors such as smoking, diabetes, and osteoporosis have been implicated as a cause of pseudoarthrosis. Recently, sophisticated technologies involving the internal fixation of the spine have been developed increasing the fusion rate. Despite modern advanced techniques, symptomatic pseudoarthrosis still occurs in 10–15% of cases [16, 62, 110]. The consequences of pseudoarthrosis include poor clinical outcomes and require extensive medical expenditure. These problems have led surgeons to devise new biological strategies, to search for alternative substitutes for autogenous bone grafting, and to stimulate bone fusion.

The biological processes involved in bone regeneration require three critical elements as follows: an osteogenic potential that is capable of directly providing cells to the newly forming bone, osteoinductive factors that are able to cause the osteoblastic differentiation of osteoprogenitor stem cells, and osteoconductive scaffold that facilitates neovascularization and supports the ingrowth of bone. The ideal bone graft material possesses all of these three properties along with an optimal biological reaction and without a risk of transmission of diseases. Autogenous bone grafts possess each of these three essential properties; therefore, they have been considered as the first choice for graft material in patients undergoing spinal fusion. However, a limited availability of bone and a frequent incidence of graft site pain that persists into the postoperative period

**Table 1** Current approach to grades of recommendations

| Grade of recommendation | Clarity of risk/benefit | Methodologic strength of supporting evidence | Implications |
|---|---|---|---|
| 1A | Clear | Randomized trials without important limitations | Strong recommendation; can apply to most patients in most circumstances without reservation |
| 1B | Clear | Randomized trials with important limitations (inconsistent results, methodologic flaws) | Strong recommendations, likely to apply to most patients |
| 1C+ | Clear | No RCTs, but RCT results can be unequivocally extrapolated, or overwhelming evidence from observation studies | Strong recommendation: can apply to most patients in most circumstances |
| 1C | Clear | Observation studies | Intermediate-strength recommendation; may change when stronger evidence available |
| 2A | Unclear | Randomized trials without important limitations | Intermediate-strength recommendation; best action may differ depending on circumstances or patients' or societal values |
| 2B | Unclear | Randomized trials with important limitations (inconsistent results, methodologic flaws) | Weak recommendation; alternative approaches likely to be better for some patients under some |
| 2C | Unclear | Observation studies | Very weak recommendations; other alternatives may be equally reasonable |

have been reported [1, 80]. In an attempt to avoid the morbidity associated with harvesting a graft and in order to increase the fusion rate, several bone substitutes have been developed. Although none of the existing bone substitutes exhibit all three of the principal properties in their current stages, various bone graft substitutes have demonstrated their capacity in basic and clinical studies. Current research in molecular biology has demonstrated new technologies for bone regeneration. Particularly, with advances in regional gene therapy as well as in osteoinductive proteins and osteoconductive carrier matrices, spinal fusion procedures are advancing into a new era of bone biology.

Along with the current advances in spinal fusion in minimally invasive surgery, an understanding of the precise biological mechanism of each bone substitute is necessary for achieving successful results. Moreover, clinical studies should be evaluated according to their levels of evidence in order to elucidate the precise effect of each bone substitute and to establish clinical guidance. Several orthopedic journal publications categorize clinical articles into 1–5 "levels" based on their respective designs [68, 105]. For this review, the authors searched the Medline database for articles in peer-reviewed journals, which contained original data on bone substitutes for spinal fusion including allograft, ceramics, demineralized bone matrix, osteoinductive factors, autogenous platelet concentrate, mesenchymal stem cells, and gene therapy. This article will review both clinical studies based on evidence and basic research on novel technologies not available yet for clinical use.

## Literature search and criteria

A Medline search was conducted via PubMed using various combinations of the following key words: spinal fusion, allograft, ceramics, hydroxyapatite, beta tricalcium phosphate, demineralized bone matrix, osteoinductive growth factors, bone morphogenic protein, autogenous platelet concentrate, mesenchymal stem cells, and gene therapy. When a new article was found, the "related articles" option of PubMed was used to further expand the search. The search was performed on articles written in English only. Inclusion criteria for clinical studies were as follows: studies evaluating efficacy and rates of fusion by use of a particular bone material, the surgical procedures and grafting technique well described; evaluation technique for fusion well-described, the number of patients more than ten and follow-up more than a year (Table 1).

## Evaluation of the level of evidence in clinical studies

The evaluation of the level of evidence in clinical studies was conducted according to previously published journal reports [68, 105]. Each article was categorized into one of five levels on the basis of its study design. Well-designed randomized controlled trials and systematic reviews of such trials were classified as Level I studies; randomized controlled trials with less than 80% follow-up or unclear randomization and prospective cohort studies with control groups, as Level II; case-control studies and retrospective cohort studies, as Level III; case series, as Level IV; and

**Table 2** Summary of clinical literatures on allograft for spinal fusion

| Ref. | Description of study |
|---|---|
| Cervical spine | |
| Brown et al. [17] | A roentgenographic evaluation of frozen allografts versus autografts in anterior cervical spine fusions |
| Young et al. [109] | A retrospective comparison of cadaveric fibular allografts and autologous iliac crest grafts for cervical anterior spinal fusion |
| Savolainen et al. [77] | A retrospective comparison of iliac crest versus artificial bone grafts in cervical fusions |
| Zhang et al. [112] | A retrospective analysis of cervical spondylotic myelopathy cases treated by anterior fusion |
| Zdeblick et al. [111] | A retrospective analysis of freeze-dried allograft bone for anterior cervical fusions |
| Bishop et al. [8] | A prospective study of anterior cervical fusion in order to compare allografts and autografts |
| Lumbar spine | |
| Jorgenson et al. [48] | A prospective analysis of autograft versus allograft in posterolateral lumbar fusion in the same patient |
| An et al. [3] | A prospective comparison of autograft versus allograft for adult posterolateral lumbar spine fusion |
| Scoliosis | |
| Aurori et al. [5] | A retrospective comparison of the incidence of pseudoarthrosis in fusions for scoliosis supplemented with autografts and frozen allografts |
| Dodd et al. [29] | A case-control study of the use of autograft versus allograft bone in the surgery of idiopathic adolescent scoliosis with instrumentation |

| Ref. | Evidence level | Conclusion |
|---|---|---|
| Cervical spine | | |
| Brown et al. [17] | III | No significant difference was noted in the fusion rates |
| Young et al. [109] | III | The use of fibular allografts for anterior cervical fusion can be performed with acceptable rates of fusion as compared to the use of autologous iliac crest grafts |
| Savolainen et al. [77] | III | There was no significant difference in the fusion rate; moreover, donor site complications were not observed in patients with allografts |
| Zhang et al. [112] | IV | Autografts yielded higher fusion rates and better overall results than did allografts |
| Zdeblick et al. [111] | III | For two-level procedures, the nonunion rate with allografts was higher than that with autografts and graft collapse was more commonly observed with allografts |
| Bishop et al. [8] | II | Autografts were found to be superior to allografts after both single- and multiple-level anterior cervical fusion procedures |
| Lumbar spine | | |
| Jorgenson et al. [48] | II | Ethylene oxide-treated allograft is inferior to autograft and should not be used for posterior lumbar fusions |
| An et al. [3] | II | Bone densitometry results also showed that autograft sites gave significantly greater bone density, followed by mixture, frozen allografts, and freeze-dried allografts |
| Scoliosis | | |
| Aurori et al. [5] | III | The incidence of pseudarthrosis was not significantly different |
| Dodd et al. [29] | III | There was no difference in a radiographic assessment of bone graft mass nor in the maintenance of the curve correction |

expert opinions, as Level V studies. Summaries of clinical literatures were provided in Tables 2, 3, 4, 5, and 6.

Grades of recommendation have been done by using Guyatt criteria [78]. This grading system has been pointed out to be the most compelling and innovative approach where the strength of recommendation depends not only on the methodology, but also on the trade-off between benefits and risks plus costs, as judged by expert opinion and the literature [34].

## Allografts

Allografts are obtained from cadaver sources and have been traditionally used as substitutes for autogenous bone

**Table 3** Summary of clinical literatures on ceramics for spinal fusion

| Ref. | Description of study |
|---|---|
| Cervical spine | |
| Thalgott et al. [84] | A retrospective study of coralline hydroxyapatite as a bone replacement in anterior interbody fusion in cervical spine |
| Lumbar spine | |
| Thalgott et al. [86] | A retrospective study of coralline hydroxyapatite as a bone replacement in anterior interbody fusion in lumbar spine |
| Chen et al. [25] | A prospective study of calcium sulfate with local autograft bone compared with autologous iliac bone graft for instrumented short-segment spinal fusion |
| Epstein et al. [31] | Case series of beta tricalcium phosphate as a bone expander for instrumented posterolateral lumbar fusions |
| Korovessis et al. [51] | A prospective randomized study comparing the efficiency using either iliac bone autograft and coralline hydroxyapatite mixed with local bone and bone marrow |
| Scoliosis | |
| Xie et al. [106] | Case series of porous biphasic ceramics in the human spine |
| Passuti et al. [69] | Case series of macroporous calcium phosphate ceramics for scoliosis surgery |
| Ransford et al. [74] | A prospective randomized study to evaluate the use of a synthetic porous ceramic as a bone graft substitute in posterior spinal fusion for idiopathic scoliosis |
| Muschik et al. [66] | A prospective study of $\beta$-tricalcium phosphate as a bone graft extender for posterior spinal fusion in scoliosis cases |
| Others | |
| Heise et al. [41] | Retrospective review of hydroxyapatite ceramic as bone extenders for spinal fusion |

| Ref. | Evidence level | Conclusion |
|---|---|---|
| Cervical spine | | |
| Thalgott et al. [84] | III | The use of coralline hydroxyapatitewith rigid anterior plating seems promising as a bone replacement in the cervical spine |
| Lumbar spine | | |
| Thalgott et al. [86] | III | Coralline hydroxyapatite is a practicable anterior lumbar interbody fusion alternative to autograft and allograft with rigid posterior fixation |
| Chen et al. [25] | II | Calcium sulfate pellets may play a role as a bone graft extender in short-segment spinal fusion |
| Epstein et al. [31] | IV | B-TCP and laminectomy autograft (50:50 mix) effectively promoted posterolateral lumbar fusion |
| Korovessis et al. [51] | I | The incorporation of coralline hydroxyapatite mixed with local bone and bone marrow needs an adequate bleeding bone surface |
| Scoliosis | | |
| Xie et al. [106] | IV | The porous biphasic ceramic should be well mixed or layered with autogenous bone in order to achieve satisfied new bone ingrowth in posterior spinal fusion |
| Passuti et al. [69] | IV | The microporous calcium phosphate ceramics were demonstrated the bioactivity and the osteoconduction in human spinal fusion |
| Ransford et al. [74] | I | Porous ceramic is a safe and effective bone substitute |
| Muschik et al. [66] | II | $\beta$-Tricalcium phosphates were a valuable alternative to allografts as a bone extender, even when large amounts of bone were needed |
| Others | | |
| Heise et al. [41] | III | There was no difference between simple autologous bone grafts and hydroxyapatite ceramic bone extenders |

graft. Allografts have an osteoconductive scaffold with minimal osteoinductive factors; however, they are not able to provide osteogenic cells because of the processing that they undergo in order to decrease their antigenicity. Despite several aseptic techniques, allografts pose potential risks of bacterial contamination. Another disadvantage of allografts is the possible spread of viral transmission diseases such as those caused by the human immunodeficiency virus and hepatitis virus. In addition, in comparison to autografts, an allograft is incorporated

**Table 4** Summary of clinical literatures on demineralized bone matrices for spinal fusion

| Ref. | Description of study |
|---|---|
| Cervical spine | |
| An et al. [4] | A prospective comparison of an allograft-demineralized bone matrix composite versus autograft in anterior cervical spinal fusion |
| Lumbar spine | |
| Thalgott et al. [85] | A case series study for anterior lumbar interbody fusion with DBM composites consisting of titanium mesh cages, coralline hydroxyapatite |
| Girardi et al. [37] | Retrospective review of allograft demineralized bone matrix as bone extenders for posterolateral lumbar spine fusion |
| Sassard et al. [76] | Retrospective review of Grafton demineralized bone matrix as bone extenders for posterolateral lumbar spine fusion |
| Cammisa et al. [21] | A multicenter prospective study that compared the effectiveness of a Grafton DBM gel composite with an iliac crest autograft in posterolateral spinal fusion |
| Vaccaro et al. [96] | A prospective comparison of bone grafting with Grafton DBM putty enriched with aspirated bone marrow, DBM putty combined with iliac crest autograft, or autograft |
| Scoliosis | |
| Price et al. [73] | A retrospective study of determining the efficacy of a DBM composite consisting of DBM and bone marrow for posterior spinal fusion in scoliosis cases |

| Ref. | Evidence level | Conclusion |
|---|---|---|
| Cervical spine | | |
| An et al. [4] | I | The allograft-demineralized bone matrix construct gives a higher rate of graft collapse and pseudarthrosis |
| Lumbar spine | | |
| Thalgott et al. [85] | IV | The DBM composite was effective for anterior interbody fusion of the lumbar spine when used as part of a rigidly instrumented circumferential fusion |
| Girardi et al. [37] | II | The use of these bone graft extenders may decrease the required amount of autologous bone graft |
| Sassard et al. [76] | III | The percentage of patients fused was similar in both groups |
| Cammisa et al. [21] | II | Grafton DBM could only extend an autograft that was smaller than is normally required to achieve a solid spinal fusion |
| Vaccaro et al. [96] | II | The DBM composite consisting of DBM putty and aspirated bone marrow offer a similar performance as the autograft in posterolateral spinal fusion |
| Scoliosis | | |
| Price et al. [73] | III | The fusion rates were comparable to those of iliac bone autografts and DBM composites |

slower and less completely with decreased vascularization and osteoconduction [87]. There are several clinical reports that discuss the efficacy of allografts as bone substitutes in posterior spinal fusion (Table 2). Jorgenson et al. [48] conducted a prospective analysis of autografts versus allografts in posterolateral lumbar fusion in the same patient and concluded that an ethylene oxide-treated allograft is inferior to an autograft and should not be used for posterior lumbar fusions (Level II). Further, An et al. [3] conducted a prospective comparison of autografts and allografts for adult posterolateral lumbar spinal fusion and reported that autografts resulted in significantly greater bone density, followed by the mixture of autografts and allografts, frozen allografts, and freeze-dried allografts (Level II). These reports indicate that allografts alone were not able to achieve a sufficient fusion rate for posterior spinal fusion in the adult patients. However, there are several reports recommending the use of allografts as bone extenders in adolescent idiopathic scoliosis. Aurori et al. [5] retrospectively compared the incidence of pseudoarthrosis in fusions for scoliosis supplemented with autografts and frozen allografts which were obtained from femoral heads and reported that the incidence of pseudoarthrosis was not significantly different (Level III). Dodd et al. conducted a case-control study of the use of autografts versus allografts which were from femoral heads in the surgery of idiopathic adolescent scoliosis. They reported that there was no difference, either in a

**Table 5** Summary of clinical literatures on osteoinductive growth factors for spinal fusion

| Ref. | Description of study |
|---|---|
| Cervical spine | |
| Baskin et al. [7] | A prospective pilot trial for the use of rhBMP-2/collagen sponge with a fibular allograft and anterior cervical plate |
| Shields et al. [79] | Retrospective review of patients who underwent an anterior cervical fusion using high-dose rhBMP-2/collagen sponge |
| Vaidya et al. [98] | Retrospective review of patients undergoing anterior cervical spinal fusion and instrumentation |
| Lumbar spine | |
| Boden et al. [14] | A prospective human pilot trial of the use of rhBMP-2/collagen inside lumbar interbody spinal fusion cages |
| Burkus et al. [18–20] | A prospective study of the use of rhBMP-2/collagen sponge with allograft dowels or tapered cylindrical fusion devices in anterior lumbar interbody fusion |
| Slosar et al. [81] | A prospective study comparing allografts for anterior lumbar interbody fusions with and without the addition of rhBMP-2 with posterior instrumentation |
| McClellan et al. [61] | Retrospective review of transforaminal lumbar interbody fusion with BMP |
| Pradhan et al. [72] | A prospective cohort study of ALIF using femoral ring allografts and rhBMP-2 |
| Boden et al. [10] | A prospective randomized clinical pilot study of the use of rhBMP-2 for posterolateral fusion |
| Dimar et al. [28] | A prospective randomized study comparing the use of iliac crest bone grafts to that of rhBMP-2 for single-level posterolateral fusions |
| Johnsson et al. [47] | A randomized clinical trial for ensuring OP-1 efficacy in noninstrumented posterolateral fusion as evaluated by radiostereometric analysis |
| Vaccaro et al. [93, 94] | Case series study using OP-1 putty for bone extenders as an adjunct to iliac crest autografts without instrumentation in posterolateral lumbar fusions |
| Vaccaro et al. [91, 92, 95] | A prospective randomized controlled study comparing OP-1 putty and iliac crest autograft without instrumentation in posterolateral spinal fusion |
| Kanayama et al. [50] | A prospective randomized controlled study of OP-1 in posterolateral lumbar fusion with instrumentation |

| Ref. | Evidence level | Conclusion |
|---|---|---|
| Cervical spine | | |
| Baskin et al. [7] | I | 100% fusion rate was observed |
| Shields et al. [79] | IV | A total of 23.2% patients suffered complications such as hematomas, dysphagia, and excessive edema |
| Vaidya et al. [98] | III | Dysphagia was shown to be significantly more frequent and more severe in patients in whom rhBMP-2 was used |
| Lumbar spine | | |
| Boden et al. [14] | I | After a 2-year follow up, fusion was observed to occur more reliably in patients treated with rhBMP-2-filled cages than in controls treated with autogenous bone graft |
| Burkus et al. [18–20] | II | The use of these rhBMP-2 composites were promising methods of facilitating anterior intervertebral spinal fusion |
| Slosar et al. [81] | II | The excellent results obtained with the use of r hBMP-2 |
| McClellan et al. [61] | IV | A high rate of bone resorption defects and assumed that the osseous remodeling potential of rhBMP-2 may lead to bone resorption within the vertebral body |
| Pradhan et al. [72] | II | The nonunion rate of femoral ring allografts with rhBMP-2 was higher than that of femoral ring allografts with iliac bone autografts |
| Boden et al. [10] | I | The fusion rate of the rhBMP-2/ceramic granules without instrumentation group was 100%, which was superior to the autograft with instrumentation group (40%) |
| Dimar et al. [28] | II | The rhBMP-2 group demonstrated increased fusion rates as compared to the autograft group |
| Johnsson et al. [47] | I | The OP-1 made reduced vertebral movement with bone formation as well as autograft bone |
| Vaccaro et al. [93, 94] | IV | They could not demonstrate the statistical superiority of OP-1 putty combined with autograft over an autograft alone |

**Table 5** continued

| Ref. | Evidence level | Conclusion |
|---|---|---|
| Vaccaro et al. [91, 92, 95] | I | OP-1 putty was able to achieve solid fusion in the absence of autograft with favorable fusion rates that were comparable to those of the autograft control group |
| Kanayama et al. [50] | I | The fusion success rate evaluated by surgical exploration was inferior to that in the control (hydroxyapatite-tricalcium phosphate/autograft) group |

**Table 6** Summary of clinical literatures on autologous platelet concentrate for spinal fusion

| Ref. | Description of study |
|---|---|
| Lumbar spine | |
| Hee et al. [40] | A prospective study of AGF in instrumented transforaminal lumbar interbody spinal fusion |
| Lowery et al. [57] | Retrospective case series using autologous growth factor concentrate in lumbar posterolateral spinal fusion |
| Weiner et al. [103] | Retrospective review of an autograft alone and an autograft with AGF in posterolateral spinal fusion |
| Carreon et al. [23] | Retrospective review of platelet gel in instrumented posterolateral spinal fusion |

| Ref. | Evidence level | Conclusion |
|---|---|---|
| Lumbar spine | | |
| Hee et al. [40] | II | The use of AGF in TLIF procedures did not increase the overall fusion rates; however, it might promote a faster rate of fusion |
| Lowery et al. [57] | IV | AGF may enhance the formation of new bone in lumbar spinal fusion when used in combination with autografts |
| Weiner et al. [103] | III | The use of AGF resulted in inferior rates of fusion compared with those of autograft alone |
| Carreon et al. [23] | III | Platelet gel failed to enhance the fusion rate when added to autograft |

radiographic assessment of bone graft mass or in the maintenance of the curve correction [29] (Level III).

*Grade of recommendation* is 2B for use of allograft alone for posterior spinal fusion in adults, while it is grade 1C+ for its use for adolescent idiopathic scoliosis cases.

One of the merits of using allografts is that cortical allografts have substantial structural strength and are suitable for anterior interbody fusion. There are several articles that report the advantage of using allografts in anterior cervical fusion. Brown et al. compared serial roentgenograms of anterior cervical spinal fusion using cadaveric iliac crest allografts and autografts. They reported that no significant difference was noted in the fusion rates [17] (Level III). Further, Young et al. retrospectively compared cadaveric fibular allografts and autologous iliac crest grafts for cervical anterior spinal fusion. They concluded that the use of fibular allografts for anterior cervical fusion can be performed with acceptable rates of fusion as compared to the use of autologous iliac crest grafts [109] (Level III). Savolainen et al. [77] recommended the use of allografts for anterior cervical fusion because there was no significant difference in the fusion rate; moreover, donor site complications were not observed in patients with allografts (Level III). However, as can be expected, there were several articles reporting that allografts were inferior to autografts for even anterior spinal fusion. Zhang et al. [112] reported that an analysis of cervical spondylotic myelopathy cases treated by anterior fusion and autografts yielded higher fusion rates and better overall results than did allografts (Level IV). Zdeblick et al. [111] reported that particularly for two-level procedures, the nonunion rate with allografts was higher than that with autografts and graft collapse was more commonly observed with allografts than with autografts (Level III). Bishop et al. [8] conducted a prospective study of anterior cervical fusion in order to compare allografts and autografts; autografts were found to be superior to allografts after both single- and multiple-level anterior cervical fusion procedures with respect to the maintenance of cervical interspace height, interspace angulation, and radiographic and clinical fusion success rates (Level II).

*Grade of recommendation* is 1C+ for use of structural allografts for single level anterior cervical interbody fusion while it is grade 2B for multilevel interbody fusion and for corpectomy defects.

## Ceramics

Ceramic scaffolds were conceived and produced as osteoconductive and biodegradable bone graft substitutes that could be supplied in unlimited quantities without donor site morbidity and infectious risk. They are nontoxic, nonimmunogenic, and easy to sterilize. However, their disadvantages are that they are brittle and have little shear strength. Therefore, ceramics are used with rigid internal fixation and protected from loading forces until they are incorporated into bone. The most commonly used ceramic scaffolds for spinal fusion are calcium phosphates such as hydroxyapatite, tricalcium phosphate, and a combination of these materials. After confirming the efficacy of osteoconductivity in animal studies [6, 35], the calcium phosphates have already been used for clinical purposes (Table 3).

Generally, the ceramic scaffolds can be used as bone graft extenders to expand an existing quantity of available local autograft bone chips for posterolateral spinal fusion. With recent rigid spinal instrumentation, several studies have reported that ceramic scaffolds are efficient bone graft extenders in posterolateral spinal fusion [25] (Level II), [31] (Level IV), [106] (Level IV). Although ceramic scaffolds appear to be established as bone graft extenders, there is an opinion that hydroxyapatite is inappropriate for intertransverse posterolateral fusion because the host bleeding bone surface in this area is small. Korovessis et al. conducted a prospective randomized study comparing the evolution of instrumented posterolateral lumbar fusion using either iliac bone autograft or coralline hydroxyapatite mixed with local bone and bone marrow. They concluded that iliac bone autografts remained the gold standard for achieving solid posterolateral fusion, that the incorporation of coralline hydroxyapatite mixed with local bone and bone marrow needs an adequate bleeding bone surface, and recommended the use of hydroxyapatite over decorticated laminae (Level I) [51].

*Grade of recommendation* is 2B for use of ceramics alone for posterior lumbar spine fusion in adult patients.

On the other hand, successful results have been reported for the implantation of ceramic scaffolds for posterior spinal fusion in scoliosis cases, which requires multiple bone grafts [69] (Level IV), [41] (Level III). Ransford et al. [74] conducted a prospective randomized study to evaluate the use of a synthetic porous ceramic as a bone graft substitute in posterior spinal fusion for idiopathic scoliosis; they concluded that porous ceramic is a safe and effective bone substitute (Level I). Muschik et al. [66] also reported a preliminary prospective study to evaluate the ability of $\beta$-tricalcium phosphate as a bone graft extender for posterior spinal fusion in scoliosis cases and reported that they were a valuable alternative to allografts as a bone extender, even when large amounts of bone were needed (Level II). However, a limitation associated with these studies is the fact that the confirmation of fusion is based on X-ray or CT scan, thus making it impossible to confirm that ceramics actually support ingrowth of bone.

*Grade of recommendation* is 1B for use of ceramics as a bone substitute in posterior spinal fusion for adolescent idiopathic scoliosis cases.

For anterior spinal fusion, ceramic scaffolds need to be used with rigid internal fixation. Thalgott et al. reported a retrospective study to evaluate the efficacy of coralline hydroxyapatite as a bone replacement in anterior interbody fusion in both the cervical and lumbar spine. They concluded that the use of coralline hydroxyapatite with rigid anterior plating appeared to be a promising bone replacement in anterior fusion but it was not recommended for stand-alone anterior interbody fusion [84] (Level III), [86] (Level III).

*Grade of recommendation* is 1C for use of ceramics as a bone substitute for anterior cervical interbody fusion when combined with interbody fusion.

Recently, several new osteoconductive agents, for example, calcium sulphate (plaster of Paris), bioactive glass, dual hydroxyapatite composite with porous and solid parts, poly (propylene glycol-co-fumaric acid), and highly porous hydroxyapatite, were tested in animal studies and demonstrated to possess an osteoconductive ability [39, 44, 49, 55, 65]. Although the implantation of ceramic scaffold alone has been associated with an efficient outcome for bone fusion, these osteoconductive scaffolds have greater effects when they are coupled with other osteogenic or osteoinductive agents. Ceramic scaffolds can become effective vehicles for the delivery of these factors.

## Demineralized bone matrices

Demineralized bone matrices (DBMs) are generated by the acid extraction of allograft bone, resulting in loss of most of the mineralized component but giving rise to type I collagen and noncollagenous proteins, including numerous growth factors. DBMs do not have structural strength but possess osteoconductivity and the osteoinductive agents. The osteoinductive ability in DBMs to stimulate bone regeneration is dependent upon the activity of the bone morphogenic proteins (BMPs). Commercially available DBMs have demonstrated the variability of their osteoinductive potential that may reflect differences in their BMP content in rat spinal fusion models [53, 71, 100].

While several studies have demonstrated the success of posterolateral spinal fusion using DBMs alone or in conjunction with autograft in a rabbit and nonhuman primate model [27, 56, 60, 108], certain clinical studies also support the efficacy of DBMs as bone graft extenders for posterolateral spinal fusion [37] (Level III), [76] (Level III) (Table 4). Cammisa et al. [21] conducted a multicenter prospective study that compared the effectiveness of a Grafton DBM gel composite with an iliac crest autograft in posterolateral spinal fusion; they reported that Grafton DBM could only extend an autograft that was smaller than is normally required to achieve a solid spinal fusion (Level II). Vaccaro et al. [96], in a prospective study, also reported that the DBM composite consisting of DBM putty and aspirated bone marrow offer a similar performance as the autograft in posterolateral spinal fusion (Level II). Spinal fusion for scoliosis surgery requires multiple bone grafts, which is a suitable indication for the use of bone substitutes as bone graft extenders. Price et al. [73] conducted a retrospective study of determining the efficacy of a DBM composite consisting of DBM and bone marrow for posterior spinal fusion in scoliosis cases and reported that the fusion rates were comparable to those of iliac bone autografts and DBM composites (Level III).

*Grade of recommendation* is 1C+ for use of DBM as a bone graft extender for posterior fusion in both adult patients and adolescent patients with scoliosis.

Although it was confirmed that DBM has an efficient osteoinductivity, the efficacy of DBM in anterior spinal fusion has not been testified enough. Commercially available DBM can be obtained in a variety of forms; these forms include injectable gel, flex strips, and putty. However, each of these forms lacks structural strength. Therefore, DBM composites should consist of materials possessing shear strength when they are to be used for anterior spinal fusion in a clinical setting. Thalgott et al. [85] conducted a case series study for anterior lumbar interbody fusion with DBM composites consisting of titanium mesh cages, coralline hydroxyapatite, and DBM; they concluded that the DBM composite was effective for anterior interbody fusion of the lumbar spine when used as part of a rigidly instrumented circumferential fusion (Level IV). On the other hand, An et al. [4] analyzed the fusion rates of allograft-demineralized bone matrix composite as compared with autograft in anterior cervical fusion prospectively and concluded that the allograft-demineralized bone matrix construct gives a higher rate of graft collapse and pseudarthrosis (Level I).

*Grade of recommendation* is 1C for use of DBM as a bone substitute when used for anterior lumbar interbody fusion with a structural carrier. However, as there is one Level I study demonstrating a higher rate of graft collapse and pseudarthrosis in anterior cervical fusion, further clinical studies are required in order to elucidate the applicability of DBM use for anterior spinal fusion.

## Osteoinductive growth factors

In 1965, Marshal Urist [88] first observed that DBM possessed an osteoinductive ability. Significant efforts in protein isolation, purification, and characterization identified osteoinductive proteins, including bone morphogenetic proteins (BMPs). BMPs are members of the transforming growth factor-beta superfamily. By binding to specific receptors present on the surface of the osteogenic progenitor, intracellular cascades—which resemble endochondral ossification—are activated. BMPs have been demonstrated to work by stimulating pleuripotent mesenchymal cells to differentiate into osteoblasts and produce a bony matrix in an in vitro study.

In the early phases, a large amount of bone is necessary for extracting BMPs even in partially purified forms. They comprise only 0.1% by weight of all bone proteins and are not accessible until the bone matrix has been demineralized [89, 90]. Therefore, BMPs remain rare and very expensive. With advances in molecular cloning and sequencing technology, it has been possible to produce large quantities of recombinant proteins as a singular molecular species and without immunogenic properties. Recombinant BMP-2 (rhBMP-2) and recombinant BMP-7 (osteogenic protein-1: OP-1) are the most widely studied in animals; moreover, they are the only BMPs currently being administered in human clinical studies (Table 5).

Recombinant BMPs (rhBMPs) are soluble, and they can diffuse away from the fusion site easily and become inactivated in vivo when they are used alone. Therefore, rhBMPs are combined with a carrier matrix that serves to retain the concentration and releases them consistently over time. These carrier matrices may also possess osteoconductive capacities or structural strength; they are now being tested in order to provide an ideal combination that may adjust to each clinical situation.

Multiple animal studies have reported the usefulness of rhBMP in spinal fusion. They have demonstrated the efficacy of rhBMP-2 and OP-1 as bone substitutes for autografts, resulting in more rapid and reliable healing than that observed in control groups using various carrier matrices in both anterior and posterolateral spinal fusion in nonhuman primate, sheep, and rabbit models [9, 11, 12, 15, 38, 46, 75].

Boden et al. conducted a prospective randomized clinical pilot study of the use of rhBMP-2 for posterolateral fusion in humans. In this study, they randomly divided the enrolled patients into three treatment groups as follows: autograft with instrumentation, rhBMP-2/ceramic granules with instrumentation, and rhBMP-2/

ceramic granules only without instrumentation. They reported that the fusion rate of the rhBMP-2/ceramic granules without instrumentation group was 100%, which was superior to the autograft with instrumentation group (40%) [10] (Level I). Following this pilot study, Dimar et al. conducted a prospective randomized study comparing the use of iliac crest bone grafts to that of rhBMP-2 combined with a carrier consisting of bovine collagen and tricalcium/hydroxyapatite for single-level posterolateral fusions. They also reported that the rhBMP-2 group demonstrated increased fusion rates as compared to the autograft group [28] (Level II).

*Grade of recommendation* is 1A for use of BMP-2 for posterior spinal fusion in adult patients.

Boden et al. also described the human pilot trial of the use of rhBMP-2/collagen inside lumbar interbody spinal fusion cages. Although the number of patients enrolled in this study was small, they reported that after a 2-year follow up, fusion was observed to occur more reliably in patients treated with rhBMP-2-filled cages than in controls treated with autogenous bone graft [14] (Level I). Burkus et al. also conducted a prospective study of the use of rhBMP-2/collagen sponge with allograft dowels or tapered cylindrical fusion devices in anterior lumbar interbody fusion and concluded that the use of these rhBMP-2 composites were promising methods of facilitating anterior intervertebral spinal fusion [18] (Level II), [19] (Level II), [20] (Level II). Further, Slosar et al. [81] reported a prospective study comparing patients treated with allografts for anterior lumbar interbody fusions with and without the addition of rhBMP-2 with posterior instrumentation and demonstrated the excellent results obtained with the use of rhBMP-2 (Level II). These reports supported the use of rhBMP-2 for anterior lumbar interbody fusion. On the other hand, there are reports that rhBMP-2 causes aggressive resorption of an implanted graft before osteoinduction in vertebral interbody fusion. McClellan et al. [61] retrospectively investigated cases with a transforaminal lumbar interbody fusion with BMP; they reported a high rate of bone resorption defects and assumed that the osseous remodeling potential of rhBMP-2 may lead to bone resorption within the vertebral body (Level IV). Pradhan et al. reported that the nonunion rate among patients who received femoral ring allografts with rhBMP-2 was higher than that in patients who received femoral ring allografts with iliac bone autografts. They concluded that this result appeared to be caused by the aggressive resorptive phase of allograft incorporation, which occurs before the osteoinduction phase [72] (Level II). These results suggest that careful use of rhBMP-2 for anterior lumbar interbody fusion is more beneficial than the use of autografts, although further clinical studies are required.

*Grade of recommendation* is 2A for use of BMP-2 for anterior lumbar interbody fusion.

With respect to anterior cervical spinal fusion, Baskin et al. reported a prospective pilot trial for the use of rhBMP-2/collagen sponge with a fibular allograft and anterior cervical plate; a total absence of adverse events and a 100% fusion rate were observed. Moreover, after a 2-year follow up, a mean improvement superior to that obtained in the iliac autograft control group was observed with respect to neck disability and arm pain scores [7] (Level I). In contrast, there are the important studies that caution against the use of high-dose rhBMP-2 for cervical anterior spinal fusion. Shields et al. reported a retrospective review of patients who underwent an anterior cervical fusion using high-dose rhBMP-2/collagen sponge. They reported that a total of 23.2% patients suffered complications such as hematomas, dysphagia, and excessive edema [79] (Level IV). Vaidya et al. [98] also reported that complications were associated with anterior cervical spinal fusion using rhBMP-2; further, dysphagia was shown to be significantly more frequent and more severe in patients in whom rhBMP-2 was used (Level III).

*Grade of recommendation* is 2A for use of BMP-2 for anterior cervical interbody fusion. Therefore, rhBMP-2 should be used cautiously for anterior cervical spinal fusions until more research is undertaken and these clinical issues are resolved.

In an animal study, another major rhBMP, OP-1, was tested in noninstrumented posterolateral fusions and demonstrated high fusion rates [15, 38, 46, 75]. Johnsson et al. performed a randomized clinical trial for ensuring OP-1 efficacy in noninstrumented posterolateral fusion as evaluated by radiostereometric analysis. Although the OP-1 implant did not yield better stabilizing bony fusion than did the autograft bone, the OP-1 made reduced vertebral movement with bone formation as well as autograft bone [47]. (Level I). Vaccaro et al. conducted a study using OP-1 putty for bone extenders as an adjunct to iliac crest autografts without instrumentation in posterolateral lumbar fusions. They could not demonstrate the statistical superiority of OP-1 putty combined with autograft over an autograft alone; however, there were no adverse events related to the use of OP-1. These studies demonstrated the clinical feasibility of using OP-1 as a bone substitute and bone extender in spinal fusion [93] (Level IV), [94] (Level IV). Vaccaro et al. also performed a prospective randomized controlled study comparing OP-1 putty and iliac crest autograft without instrumentation in posterolateral spinal fusion, and they reported that OP-1 putty was able to achieve solid fusion in the absence of autograft with favorable fusion rates that were comparable to those of the autograft control group after a 4-year follow up [91] (Level I), [92] (Level I), [95] (Level I). These

reports supported the use of OP-1 for posterolateral fusion. However, there was also a report with findings conflicting with those of the above study. Kanayama et al. conducted a prospective randomized controlled study of OP-1 in posterolateral lumbar fusion with instrumentation; further, they performed the removal of instrumentation along with the surgical exploration of the fusion site and collected biopsy specimens after ensuring radiographic evidence of fusion. Their histological assessment demonstrated that OP-1 reliably induced viable amounts of new bone formation; however, the fusion success rate evaluated by surgical exploration was inferior to that in the control (hydroxyapatite-tricalcium phosphate/autograft) group [50] (Level I).

*Grade of recommendation* is 1A for use of rhBMP-7 for posterior lumbar spinal fusion. However, as there is one Level I study demonstrating lower fusion rates in contradiction to many other Level I studies, further clinical data are needed to determine the exact grade of recommendation.

### Autologous platelet concentrate

Platelet degranulation and the release of several growth factors, such as platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF-$\beta$), to the fracture healing site are well known as part of the normal cascade. These growth factors promote chemotaxis and proliferation of mesenchymal stem cells and osteoblasts and enhance bone healing [33, 54]. An autologous growth factor concentrate (AGF) prepared by the ultraconcentration of platelets contains such growth factors, and it has been reported that AGF may enhance the formation of new bone in lumbar spinal fusion when used in combination with autografts [57] (Level IV).

Recently, several reports have addressed in detail the efficacy of AGF for spinal fusion (Table 6). Weiner et al. [103] compared an autograft alone and an autograft with AGF in posterolateral spinal fusion retrospectively; they reported that the use of AGF resulted in inferior rates of fusion compared with those of autograft alone (Level III). Hee et al. [40] conducted a prospective study of AGF in instrumented transforaminal lumbar interbody spinal fusion (TLIF) and concluded that the use of AGF in TLIF procedures did not increase the overall fusion rates; however, it might promote a faster rate of fusion (Level II). Carreon et al. [23] investigated the effectiveness of platelet gel in instrumented posterolateral fusion retrospectively and reported that platelet gel failed to enhance the fusion rate when added to autograft (Level III).

*Grade of recommendation* is 2B for use of platelet gel as an enhancer of the effect of autografts for both posterior lumbar fusion and anterior lumbar interbody fusion.

### Mesenchymal stem cells

Mesenchymal stem cells (MSCs) have attracted much interest because of their self-renewing potential and multipotentiality for possible clinical uses. In addition, they have been identified in a variety of tissues including bone marrow [22], muscle [45, 104], periosteum [67], and adipose tissue [115]. Among these tissues, bone marrow has been well established as a source of MSCs. In a variety of animal models, bone marrow-derived MSCs have demonstrated an efficacy in spinal fusion. Minamide et al. cultured MSCs derived from bone marrow and implanted these cells into the posterolateral lumbar transverse process with a hydroxyapatite-granule carrier on a rabbit model and 5 of 7 in the high-number-cultured cell group were fused in manual palpation. They demonstrated that these cells acted as a substitute for the autograft in spinal fusion [63]. Using a rhesus monkey model, Wang et al. expanded autologous MSCs derived from bone marrow in culture, stimulated them with osteogenic supplements, and constructed calcium phosphate ceramic composites with MSCs. They demonstrated that autologous MSCs composites could enhance bone regeneration and achieve osseous spinal fusion in an anterior interbody fusion model [102].

Clinically, Gan et al. [36] used bone-marrow-derived MSCs combined with porous beta-TCP for posterior spinal fusion, and reported that 95.1% cases had positive spinal fusion results (Level IV). The use of autologous MSCs to treat spinal fusion is an effective alternative to autogenous bone grafts. Successful spinal fusion is largely mediated by endogenous osteoblasts; therefore, MSC therapy may be particularly beneficial for elder patients and other patients with reduced cellular stores.

With advances in tissue engineering technology, MSCs have been considered as ideal vehicles for target gene in regional gene therapy. MSCs themselves have an ability to differentiate as osteoblasts; moreover, transfected MSCs are used for secreting target proteins in an autocrine and paracrine fashion, thereby stimulating sufficient spinal fusion. It is remarked that ex vivo gene therapy is the most innovative and feasible technology for spinal fusion in the future.

### Gene therapy

Recombinant BMPs have been used successfully in several clinical trials [7, 10, 14, 18–20, 28, 81, 91–95]. However, high doses of these recombinant proteins are required in humans because the molecules are soluble and can diffuse away or become inactivated in vivo. Therefore, their usefulness is limited by their enormous expense along with the problems of local adverse effects such as unwanted ectopic bone formation and inflammation. A number of strategies

are being developed to provide safer, cheaper, and more efficacious rhBMP therapy. Currently, an attempt is being made to design carriers that would allow a more controlled and sustained release of the protein so that growth factor concentration is maintained locally within a therapeutic range. Further, the alternative evolutional technique—gene therapy for spinal fusion—has been developed.

Gene therapy was originally used for hereditary disorders; however, more recent studies have been applying these gene transfer technologies to situations that require the sustained production of large amounts of a biologically active target gene protein. For spinal fusion, one or more of the genes coding for osteoinductive factors can be transferred to the local fusion environment. Transduced cells can then secrete the target protein extracellularly and deliver it to the environment in physiologically appropriate doses for a sustained period of time, thereby maximizing the osteoinductive potential of these growth factors.

The transduction of a gene can be performed via either an in vivo or an ex vivo approach. The in vivo genetic transfer involves directly introducing the target gene-containing vector into the body. The potential benefits of this strategy include its relatively simple technical application and its potentially lower costs; moreover, it is convenient because the process does not involve harvesting autogenous cells. However, the disadvantages of the in vivo approach are limited by inefficient transduction. There are a few reports that describe successful results in vivo gene therapy in animal spinal fusion models. Alden et al. [2] attempted to induce spinal fusion in an in vitro gene transfer in an athymic nude rat model and injected an adenoviral vector containing the BMP-2 gene into the paraspinal region percutaneously; at 12 weeks after injection, the evidence of new endochondral bone formation was observed on three-dimensionally reconstructed CT scans and histological examination. Following this study, Helm et al. injected an adenoviral vector containing BMP-9 into the paraspinal muscles using the same in vivo gene therapy technique in an athymic nude rat model. At 16 weeks after injection, a CT scan and histological analysis demonstrated massive bone induction at the injection sites, clearly leading to solid spinal fusion [42]. These studies showed that the generation of a spinal fusion is possible with a percutaneous in vivo gene therapy technique.

The ex vivo approach is technically more demanding. The autogenous target cells must be harvested from the donor site and the harvested cells are expanded in tissue culture before being transduced with the desired gene. These cells are reimplanted either at a specific anatomic site or systemically. The advantages of the ex vivo strategy are that no viral particles or DNA complexes are injected directly into the patient and that the cell type to be used for delivery can be selected. Ex vivo techniques have a higher efficiency of cell transduction, and this allows for the preferential selection of target cells. Although the disadvantages of this strategy include the requirement of an extra harvesting step and the increased time and cost of the process, ex vivo gene therapy is considered to be safer than in vivo gene therapy. MSCs are suitable as vehicles for ex vivo gene therapy because this selection contributes to both the osteogenic potential and the production of osteoinductive factors at the spinal fusion site.

Boden et al. [13] reported successful ex vivo gene therapy for posterior spinal fusion in rats by supplying bone marrow cells transfected with genes encoding the LIM mineralization protein (LMP-1), which stimulates the expression of multiple osteoinductive factors. In a related study, Viggeswarapu et al. [99] demonstrated posterolateral fusions in an immune-competent rabbit model with bone-marrow-derived buffy-coat cells transfected with the adenoviral vector gene encoding LMP-1 (Ad-LMP-1). Wang et al. [101] performed ex vivo gene therapy for posterolateral spinal fusion in a Lewis rat model and demonstrated that rat bone marrow cells transfected with Ad-BMP-2 induced solid fusion masses. Hidaka et al. [43] conducted posterolateral fusion on Lewis rats using rat bone marrow cells transfected with Ad-BMP-7 and allografts and reported an 80% fusion rate.

These studies demonstrate the feasibility of ex vivo gene therapy for spinal fusion for clinical use. Further, Dumont et al. treated human MSCs transfected with Ad-BMP-9 and injected them into the paraspinal muscle in athymic nude rats. At 8 weeks postinjection, CT scans and histological analysis clearly demonstrated large volumes of ectopic bone at the injection sites, resulting in successful spinal fusion [30]. Moreover, Peterson et al. [70] performed posterolateral fusion surgically using human-derived bone marrow cells transfected with Ad-BMP-2 in athymic nude rats and demonstrated sufficient spinal fusions.

In order to increase the efficacy of gene therapy, various experiments have been performed. Zhu et al. [113] tested a combination of Ad-BMP-2 and Ad-BMP-7 gene transfers in an example of in vitro gene therapy in a rat posterolateral spinal fusion model in order to enhance the osteogenic activity of BMP; they concluded that the combined Ad-BMP-2 and Ad-BMP-7 gene transfer was significantly more effective than individual Ad-BMP gene transfer. Lee et al. [52] used fibrin gel as a scaffold for ex vivo gene therapy in a rabbit spinal fusion model and reported its effectiveness. Lu et al. [58] tested a new osteoinductive factor, Nell-1(Nel-like molecule-1), for in vivo gene therapy in a rat spinal fusion model and concluded that it may be a potent osteoinductive molecule.

For these gene therapy studies, adenoviruses have been the most commonly tested viral delivery vehicles for bone healing because they can easily transfect target cells and

produce large quantities of the cytokine and have demonstrated successful results in animal experiments. However, there are several potential limitations of using adenoviral vectors in a clinical setting. Although these vectors transfect both dividing and nondividing cells, they cannot integrate into the host genome; thus, protein production by the transfected cells is limited to 3 weeks in even an immunocompromised animal model [32]. This is probably due to the episomal nature of the adenoviral DNA that makes it susceptible to degeneration by host nucleases. Furthermore, adenoviral vectors generally retain their ability to synthesize adenoviral proteins, which stimulate the host immune response [59, 107]. Host immunity destroys the transduced cells and reduces the effect of transgene expression. Recently, in order to compensate the disadvantage of adenoviral vectors, various viral vectors such as adeno-associated viral vector and lentiviral vector, have been tested [26, 64, 83].

Although viral based gene therapy promises several advantages, there are major concerns regarding the safety of using viral vectors in clinical scenarios. Various improvements have been implemented to ensure such safety [82, 97, 114], and gene therapy has been validated as a safe technique in preclinical animal experiments [13, 30, 43, 64, 70, 99, 101]. However, long-term results have not yet been elucidated, and further studies are required before these vectors can be used in clinical practice.

## Conclusion

Significantly advanced bone substitutes have been developed for achieving successful spinal fusion. Many studies are attempting to elucidate the evidence for the usefulness and the advantages of each substitute. Among many alternatives, there seems to be strong evidence only for osteoinductive proteins (rhBMP-2 and OP-1) to show that they can be used as bone enhancers and substitutes for any kind of spinal fusion. All other alternatives seem to work successfully as bone graft extenders based on the available evidence in the literature. On the other hand, the new innovational technologies such as stem cells and gene therapy have been investigated extensively with animal studies providing promising results. Efforts are under way for their further clinical use. Understanding the biology and the specificity of each bone substitute seems to be the most important issue necessary for achieving successful spinal fusion.

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