Gene Therapy and Spinal Fusion: Systematic Review and Meta-Analysis of the Available Data

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Key words
- Animal models
- Bone tissue engineering
- Gene therapy
- Spinal fusion

Abbreviations and Acronyms
BMP: Bone morphogenetic protein
CI: Confidence interval
MSC: Mesenchymal stem cell
OR: Odds ratio

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OBJECTIVE: **To analyze the extant literature describing the application of gene therapy to spinal fusion.**

METHODS: **A systematic review of the English-language literature was performed. The search query was designed to include all published studies examining gene therapy approaches to promote spinal fusion. Approaches were classified as ex vivo (delivery of genetically modified cells) or in vivo (delivery of growth factors via vectors). The primary endpoint was fusion rate. Random effects meta-analyses were performed to calculate the overall odds ratio (OR) of fusion using a gene therapy approach and overall fusion rate. Subgroup analyses of fusion rate were also performed for each gene therapy approach.**

RESULTS: **Of 1179 results, 35 articles met criteria for inclusion (all preclinical), of which 26 utilized ex vivo approaches and 9 utilized in vivo approaches. Twenty-seven articles (431 animals) were included in the meta-analysis. Gene therapy use was associated with significantly higher fusion rates (OR 77; 95% confidence interval {CI}: [31, 192]; $P < 0.001$); ex vivo strategies had a greater effect (OR 136) relative to in vivo strategies (OR 18) ($P = 0.017$). The overall fusion rate using a gene therapy approach was 80% (95% CI: [62%, 93%]; $P < 0.001$); overall fusion rates were significantly higher in subjects treated with ex vivo compared to in vivo strategies (90% vs. 42%; $P = 0.011$). For both ex vivo and in vivo approaches, the effect of gene therapy on fusion was independent of animal model.**

CONCLUSIONS: **Gene therapy may augment spinal fusion; however, future investigation in clinical populations is necessary.**

INTRODUCTION

Each year, more than a half-million spinal fusion procedures are performed in the United States,[1] at a cost of approximately $13 billion—the highest of any inpatient procedure.[2] Previous reports have suggested that long-term symptomatic improvement following spinal fusion correlates with successful bony union across the fusion site.[3,4] For this reason, there is great interest in maximizing fusion rates through preoperative patient optimization,[5,6] use of efficacious bone grafts,[7-9] and postoperative adjuvant therapies.[10,11] Nonetheless, nonunion is noted in about 20% of patients undergoing spinal fusion.[12,13]

Recent preclinical reports have suggested gene therapy may augment fusion rates by creating a sustained release of osteoinductive agents (e.g., bone morphogenetic protein [BMP]-2) at the fusion site.[14,15] Broadly, gene therapy for spinal fusion can be divided into two categories: 1) in vivo approaches, where a vector (e.g., a virus) encoding an osteoinductive gene is delivered at the fusion site and taken up by host cells, which then express the gene; and 2) ex vivo approaches, where genetically modified cells (e.g., mesenchymal stem cells [MSCs]) encoding an osteoinductive gene are delivered at the fusion site and express the gene of interest (Figure 1).[16]

Despite the interest in gene therapy for spinal fusion, there has yet to be a systematic review evaluating the efficacy of gene therapy on improving fusion rates. Here we sought to 1) systematically review the available data describing the use of gene therapy on spinal fusion and 2) perform a meta-analysis of the available data to evaluate the effect of gene therapy on spinal fusion rates.

MATERIAL AND METHODS

Electronic Literature Search

Using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines, a systematic review of the English-language literature was performed using PubMed, Web of Science, and Embase databases on July 1, 2023. The search query was designed to include all published studies examining gene therapy approaches on promoting spinal fusion. The query for the PubMed database was as

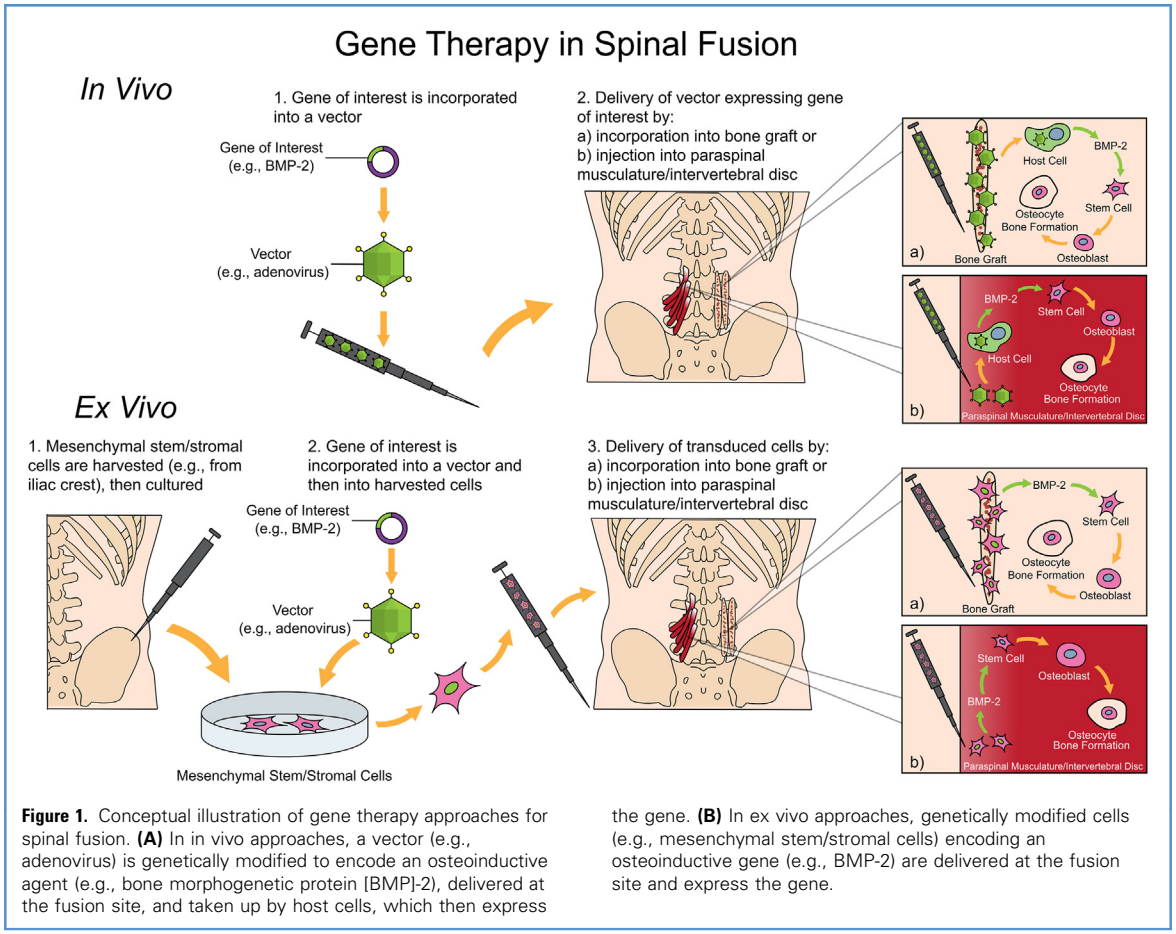

Figure 1. Conceptual illustration of gene therapy approaches for spinal fusion. **(A)** In in vivo approaches, a vector (e.g., adenovirus) is genetically modified to encode an osteoinductive agent (e.g., bone morphogenetic protein [BMP]-2), delivered at the fusion site, and taken up by host cells, which then express the gene. **(B)** In ex vivo approaches, genetically modified cells (e.g., mesenchymal stem/stromal cells) encoding an osteoinductive gene (e.g., BMP-2) are delivered at the fusion site and express the gene.

follows: ("gene therapy" OR "viral" OR "non-viral" OR "plasmid" OR "virus*" OR "vector*" OR "viral vector*" OR "baculovir*" OR "BAC" OR "adenovir*" OR "Ad5" OR "adeno-associated vir*" OR "AAV" OR "AAV6" OR "rAAV2" OR "cytomegalovir*" OR "CMV" OR "pCMV*" OR "murine leukemia vir*" OR "MLV" OR "lentivir*" OR "retrovir*" OR "herpes*" OR "HSV" OR "parvovir*" OR "pVR1055" OR "lipid vector*" OR "cationic lipid*" OR "gene-activated matri*" OR "dendrimer*" OR "chitosan" OR "polyethylenimine" OR "transduc*" OR "transfect*" OR "gene delivery" OR "gene insertion" OR "gene transfer" OR "gene gun" OR "liposom*" OR "lipid vesicle*" OR "transgene" OR "microprojectile*" OR "microinjection" OR "polycation" OR "lipoplex*" OR "polyplex*" OR "lipofectamine" OR "cDNA" OR "tetracycline" OR "doxycycline" OR "Tet-off" OR "sonoporation" OR "electroporation" OR "nucleofection" OR "miRNA" OR "microRNA" OR "siRNA") AND ("spinal fusion" OR "spine fusion" OR "spinal arthrodes*" OR "spine arthrodes*" OR "cervical fusion" OR "thoracic fusion" OR "lumbar fusion" OR "thoracolumbar fusion" OR "cervical arthrodes*" OR "thoracic arthrodes*" OR "lumbar arthrodes*" OR "thoracolumbar arthrodes*" OR "posterior fusion" OR "posterolateral fusion" OR "interbody fusion"). This query was stylistically modified for use in the Web of Science and Embase databases. Additional sources were identified by reviewing the bibliographies of the included studies.

Included studies were those that examined the effect of any gene therapy approach on spinal fusion with full English-language text availability. We defined gene therapy as the use of genetically modified cells or vectors encoding one or more osteoinductive genes for the purpose of promoting spinal fusion. Studies were excluded if they examined a model other than spinal fusion, lacked English full-text availability, or failed to present primary data. Eligible studies were screened against these criteria by two independent reviewers (E.C. and M.D.), with a third reviewer (Z.P.) resolving any discrepancies between the first two reviewers.

Data Extraction

Studies meeting the inclusion criteria were reviewed to extract details on the type of gene therapy (ex vivo or in vivo), animal model utilized, spinal fusion technique, bone graft/carrier used, growth factor examined, and—for ex vivo approaches—type of genetically modified cells employed. In addition, fusion rates at the end timepoint—the primary outcome measure—were also recorded. When multiple fusion rates were reported based on different assessment modalities (e.g., manual palpation, computerized tomography, and histology) in the same study, fusion rates were recorded based on the

following convention[10,11] in order of decreasing preference: manual palpation, computerized tomography, plain radiography, and histology.

Quality Assessment

The methodological quality of the studies included in the meta-analysis was assessed using a modified version of the Systematic Review Centre for Laboratory Animal Experimentation risk of bias tool, an established method for evaluating bias in animal studies.[10,17] Two reviewers (C.J. and D.S.) independently assessed all studies included in the quantitative analysis using this fourteen-item tool, while a third reviewer (E.C.) resolved any discrepancies between the first two reviewers.

Statistical Analysis

All statistical analyses were performed using R version 4.3.1 (The R Foundation for Statistical Computing; Vienna, Austria). The overall odds of fusion using gene therapy was calculated from all studies reporting fusion data for the experimental (i.e., gene therapy) and control (null) groups using the Freeman-Tukey double arcsine transformation and random-effects modeling, as previously described.[11,18] Similarly, the overall fusion rate using gene therapy was calculated. Because of residual heterogeneity in the estimated fusion rates for both in vivo and ex vivo approaches, subgroup meta-analyses were performed to evaluate the degree to which fusion rates were affected by animal model, type of surgery, vector, growth factor, and (for ex vivo approaches) cell type employed. For studies including multiple experimental groups (e.g., multiple vectors, multiple growth factors), each experimental group was treated as a unique treatment arm in the subgroup meta-analyses. For all analyses, an α of 0.05 was used as the definition of statistical significance.

RESULTS

Our search identified 1179 unique articles, of which 46 underwent full-text review. After review, 35 studies were included in the qualitative synthesis,[14,15,19-51] of which 26 evaluated ex vivo approaches and 9 evaluated in vivo approaches. Among the 11 excluded studies, 8 were excluded because they were available in abstract form only and 3 were excluded because the full English-language text was unavailable (Figure 2). Twenty-seven studies (431 animals) reported data on fusion rates and were included in the meta-analysis. Descriptive summaries of the included studies are included in Tables 1 and 2; they ranged in year of publication from 1998 to 2022.

Quality Assessment

Quality assessment of the studies included in the meta-analysis is shown in Supplementary Figure 1. All studies were determined to have a low risk of bias within attrition and reporting categories, and most were deemed to have a low risk of bias among detection categories. However, there was unclear risk of bias among two of the three selection categories, and only 63% of studies reported blinding or randomization during any portion of the experimental protocol. To this end, there was an overall unclear risk of bias among the included studies.

Overall Effect of Gene Therapy on Spinal Fusion

Use of gene therapy was found to significantly increase fusion rates across all studies (odds ratio [OR] 77.4; 95% confidence interval {CI}: [31.2, 192.0]; $P < 0.001$), which was significantly greater among ex vivo strategies (OR 135.7; 95% CI: [50.1, 367.3]) compared to in vivo strategies (OR 18.1; 95% CI: [4.9, 67.8]) ($P = 0.017$) (Figure 3). In both subgroups $I^2 = 0\%$, suggesting a high degree of heterogeneity in the results. The overall fusion rate using a gene therapy approach was 80% (95% CI: [62%, 93%]; $P < 0.001$); fusion rates were again significantly higher in animals treated with ex vivo strategies (90%; 95% CI: [72%, 100%]) compared to in vivo

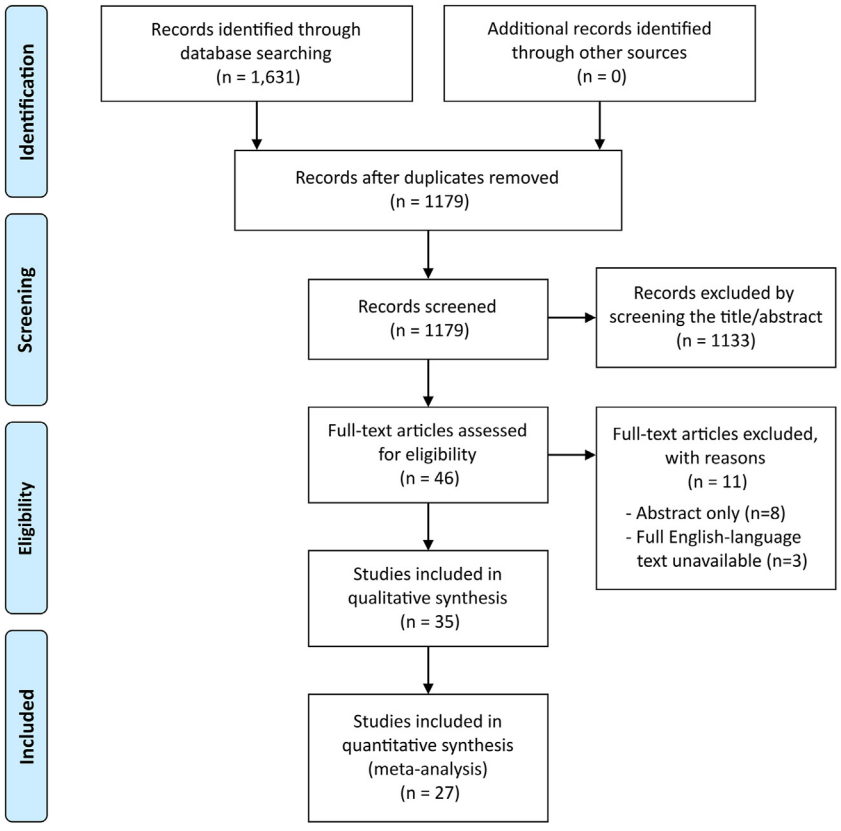

Figure 2. Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) 2009 flow diagram for article selection.

Table 1. Summary of Preclinical Studies Employing Ex Vivo Gene Therapy Approaches for Spinal Fusion

Study (Author, Year)	Animal, Surgical, and Gene Therapy Details	Study Groups	Fusion Rate by Assessment Technique and Time Point (No. Fused/No. Fusion Sites)
Boden et al., 1998[21]	Animal: Rats (n = 9) Surgery: Posterior (spinous process and laminae) L5-L6 or T11-T12 w/o decortication Graft: DBM Cells: Rat BMSCs from hind limb long bones; 3 M/level Vector (promoter): pCMV2 (CMV) Growth factor: LMP-1	A. pCMV2-LMP-1-transduced BMSCs (n = 9 levels; 4 thoracic and 5 lumbar) B. pCMV2-null-transduced BMSCs (LMP-1 protein not expressed; n = 9 levels; 5 thoracic and 4 lumbar)	Time point: 4 weeks Manual palpation: A. 100% (9/9 levels) *$P < 0.05$ versus B B. 0% (0/9 levels)
Riew et al., 1998[45]	Animal: Rabbits (n = 5) Surgery: L5-L6 PLF Graft: Collagen Cells: Rabbit BMSCs; 3 M/side Vector (promoter): Ad5 (CMV) Growth factor: BMP-2	A. Ad5-BMP-2-transduced cells on left side (n = 5 sides) B. Ad5-β-gal-transduced cells on right side (n = 5 sides)	Time point: 5 weeks Radiography A. 20% (1/5 sides; *0.5/2.5 levels**) B. 0% (0/5 sides; *0/2.5 levels**)
Cheng et al., 2001[23]	Animal: Rabbits (n = 3) Surgery: L5-L6 PLF Graft: Collagen Cells: Rat BMSCs; 3 M/side Vector (promoter): Ad5 (CMV) Growth factor: BMP-2	A. Ad5-BMP-2-transduced cells on left side (n = 3 sides) B. Ad5-β-gal-transduced cells on right side (n = 3 sides)	Time point: 4 weeks Fusion rates were not reported; however, via radiographic evaluation, new bone formation was visualized in 100% of sides (3/3) in A and 0% of sides (0/3) in B
Viggeswarapu et al., 2001[48]	Animal: Rabbits (n = 20) Surgery: L5-L6 PLF Graft: 15% HA/85% TCP Cells: Peripheral blood buffy coat cells; 0.5 M/side Vector (promoter): Ad5 (CMV) Growth factor: LMP-1	A. Ad5-LMP-1-transduced cells (n = 10 rabbits) B. Ad5-β-gal-transduced cells (n = 10 rabbits)	Time point: 4 weeks Manual palpation A. 100% (10/10 levels) B. 0% (0/10 levels)
Dumont et al., 2002[25]	Animal: Rat (n = 16) Surgery: Lumbar paraspinal, percutaneous injection Graft: None Cells: Human MSCs; 1 M/side Vector (promoter): Ad5 (CMV) Growth factor: BMP-9	A. Ad5-BMP-9-transduced cells on left side (n = 16 sides) B. Ad5-β-gal-transduced cells on right side (n = 16 sides)	Time point: 8 weeks Radiography A. 100% (16/16 sides; *8/8 levels**) B. 0% (0/16 sides; *0/8 levels**)
Hidaka et al., 2003[29]	Animal: Rat (n = 33) Surgery: L4-L5 PLF Graft: Allograft bone (50 mg/side) and type 1 collagen gel Cells: Rat BMSCs from hindlimb long bones; 1.5 M/side Vector (promoter): Ad5 (CMV) Growth factor: BMP-7	A. Ad5-BMP-7-transduced cells (n = 10) B. Ad5-β-gal-transduced cells (n = 11) C. Non-transduced BMSCs (n = 12)	Time point: 8 weeks Manual palpation: A. 80% (8/10 levels) *$P < 0.001$ versus B/D B. 0% (0/11 levels) C. 0% (0/12 levels)
Kim et al., 2003[32]	Animal: Rabbit (n = 7) Surgery: L5-L6 PLF Graft: Collagen Cells: Autologous buffy coat cells; 4 M/side Vector (promoter): Ad5 (CMV) Growth factor: LMP-1	A. Ad5-LMP-1-transduced cells (no pre-immunization with Ad5-lacZ; n = 2) B. Ad5-LMP-1-transduced cells and pre-immunization with Ad5-lacZ (16 weeks prior to fusion surgery; 10^8 M viral particles; (n = 3) C. Ad5-LMP-1-transduced cells and pre-immunization with Ad5-lacZ (16 weeks prior to fusion surgery; 10^9 viral particles; n = 2)	Time point: 4 weeks Manual palpation: A. 100% (2/2 levels) B. 100% (3/3 levels) C. 0% (2/2 levels)

Continues

Table 1. Continued

Study (Author, Year)	Animal, Surgical, and Gene Therapy Details	Study Groups	Fusion Rate by Assessment Technique and Time Point (No. Fused/No. Fusion Sites)
Riew et al., 2003[44]	Animal: Pig (n = 3) Surgery: Thoracoscopic intradiscal fusion (1 cm^3 disc removed for cell implantation) Graft: None Cells: Pig BMSCs; 50 M/disc space Vector (promoter): Ad5 (CMV) Growth factor: BMP-2	A. Ad5-BMP-2-transduced cells (n = 6 discs) B. Ad5-β-gal-transduced cells (n = 3 discs) C. Sham-operated control (no fusion or cells) (n = 3 discs)	Time point: 6 weeks Histology: A. 100% (6/6 levels) B. 0% (0/3 levels) C. 0% (0/3 levels)
Wang et al., 2003[49]	Animal: Rat (n = 85) Surgery: L4-L5 PLF Graft: DBM (Groups A, C, F, H, and I) or collagen (Groups B, D, and J) Cells: BMSCs from hindlimb long bones; 5 M/side (Groups A, B, F, and H) Vector (promoter): Ad5 (CMV) Growth factor: BMP-2	A. Ad5-BMP-2-transduced cells and DBM (n = 8) B. Ad5-BMP-2-transduced cells and collagen (n = 7) C. 10 μg rhBMP-2 and DBM (n = 8) D. 10 μg rhBMP-2 and collagen (n = 7) E. AIBG (n = 15) F. Ad-LacZ-transduced cells and DBM (n = 8) G. Decortication only (n = 8) H. Non-transduced cells and DBM (n = 8) I. DBM only (n = 8) J. Collagen only (n = 8)	Time point: 12 weeks Radiography: A. 100% (8/8 levels) B. 100% (7/7 levels) C. 100% (8/8 levels) D. 100% (7/7 levels) *$P < 0.001$ (A-D vs. E-J) E. 0% (0/15 levels) F. 0% (0/8 levels) G. 0% (0/8 levels) H. 0% (0/8 levels) I. 0% (0/8 levels) J. 0% (0/8 levels)
Hasharoni et al., 2005[27]	Animal: Mouse (n = 30) Surgery: Posterior lumbar paravertebral muscle injection Graft: None Cells: MSCs; 2 M/mouse Vector (promoter): ptTATop-BMP-2 plasmid vector (TATA) with a tetracycline-sensitive transactivator (presence of tetracycline prevents expression of BMP-2) Growth factor: BMP-2	A. ptTATop-BMP-2-transduced cells; DOX withheld for the first 3 days postoperatively, then provided in drinking water until 30 days postoperatively (n not reported) B. ptTATop-BMP-2-transduced cells; DOX withheld for the first 7 days postoperatively, then provided in the drinking water until 30 days postoperatively (n not reported) C. ptTATop-BMP-2-transduced cells; no DOX provided (n not reported) D. ptTATop-BMP-2-transduced cells; DOX provided in the drinking water for the first 30 days postoperatively (n = 10)	Time point: 8 weeks CT: A. 0% (n not reported) B. 100% (n not reported) C. 100% (n not reported) n = 20 mice total for A, B, and C D. 0% (0/10 levels)
Peterson et al., 2005[43]	Animal: Rat (n = 23) Surgery: L4-L5 PLF Graft: Collagen Cell type: human BMSCs; 2.5 M/side Vector (promoter): Ad5 (CMV) Growth factor: BMP-2	A. Ad5-BMP-2-transduced cells (n = 8) B. Ad5-LacZ-transduced cells (n = 5) C. Non-transduced cells (n = 5) D. Carrier alone (n = 5)	Time point: 12 weeks Manual palpation: A. 100% (8/8 levels) *$P < 0.005$ versus B-D B. 0% (0/5 levels) C. 0% (0/5 levels) D. 0% (0/5 levels)
Lee et al., 2006[36]	Animal: Rabbit (n = 12) Surgery: L5-L6 PLF Graft: Fibrin or surgical gelatin Cells: Normal rat kidney (NRK) cells; 15 M/side Vector (promoter): pCMV script vector (CMV); transfection via nonviral reagent FuGENE Growth factor: BMP-2	A. pCMV-BMP-2-transfected cells and fibrin (n = 6) B. pCMV-BMP-2-transfected cells and a gelatin sponge (n = 6)	Time point: 12 weeks Radiography: A. 100% (6/6 levels) B. 0% (0/6 levels)

n = 24

β-gal, beta-galactosidase; Ad5, adenovirus type 5; ASC, adipose-derived mesenchymal stem/stromal cell; B, billion; BMP, bone morphogenetic protein; BMSC, bone marrow-derived mesenchymal stem/stromal cell; CMV, cytomegalovirus; CT, computed tomography; DBM, devitalized/demineralized bone matrix; DOX, doxycycline; GFP, green fluorescent protein; HA, hydroxyapatite; HBMC, human bone marrow cells; k, thousand; Lenti, lentivirus; LMP-1, Lim mineralization protein 1; M, million; MLV, murine leukemia virus; pCMV, plasmid CMV; pfu, plaque-forming units; PLF, posterolateral lumbar intertransverse process fusion; pu, particle units; rh, recombinant human; VEGF, vascular endothelial growth factor; VP, viral particles.

*Converted to levels for statistical analysis.

Continues

Table 1. Continued

Study (Author, Year)	Animal, Surgical, and Gene Therapy Details	Study Groups	Fusion Rate by Assessment Technique and Time Point (No. Fused/No. Fusion Sites)
Hsu et al., 2008[30]	Animal: Rat (n = 40) Surgery: L4-L5 PLF Graft: Collagen Cell type: Human ASCs; 5 M/level Vector (promoter): Ad5 (CMV) Growth factor: BMP-2	A. Ad5-BMP-2-transduced cells (n = 8) B. Non-transduced cells and 1 μg BMP-2 (n = 8) C. 10 μg BMP-2 (n = 8) D. 1 μg BMP-2 (n = 8) E. Nontransduced cells (n = 8)	Time point: 4 weeks Manual palpation: A. 100% (8/8 levels) *$P < 0.0001$ versus B *$P < 0.0001$ versus C *$P < 0.02$ versus D *$P < 0.0001$ versus E B. 0% (0/8 levels) C. 100% (8/8 levels) D. 50% (4/8 levels) E. 0% (0/8 levels)
Miyazaki et al. (a), 2008[39]	Animal: Rat (n = 18) Surgery: L4-L5 PLF Graft: Collagen Cells: Rat BMSCs from hindlimb long bones; 2.5 M/side Vector (promoter): Lentivirus (MLV) Growth factor: BMP-2	A. Lenti-BMP-2-transduced cells (n = 6) B. Lenti-GFP-transduced cells (n = 3) C. Nontransduced cells (n = 6) D. Carrier only (n = 3)	Time point: 8 weeks Manual palpation: A. 100% (6/6 levels) *$P < 0.001$ versus B-D B. 0% (0/3 levels) C. 0% (0/6 levels) D. 0% (0/3 levels)
Miyazaki et al. (b), 2008[40]	Animal: Rat (n = 39) Surgery: L4-L5 PLF Graft: Collagen Cells: Rat BMSCs from allogeneic hindlimb long bones; 2.5 M/side Vector (promoter): Lentivirus (MLV); Ad5 (CMV) Growth factor: BMP-2	A. Lenti-BMP-2-transduced cells (n = 6) B. Ad5-BMP-2-transduced cells (n = 6) C. Lenti-GFP-transduced cells (n = 3) D. Ad5-LacZ-transduced cells (n = 6) E. 10 μg BMP-2 (n = 6) F. Nontransduced cells (n = 6) G. Carrier alone (n = 6)	Time point: 8 weeks Manual palpation: A. 100% (6/6 levels) B. 100% (6/6 levels) C. 0% (0/3 levels) D. 0% (0/6 levels) E. 100% (6/6 levels) F. 0% (0/6 levels) G. 0% (0/6 levels)
Miyazaki et al. (c), 2008[41]	Animal: Rat (n = 48) Surgery: L4-L5 PLF Graft: Collagen Cells: Human ASCs or BMSCs; 2.5 M/side Vector (promoter): Ad5 (CMV) Growth factor: BMP-2	A. Ad5-BMP-2-transduced ASCs (n = 10) B. Ad5-BMP-2-transduced BMSCs (n = 10) C. 10 microg rhBMP-2 (n = 10) D. Ad5-LacZ-transduced ASCs (n = 6) E. Ad5-LacZ-transduced BMSCs (n = 6) F. Collagen only (n = 6)	Time point: 8 weeks Manual palpation: A. 100% (10/10 levels) B. 100% (10/10 levels) C. 100% (10/10 levels) D. 0% (0/6 levels) E. 0% (0/6 levels) F. 0% (0/6 levels)
Sheyn et al., 2008[46]	Animal: Mice (immunodeficient; n = 5) Surgery: Posterior lumbar paravertebral muscle injection Graft: Fibrin gel Cells: Porcine ASCs; 5 M/level Vector (promoter): pCMV (CMV) Growth factor: rhBMP-6	A. pCMV-BMP-6-transduced cells (n = 5 levels) B. Nontransduced cells (n = 3 levels)	Time point: 5 weeks CT: A. 100% (5/5 levels) B. 0% (0/3 levels)

Continues

Table 1. Continued

Study (Author, Year)	Animal, Surgical, and Gene Therapy Details	Study Groups	Fusion Rate by Assessment Technique and Time Point (No. Fused/No. Fusion Sites)
Kimelman-Bleich et al., 2009[33]	Animal: Mice (n = 20) Surgery: Posterior lumbar paravertebral muscle injection Graft: Fibrin gel with or without 10% PFTBA (used as a synthetic oxygen carrier) Cells: Tet-off BMP-2 MSC (C3H10T1/2 murine embryonic MSC cell line); 5 M/animal Vector (promoter): ptTATop-BMP-2 plasmid vector (TATA) with a tetracycline-sensitive transactivator (presence of tetracycline prevents expression of BMP-2) Growth factor: BMP-2	A. Tet-off BMP-2 MSCs *with* 10% PFTBA (n = 10 mice) B. Tet-off BMP-2 MSCs *without* 10% PFTBA (n = 10 mice)	Time point: 6 weeks Fusion rates were not reported; however, via micro-computed tomographic analysis, significantly greater bone formation was seen in A versus B (70.71 ± 2.7 mm^3 vs. 50.86 ± 4.27 mm^3; $P < 0.05$).
Douglas et al., 2010[24]	Animal: New Zealand white rabbits (n = 12) Surgery: L6-L7 PLF Graft: Absorbable gelatin powder Cell type: BMSCs; 100 k/side Vector (promoter): Ad5 (CMV); RGD variants are integrin-targeted Growth factor: Smad1C	A. Ad5-Smad1C-transduced cells (n = 2 levels) B. Ad5-Smad1C-RGD-transduced cells (n = 2 levels) C. Ad5-BMP-2-transduced cells (n = 2 levels) D. Ad5-BMP-2-RGD-transduced cells (n = 2 levels) E. Ad5-β-gal-transduced cells (n = 2 levels) F. Ad5-β-gal-RGD-transduced cells (n = 2 levels)	Time point: 4 weeks Fusion rates were not reported; however, via histological analysis, areas of new bone formation were determined as follows: A. 2–3 mm^2 B. 9–10 mm^2 *$P < 0.0007$ versus A *$P < 0.02$ versus all others C. 2–3 mm^2 D. 4–5 mm^2 *$P < 0.04$ A-D versus F E. Not reported F. 1–2 mm^2
Sheyn et al., 2010[47]	Animal: C3H/HeN mice (n = 23) Surgery: Posterior lumbar paravertebral muscle injection Graft: None Cells: MSCs; 5 M/side Vector (promoter): ptTATop-BMP-2 plasmid vector (TATA) with a tetracycline-sensitive transactivator (presence of tetracycline prevents expression of BMP-2) Growth factor: BMP-2	A. ptTATop-BMP-2-transduced cells (n = 9) B. ptTATop-BMP-2-transduced cells and DOX (n = 7) C. Carrier alone (n = 7)	Time point: 5 weeks CT: A. 100% (9/9 levels) B. 0% (0/7 levels) C. 0% (0/7 levels)
Olabisi et al., 2011[42]	Animal: Mouse; immunodeficient (ID; n = 44) and immunocompetent (IC; n = 43) Surgery: Right paraspinous muscle injection Graft: None Cells: Murine osteoblast cell line MC3T3-E1; 5 M/level Vector (promoter): Ad5 (CMV); 5000 VP/cell Growth factor: BMP-2	A. Ad5-BMP-2-transduced cells in ID mice (n = 9) B. Ad5-null-transduced cells in ID mice (n = 9) C. Ad5-BMP-2-transduced cells in IC mice (n = 8) D. Ad5-null-transduced cells in IC mice (n = 8)	Time point: 6 weeks Manual Palpation: A. 89% (8/9 levels) B. 0% (0/9 levels) C. 100% (8/8 levels) D. 0% (0/8 levels)

n = 24

β-gal, beta-galactosidase; Ad5, adenovirus type 5; ASC, adipose-derived mesenchymal stem/stromal cell; B, billion; BMP, bone morphogenetic protein; BMSC, bone marrow-derived mesenchymal stem/stromal cell; CMV, cytomegalovirus; CT, computed tomography; DBM, devitalized/demineralized bone matrix; DOX, doxycycline; GFP, green fluorescent protein; HA, hydroxyapatite; HBMC, human bone marrow cells; k, thousand; Lenti, lentivirus; LMP-1, Lim mineralization protein 1; M, million; MLV, murine leukemia virus; pCMV, plasmid CMV; pfu, plaque-forming units; PLF, posterolateral lumbar intertransverse process fusion; pu, particle units; rh, recombinant human; VEGF, vascular endothelial growth factor; VP, viral particles.

*Converted to levels for statistical analysis.

Continues

Table 1. Continued

Study (Author, Year)	Animal, Surgical, and Gene Therapy Details	Study Groups	Fusion Rate by Assessment Technique and Time Point (No. Fused/No. Fusion Sites)
Kaito et al., 2013[31]	Animal: Rat (n = 44) Surgery: L4-L5 PLF Graft: Collagen Cells: Human ASCs; 2.5 M/side Vector (promoter): Lentivirus (CMV) Growth factor: BMP-2 and BMP-7	A. Nontransduced ASCs (n = 10) B. Lenti-GFP-transduced cells (n = 10) C. Lenti-BMP-2-transduced cells (n = 8) D. Lenti-BMP-7-transduced cells (n = 8) E. Lenti-BMP-2- and BMP-7-transduced cells (n = 8)	Time point: 8 weeks Manual palpation: A. 0% (0/10 levels) B. 0% (0/10 levels) C. 100% (8/8 levels) D. 100% (8/8 levels) E. 100% (8/8 levels)
Fu et al., 2015[26]	Animal: Rabbit (n = 18) Surgery: L4-L5 PLF Graft: 60% HA/40% β-TCP Cells: BMSCs from allogeneic iliac crest; 20 M/side Vector (promoter): Baculovirus (CMV) Growth factor: BMP-2 and VEGF	A. Bac-BMP-2- and VEGF-transduced cells (n = 6) B. Nontransduced BMSCs (n = 6) C. Carrier alone (n = 6)	Time point: 12 weeks Manual palpation A. 83% (5/6 levels) *P = 0.003 versus B/C B. 33% (2/6 levels) *P < 0.003 versus C C. 0% (0/6 levels)
Liao, 2016[37]	Animal: Rabbit (n = 18) Surgery: L4-L5 PLF Graft: 88% ceramic, 12% collagen Cells: BMSCs from allogeneic iliac crest; 20 M/side Vector (promoter): Baculovirus (CMV) Growth factor: BMP-7	A. Bac-BMP-7-transduced cells (n = 6) B. Non-transduced cells (n = 6) C. Carrier alone (n = 6)	Time point: 12 weeks Manual palpation A. 83% (5/6 levels) *P = 0.012 versus B/C B. 0% (0/6 levels) C. 0% (0/6 levels)
Yu et al., 2021[14]	Animal: Mouse (n = 48) Surgery: paravertebral muscle injection at L3-L5 Graft: Fibrin gel (FG) Cells: BMSCs from isogenic mice; 1 M/side Vector (promoter): lipofectamine, non-viral (CMV) Growth factor: PIGF-2	A. FG only (n = 8) B. FG and BMSC-null (n = 8) C. FG and BMSC-PIGF-2 (n = 8) D. FG and BMSC-PIGF-2 (n = 8) E. FG and BMSC-PIGF-2, plus weekly liposome (n = 8) F. FG and BMSC, plus weekly clodronate (n = 8)	Time point: 6 weeks Fusion rates were not reported; however, via micro-computed tomographic analysis, a significantly greater number of fused vertebrae was observed in C compared to A and B.
Cunningham et al., 2022[15]	Animal: Rat (n = 78) Surgery: Percutaneous ventral transperitoneal injection into L4-L6 disc spaces Graft: None Cells: BMSCs from femurs and tibia; 1 M/disc space Vector (promoter): Ad5 Growth factor(s): BMP-2, BMP-7	A. Ad5-BMP-2-transduced cells (n = 15) B. Ad5-BMP-7-transduced cells (n = 15) C. Mix of Ad5-BMP-7 and Ad5-BMP-2 transduced cells (homodimer group) (n = 17) D. Double Ad5-BMP-7 and Ad5-BMP-2 transduced cells (heterodimer group) (n = 16) E. Ad5-LACZ cells (control) (n = 15)	Time point: 12 weeks Manual palpation A. 6.67% (2/30 levels) B. 0% (0/30 levels) C. 0% (0/34 levels) D. 56.25% (18/32 levels) E. 0% (0/30 levels)

n = 24

β-gal, beta-galactosidase; Ad5, adenovirus type 5; ASC, adipose-derived mesenchymal stem/stromal cell; B, billion; BMP, bone morphogenetic protein; BMSC, bone marrow-derived mesenchymal stem/stromal cell; CMV, cytomegalovirus; CT, computed tomography; DBM, devitalized/demineralized bone matrix; DOX, doxycycline; GFP, green fluorescent protein; HA, hydroxyapatite; HBMC, human bone marrow cells; k, thousand; Lenti, lentivirus; LMP-1, Lim mineralization protein 1; M, million; MLV, murine leukemia virus; pCMV, plasmid CMV; pfu, plaque-forming units; PLF, posterolateral lumbar intertransverse process fusion; pu, particle units; rh, recombinant human; VEGF, vascular endothelial growth factor; VP, viral particles.
*Converted to levels for statistical analysis.

strategies (42%; 95% CI [13%, 75%]; P = 0.011) (Figure 4).

Subgroup Meta-Analyses

Animal Model. Most studies (n = 16) evaluated rat models. Rabbit (n = 7), mouse (n = 3), and pig models (n = 1) were also reported. For both ex vivo (P = 0.37) and in vivo (P = 0.16) approaches, the effect of gene therapy on fusion was found to be independent of animal model (Figure 5).

Surgical Model. Among ex vivo gene therapy approaches, fusion via intervertebral disc injection was associated with significantly lower fusion rates (4 studies; fusion in 25%; 95% CI: [1%, 61%]) compared to posterior/posterolateral fusion (19 studies;

Table 2. Summary of Preclinical Studies Employing In Vivo Gene Therapy Approaches for Spinal Fusion

Study (Author, Year)	Animal, Surgical, and Gene Therapy Details	Study Groups	Fusion Rate by Assessment Technique and Time Point (No. Fused/No. Fusion Sites)
Alden et al., 1998[19]	Animal: Rat (n = 12) Surgery: Percutaneous, paraspinal injection at lumbosacral junction (b/w spinous process and lamina on each side) Graft: None Vector (promoter): Ad5 (CMV) Growth factor: BMP-2	A. Ad5-BMP-2 bilateral (n = 4) B. Ad5-BMP-2 on right side and Ad5-β-gal on left side (n = 4) C. Ad5-β-gal bilateral (n = 4)	Time point: 12 weeks Fusion rates were not reported; however, radiographic evaluation revealed bone formation adjacent to the spinous processes and lamina at each Ad5-BMP-2 injection site and no bone formation at the control Ad5-β-gal injection sites.
Alden et al., 1999[20]	Animal: Rat (n = 12) Surgery: Paraspinal, percutaneous injection at lumbosacral junction (b/w spinous process and lamina on each side) Graft: None Vector (promoter): Ad5 (CMV) Growth factor: BMP-2	A. Ad5-BMP-2 bilateral (n = 4) B. Ad5-BMP-2 on right side and Ad5-β-gal on left side (n = 4) C. Ad5-β-gal bilateral (n = 4)	Time point: 12 weeks CT: A. 100% (4/4 levels) B. Ad5-BMP-2: 100% (4/4 sides; *2/2 levels**); Ad5-β-gal: 0% (0/4 sides; *0/2 levels**) C. 0% (0/4 levels)
Helm et al., 2000[28]	Animal: Rat (n = 8) Surgery: Paraspinal, percutaneous injection at the lumbosacral junction (b/w spinous process and lamina on each side) Graft: None Vector (promoter): Ad5 (CMV) Growth factor: BMP-9	A. Ad5-BMP-9 on right side (n = 8) B. Ad5-β-gal on left side (n = 8)	Time point: 16 weeks CT: A. 100% (8/8 sides; *4/4 levels**) B. 0% (0/8 sides; *0/4 levels**)
Laurent et al., 2004[35]	Animal: Rabbit (n = 5) Surgery: Lumbar (L3/L4 and L5-L6) intertransverse process percutaneous injections Graft: None Vector (promoter): Ad5 (CMV) Growth factor: BMP-6	A. Ad5-BMP-6 at L3/L4 (n = 2) B. Ad5-BMP-6 at L5-L6 (n = 3) C. Ad5-β-gal at L3/L4 (n = 3) D. Ad5-β-gal at L5-L6 (n = 2)	Time point: 14 weeks CT: A + B. 80% (4/5 levels) C + D. 0% (0/5 levels)
Zhu et al., 2004[51]	Animal: Rat (*n* = 78) Surgery: L4-L5 PLF Graft: Allograft bone (50 mg/side) & carboxy-methylcellulose hemostatic foam (30 mg/side) Vector (promoter): Ad5 (CMV) Growth factor: BMP-2 and/or BMP-7	A. Saline (n = 12) B. Null vector (n = 19) C. Ad5-BMP-2 (n = 13) D. Ad5-BMP-7 (n = 19) E. Ad5-BMP-2 + BMP-7 (n = 15)	Time point: 8 weeks Manual palpation A. 0% (0/12 levels) B. 0% (0/19 levels) C. 8% (1/13 levels) **P* < 0.05 versus A/B D. 16% (3/19 levels) E. 73% (11/15 levels) **P* < 0.05 versus A-D

n = 9.

β-gal, beta-galactosidase; B, billion; BMP, bone morphogenetic protein; CMV, cytomegalovirus; DBM, demineralized bone matrix; DOX, doxycycline; CT, computed tomography; PBS, phosphate-buffer saline; PLF, posterolateral lumbar intertransverse process fusion; pfu, plaque-forming units; pu, particle units; rh, recombinant human; siRNA, small interfering ribonucleic acid; VP, viral particles.

*Converted to level for statistical analysis.

Continues

Table 2. Continued

Study (Author, Year)	Animal, Surgical, and Gene Therapy Details	Study Groups	Fusion Rate by Assessment Technique and Time Point (No. Fused/No. Fusion Sites)
Bright et al., 2006[22]	Animal: Rat (n = 64) Surgery: L5-L6 PLF by percutaneous paraspinal muscle injection (groups A-D) and by open surgery (groups E-G) Graft: Collagen Vector (promoter): pVR1055 (CMV) Growth factor: BMP-7	Percutaneous injection: A. pVR1055-BMP-7 (25 μg) in collagen solution (n = 8) B. pVR1055-BMP-7 (25 μg) in PBS (n = 8) C. BMP-7 protein (30 μg) in collagen solution (n = 8) D. BMP-7 protein (30 μg) in PBS (n = 8) Open surgery: E. pVR1055-BMP-7 (500 μg) and non-crosslinked collagen scaffold (n = 8) F. pVR1055-BMP-7 (500 μg) and crosslinked collagen scaffold (n = 8) G. BMP-7 protein (40 μg) and PBS (n = 8)	Time point: 4 weeks Manual palpation: A. 0% (0/8 levels) B. 0% (0/8 levels) C. 100% (8/8 levels) D. 0% (0/8 levels) E. 0% (0/8 levels) F. 0% (0/8 levels) G. 100% (8/8 levels)
Zhao et al., 2007[50]	Animal: Rabbit (n = 9) Surgery: L5-L6 posterior (laminae and facet joint only) fusion without decortication Graft: Collagen Vector (promoter): Ad5 (CMV) Growth factor: BMP-4	A. Ad5-BMP-4 (n = 6) B. Ad5-β-gal (n = 3)	Time point: 12 weeks Fusion rates were not reported; however, radiographic evaluation revealed bone formation around the L5-L6 laminae, facet joints, and spinous processes in the Ad5-BMP-4 group and no bone formation in the Ad5-β-gal group.
Lu et al., 2007[38]	Animal: Rat (n = 20) Surgery: L4-L5 PLF Graft: DBM Vector (promoter): Ad5 (CMV) Growth factor: Nell-1	A. Ad5-Nell-1 (n = 10) B. Ad5-LacZ (n = 10)	Time point: 6 weeks Manual palpation A. 60% (6/10 levels) *$P < 0.01$ versus B B. 20% (2/10 levels)
Klineberg et al., 2014[34]	Animal: Rabbit (n = 12) Surgery: L5-L6 PLF Graft: AIBG Gene therapy: Noggin siRNA Approach: Electroporation Electric pulse: 8 cycles/s rectangular current at 200V for 20 ms with 200 ms intervals	A. Noggin siRNA (n = 12)	Time point: 6 weeks Manual palpation A. 50% (6/12 levels)

n = 9.

β-gal, beta-galactosidase; B, billion; BMP, bone morphogenetic protein; CMV, cytomegalovirus; DBM, demineralized bone matrix; DOX, doxycycline; CT, computed tomography; PBS, phosphate-buffer saline; PLF, posterolateral lumbar intertransverse process fusion; pfu, plaque-forming units; pu, particle units; rh, recombinant human; siRNA, small interfering ribonucleic acid; VP, viral particles.

*Converted to level for statistical analysis.

fusion in 97%; 95% CI: [91%, 100%]) or paraspinal muscle injection (4 studies; fusion in 99%; 95% CI: [90%, 100%]) ($P < 0.01$). Among in vivo approaches, there was no difference in fusion rate among surgical techniques (Figure 6).

Vector. Most studies (n = 18) in both the ex vivo (n = 13) and in vivo (n = 5) groups utilized an adenovirus type-5 vector. Among the remaining 8 ex vivo studies, 3 utilized plasmid cytomegalovirus, 2 utilized lentivirus, 2 utilized baculovirus, and 1 utilized a ptTATop system. The plasmid pVR1055 was utilized in the remaining in vivo study. There was no significant difference in fusion rates based on type of vector utilized among the ex vivo studies; however, among the in vivo studies, the adenovirus type-5 system was associated with superior fusion rates relative to the pVR1055 vector, which showed no evidence of fusion in any of the 32 treated animals (62% vs. 0%; $P < 0.01$) (Supplementary Figure 2).

Growth Factor. Twenty-three of the 27 studies utilized bone morphogenetic proteins, including BMP-2 (13 ex vivo studies; 2 in vivo studies), BMP-6 (1 ex vivo study; 1 in vivo study), BMP-7 (4 ex vivo studies), and BMP-9 (1 ex vivo study; 1 in vivo study). The combination of BMP-2 and

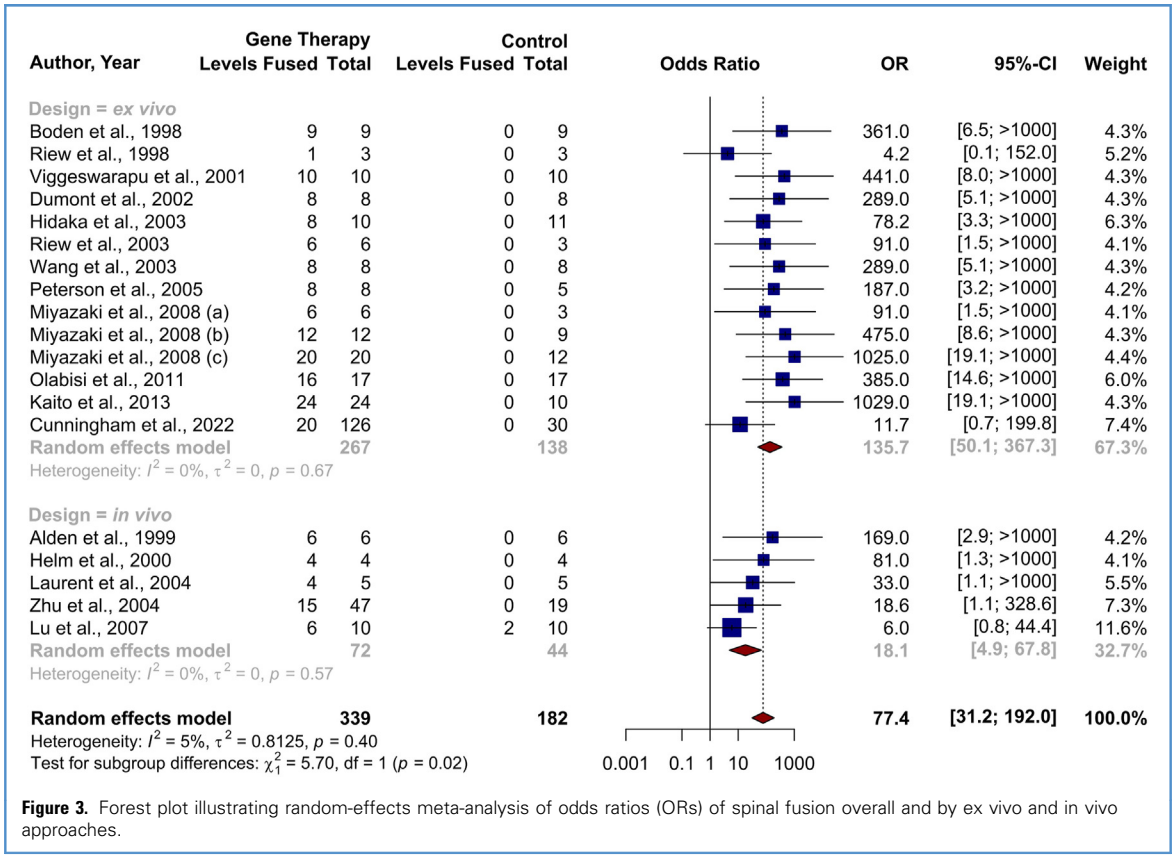

Figure 3. Forest plot illustrating random-effects meta-analysis of odds ratios (ORs) of spinal fusion overall and by ex vivo and in vivo approaches.

BMP-7 was utilized in two ex vivo studies and 1 in vivo study, while the combination of BMP-2 and vascular endothelial growth factor was examined in 1 ex vivo study. The remaining four studies utilized Lim mineralization protein 1 (3 ex vivo studies) and Nell-1 (1 in vivo study). Among ex vivo studies, there was no significant difference in fusion rates based on the type of growth factor utilized; use of BMP-7 was associated with a significantly lower rate of fusion among the in vivo studies (Supplementary Figure 3).

Cell Type. Twelve ex vivo studies utilized bone marrow-derived MSCs, and four utilized adipose-derived mesenchymal stem cells. The remaining studies utilized other stem/stromal cell lines, including buffy coat cells (n = 2), nontypified MSCs (n = 1), normal rat kidney (n = 1), embryonic MSCs (n = 1), and an osteoblast cell line (n = 1). There was no significant difference in fusion rates based on the cell type utilized (Supplementary Figure 4).

DISCUSSION

Nonunion following instrumented fusion remains a significant unsolved problem in spine surgery that is associated with suboptimal patient reported outcomes, increased rates of reoperation, and increased health care spending. Gene therapy represents one possible avenue for enhancing fusion rates and hence improving patient outcomes. Here, we systematically reviewed the existing literature examining the effect of gene therapy, including the use of both in vivo and ex vivo approaches, to promote spinal fusion and provide a meta-analysis of the available data. We found that both in vivo and ex vivo approaches were associated with significantly higher rates of spinal fusion when compared to treatment with null (biologically inactive) vectors (OR 77; $P < 0.001$). Additionally, we found ex vivo approaches—those inoculating the operative site with foreign cells expressing osteoinductive genes—were significantly more effective at facilitating fusion relative to in vivo strategies—those employing vectors to promote the expression of osteoinductive genes by host cells ($P = 0.017$). The beneficial effects of gene therapy on facilitating bony union were found to be independent of animal model, suggesting that such approaches may be efficacious in clinical populations.

The major mechanistic benefit to gene therapy in spinal fusion is the ability to create a localized and sustained concentration of osteoinductive agent (e.g., BMP-2) at the fusion site. This is in contrast to current strategies (e.g., BMP-2-infused

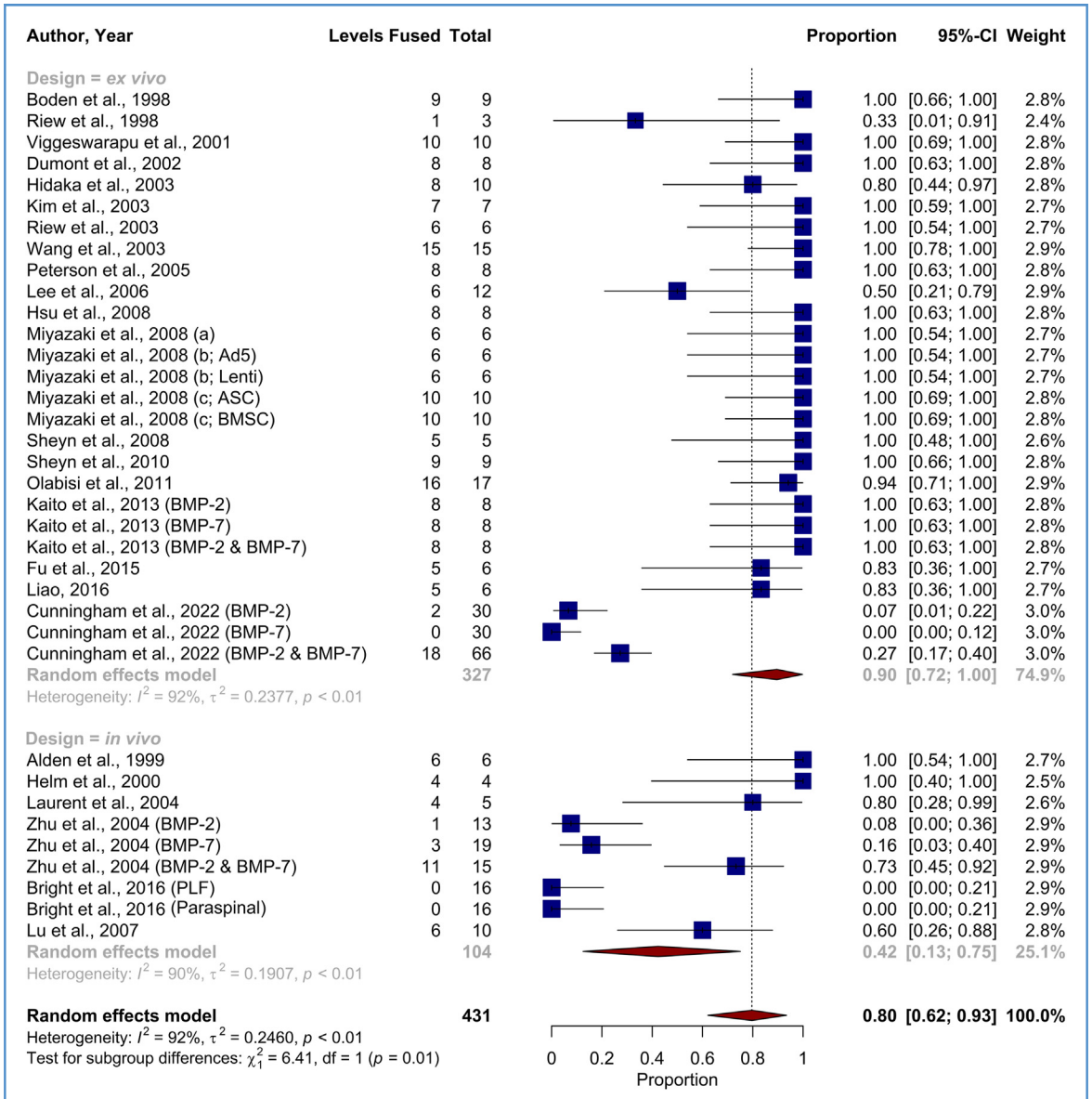

Figure 4. Forest plot illustrating random-effects meta-analysis of fusion rates overall and by ex vivo and in vivo approaches.

collagen sponge) that deliver supraphysiologic levels of growth factors that rapidly diminish in local concentration.[52,53] To this end, prior studies have suggested that the slow, sustained release of growth factors leads to greater quality and quantity of bone formation relative to burst release kinetics.[54,55] For similar reasons, the sustained and controlled local release of growth factors offered by gene therapy may also help to lower the incidence of heterotopic/ectopic bone formation and associated radiculopathy seen with current graft options that utilize osteoinductive factors.[56]

The largest unanswered questions regarding the clinical translatability of gene therapy are tied to safety and costs. Feared complications of gene therapy are often tied to the vector employed and include gene silencing, genotoxicity (insertional mutagenesis), phenotoxicity (overexpression, ectopic, or dysregulated expression of the inserted gene), immunotoxicity, horizontal transmission, and vertical transmission.[57] Of these, immunotoxicity, horizontal, and vertical transmission are likely to be of greatest concern as the most effective therapies

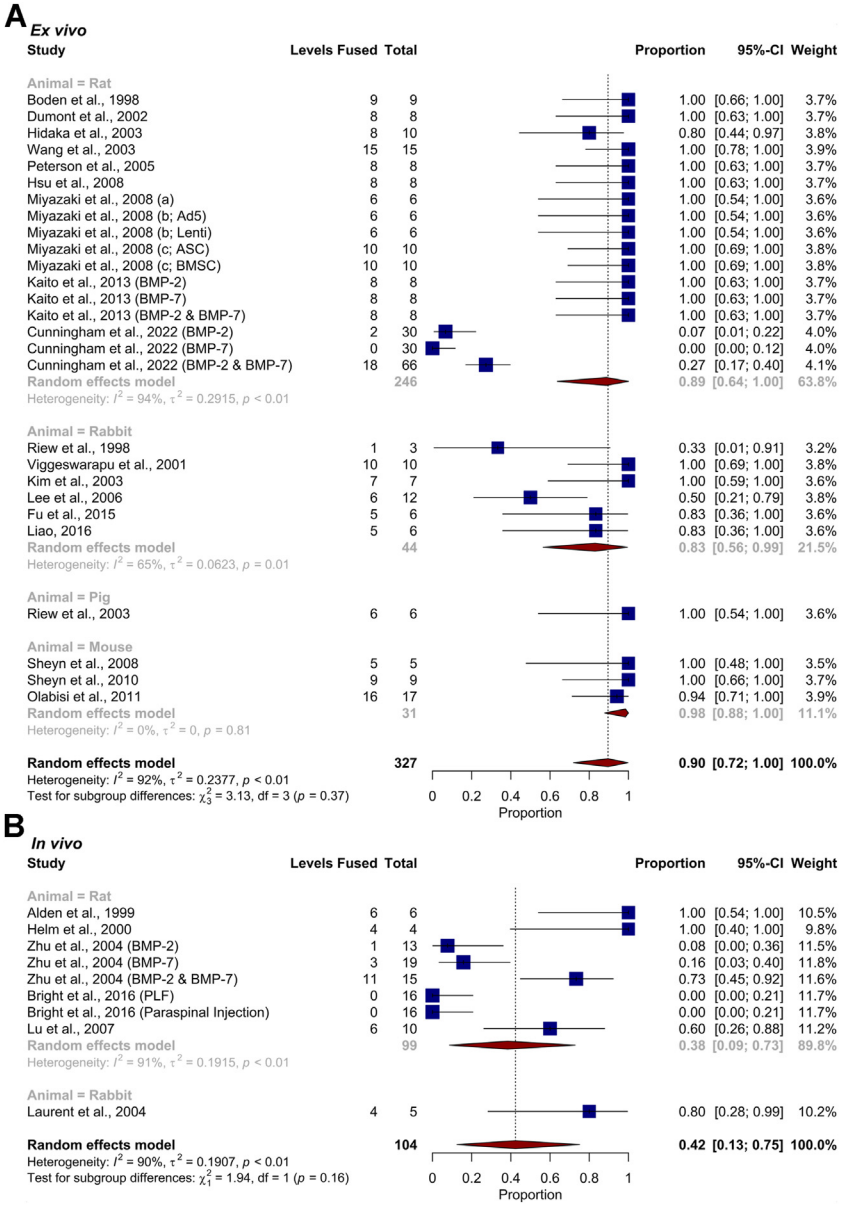

Figure 5. Forest plot illustrating subgroup random-effects meta-analysis of fusion rates by animal model.

described employed adenovirus vectors. These vectors are known for high transduction efficiencies and long expression times.[58] And while both horizontal and vertical transmission remains theoretical risks, immunotoxicity has been observed in prior experiments involving patients with spinal muscular atrophy, hemophilia, and Leber's hereditary optic neuropathy.[57] As our search failed to document the existence of a single published clinical trial examining the effects of gene therapy in spinal fusion, it is unclear to what degree these risks may manifest in human populations. Nonetheless, as many of the toxicities appear related to the vector, the relative safety of such therapies are likely to be better understood in the near future as these technologies continue to rapidly advance, with the Food and Drug Administration announcing its expectations to approve upwards of twenty cell and gene therapies per year by 2025.

Additionally, the enormous current costs associated with gene therapies will need to be substantially reduced for gene therapy to have any commercial realization in patients undergoing elective spine fusion. As a reference for the potential cost of gene therapy, etranacogene dezaparvovec (CSL Behring LLC; King of Prussia, PA)—an in vivo gene therapy for the treatment of hemophilia B—has a list price of approximately $3.5 million, making it the world's most expensive single-use medication.[59] Such high costs are driven by the unique manufacturing processes associated with gene therapies, including vector- and cell-based production.[60] To this end, the higher costs associated with ex vivo spine fusion gene therapies relative to in vivo approaches may be offset by their increased efficacy, as our findings suggest, although additional research is needed to establish the superiority of ex vivo strategies in spinal fusion. Nonetheless, further refinement of gene therapy technologies over the next decade may lower the unit cost to the point that neoadjuvant or adjuvant gene therapy strategies in spinal fusion may prove cost effective in clinical populations.

There are several limitations to the present study, the foremost of which is the reliance on heterogeneous animal data. We attempted to address this limitation by employing random-effects versus fixed-effects statistical modeling and performing subgroup meta-analyses.

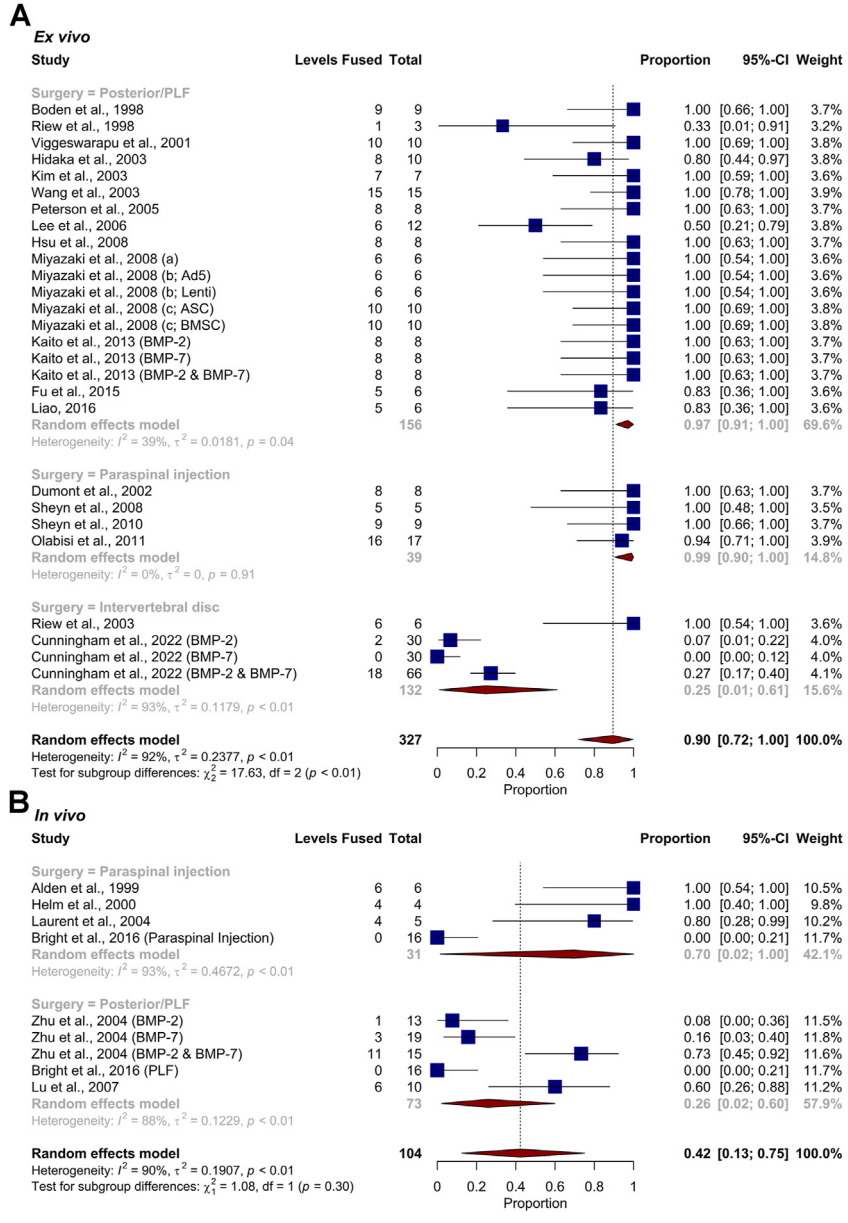

Figure 6. Forest plot illustrating subgroup random-effects meta-analysis of fusion rates by surgical technique.

We also evaluated the quality of the included studies against established criteria, leading us to conclude that the overall risk of bias among the included studies was unclear. Additionally, as with most animal models of spinal fusion, the present models did not employ instrumentation to stabilize the fusion site during bony healing, as is standard of care in human patients. The addition of instrumentation to stabilize the fusion site is held to improve fusion rates, and so it is unclear whether such marked differences in fusion rates would be seen had the gene therapy been added as an adjuvant to instrumented fusion as opposed to the in situ fusion conducted in animal models. Nevertheless, the models employed by the accrued studies are considered standard in animal work and there is a total lack of human studies at present, suggesting that the present evidence is the best available and highlighting the need for translation to clinical populations. Additionally, most of the animal models described herein compare the active vector to a null vector—one that does not express an osteoinductive gene—as compared to the current therapeutic standard for enhancing the osteoinductive properties of the fusion site—namely, the addition of graft substitutes, including biologically active glass and BMP-2-impregnated collagen sponges. Despite these limitations, this represents the first systematic review of a novel technology with the potential to improve outcomes in humans undergoing spinal fusion. We believe that the present data support the use of further investigation towards clinical translation.

CONCLUSIONS

We present the first systematic review and meta-analysis evaluating the effect of gene therapy on promoting spinal fusion. We found the use of gene therapy to significantly increase fusion rates (OR 77; $P < 0.001$) relative to inactive (null vector) controls and that ex vivo gene therapy strategies were more efficacious than in vivo strategies ($P = 0.017$). The effect of gene therapy on fusion was independent of animal model, suggesting that such approaches may be efficacious in clinical populations. Future validation in human populations is needed.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Ethan Cottrill: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Writing — original draft, Writing — review & editing. **Zach Pennington:** Data curation, Formal analysis, Methodology, Writing — review & editing. **Nathan Sattah:** Data curation, Formal analysis, Writing — review & editing. **Crystal Jing:** Data curation, Formal analysis, Writing — review & editing. **Dave Salven:** Data curation, Formal analysis, Writing — review & editing. **Eli Johnson:** Data curation, Formal analysis, Writing — review & editing. **Max Downey:** Data curation, Formal analysis, Writing — review & editing. **Shyni Varghese:** Resources, Supervision, Writing — review &

editing. **Brett Rocos:** Resources, Supervision, Writing — review & editing. **William Richardson:** Conceptualization, Project administration, Resources, Supervision, Writing — review & editing.

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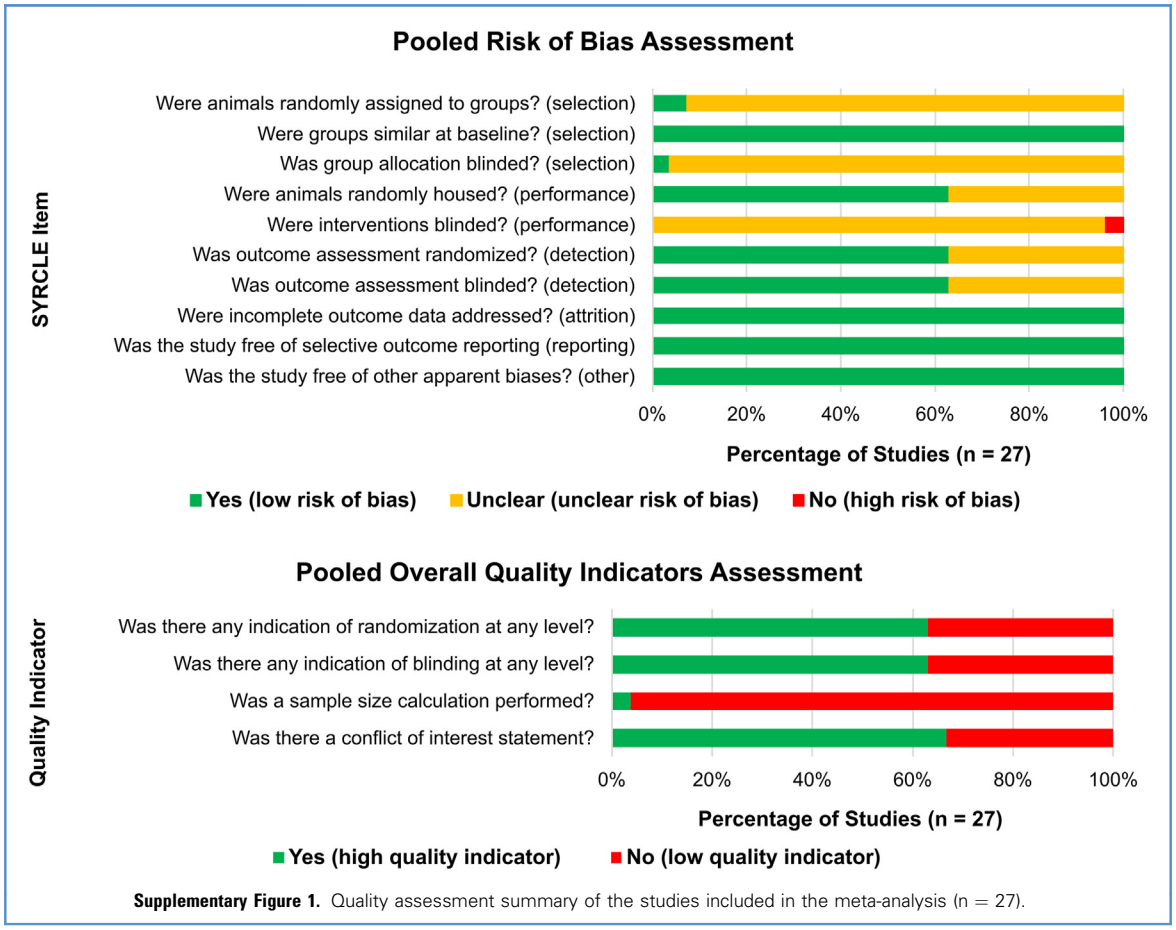
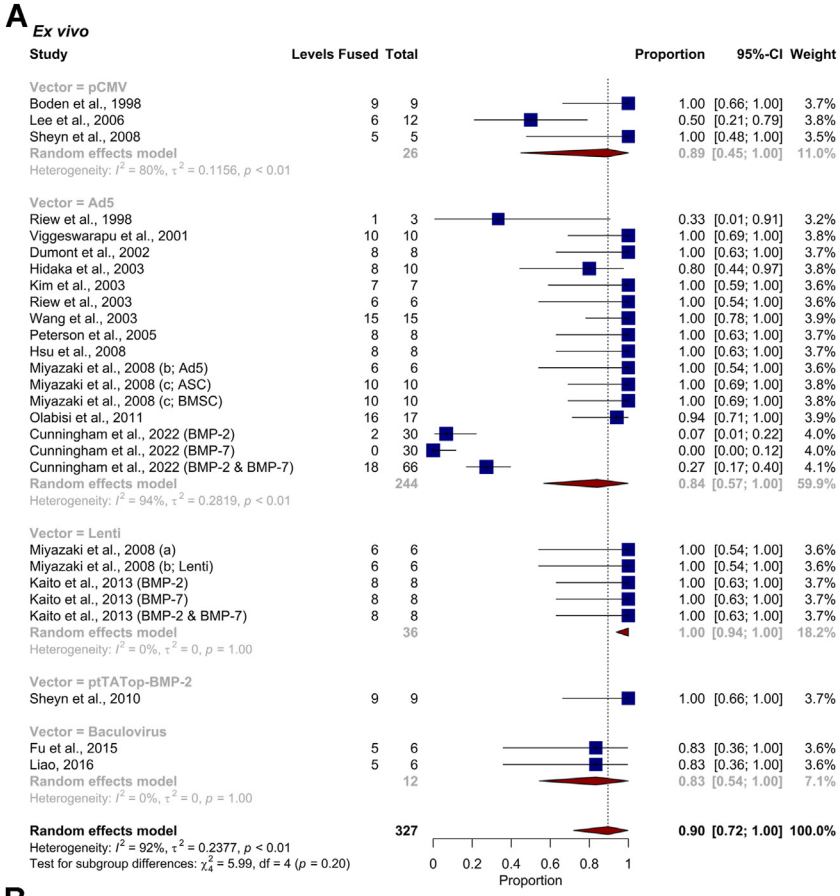
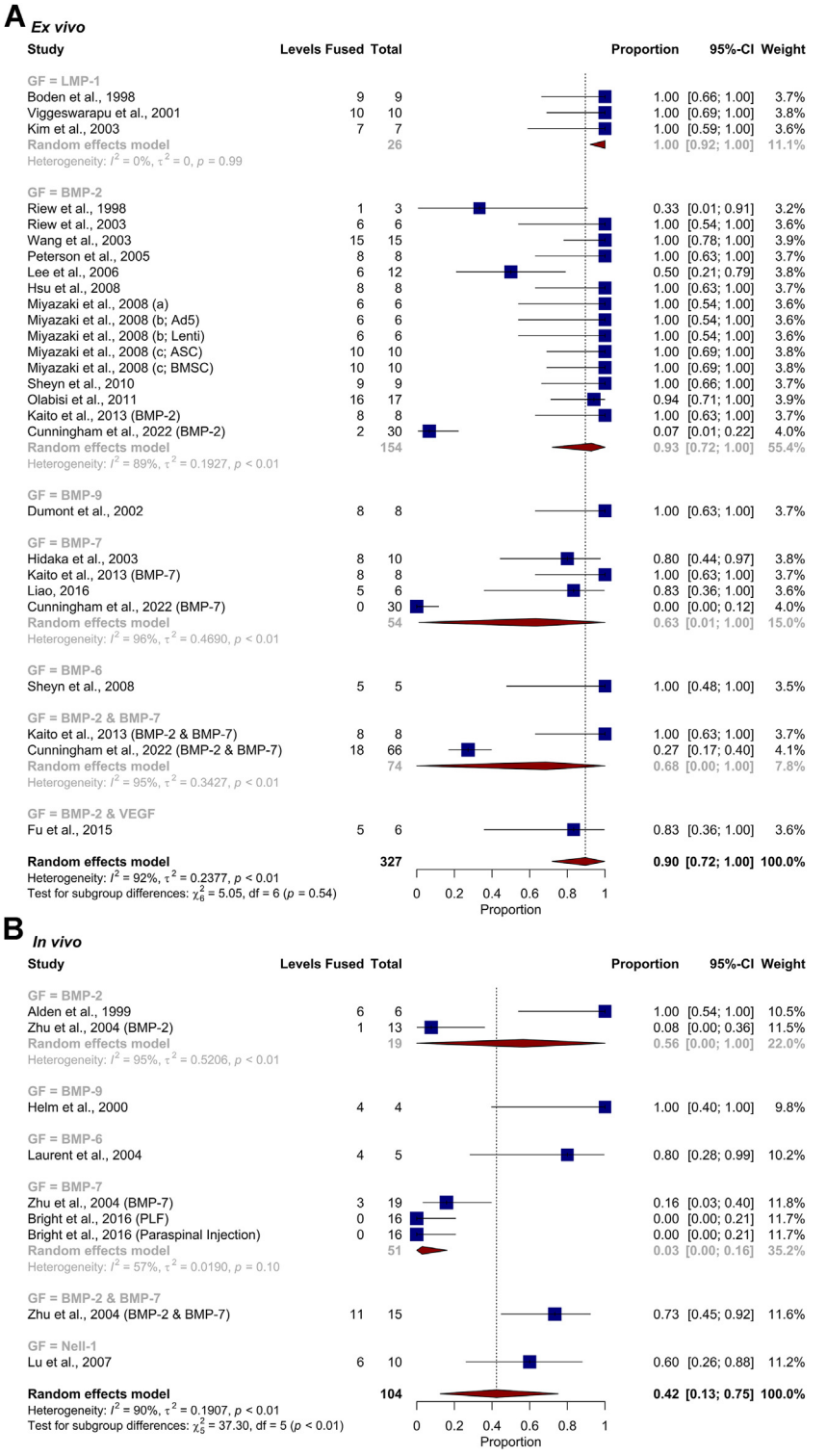
SUPPLEMENTARY DATA



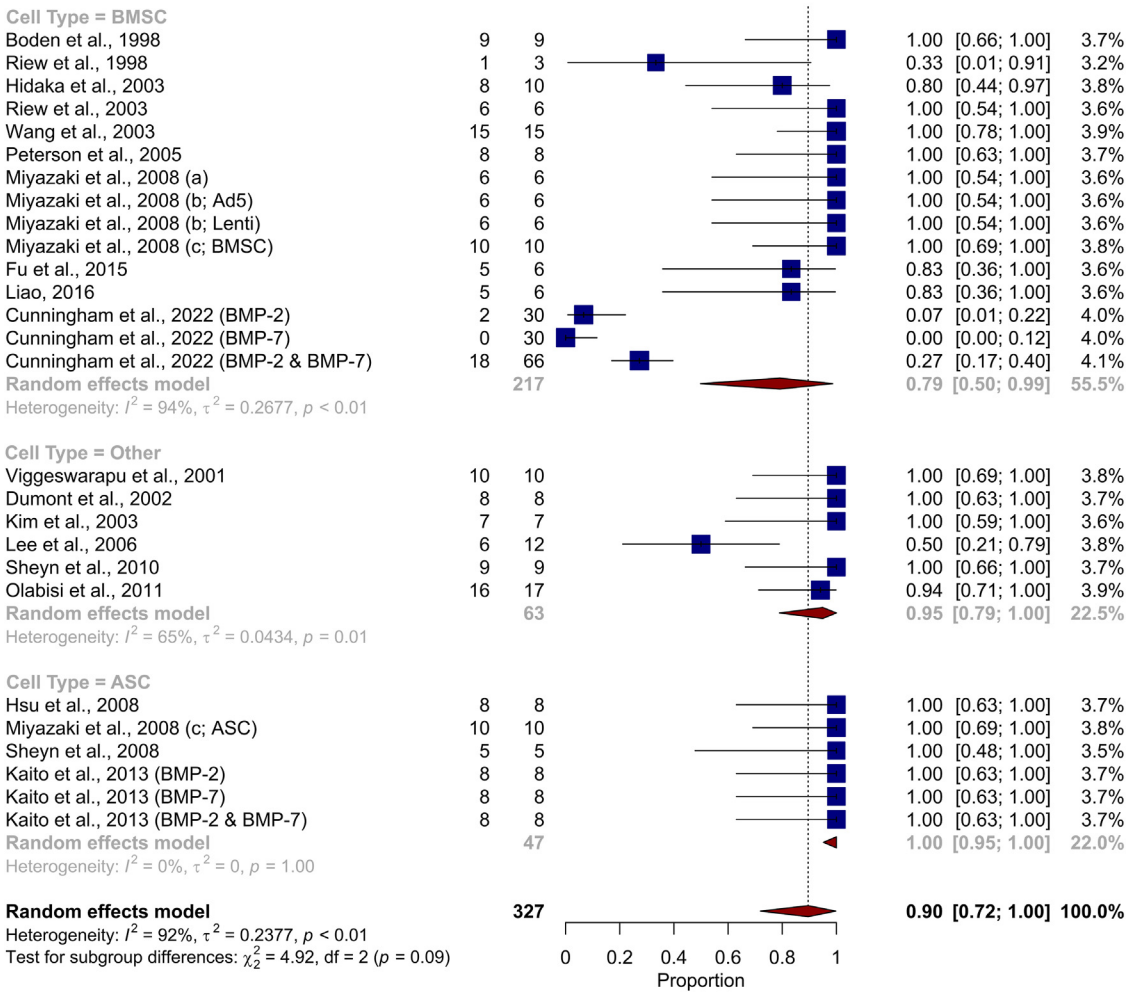
Supplementary Figure 1. Quality assessment summary of the studies included in the meta-analysis (n = 27).



Supplementary Figure 2. Forest plot illustrating subgroup random-effects meta-analysis of fusion rates by vector type.



Supplementary Figure 3. Forest plot illustrating subgroup random-effects meta-analysis of fusion rates by growth factor.



Supplementary Figure 4. Forest plot illustrating subgroup random-effects meta-analysis of fusion rates by cell type.