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Clinical Study

Fusion rate of *Escherichia coli*-derived recombinant human bone morphogenetic protein-2 compared with local bone autograft in posterior lumbar interbody fusion for degenerative lumbar disorders

Sangman Park, MD, Yeong ha Jeong, MD, Byeong Jin Ha, MD, Beom seok Yoo, MD, Soo-Heon Kim, MD, Chang Kyu Lee, MD, PhD, Seong Yi, MD, PhD, Yoon Ha, MD, PhD, Keung Nyun Kim, MD, PhD, Dong Ah Shin, MD, PhD*

Department of Neurosurgery, Spine and Spinal Cord Institute, Severance Hospital, Yonsei University College of Medicine, 50-1, Yonsei-ro, Seodaemun-gu, Seoul, Republic of Korea

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Abstract

BACKGROUND CONTEXT: The use of recombinant human bone morphogenetic proteins-2 (rhBMP-2) for spinal fusion has been reported to be effective. However, most studies have focused on posterolateral and anterior lumbar interbody fusion, and few have investigated posterior lumbar interbody fusion (PLIF).

PURPOSE: This study aimed to determine the effectiveness and safety of the delivery of *Escherichia coli*-derived rhBMP-2 (E.BMP-2) with hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) poloxamer hydrogel composite carriers for PLIF.

STUDY DESIGN: A retrospective study.

PATIENT SAMPLE: Patients who underwent 1 to 3 levels of PLIF for lumbar degenerative disc disorders between 2015 and 2020 with a follow-up of ≥1 year were enrolled. In total, 254 patients (357 levels) were included in the analysis. The evaluation was performed at each segment level. In the E.BMP-2 group, 160 patients (221 levels) received autologous local bone with E.BMP-2 (maximum 0.5 mg/level), and in the control group, 94 patients (136 levels) received only local bone graft.

OUTCOME MEASURES: The primary outcome of this study was to compare the X-ray and CT fusion rates between the two groups. Secondary outcomes included analysis of the patients' clinical outcomes and postoperative complications on CT scans.

METHODS: Clinical evaluations were performed using a visual analog scale for back pain, the Oswestry Disability Index for disability, and physical and mental component summaries of the Short Form 36-Item Form Health Survey to assess functional effects and quality of life. The fusion was evaluated using radiography and CT. On radiography, solid fusion was defined when the difference between extension and flexion was less than 5°. On CT, solid fusion was defined when the upper and lower vertebral bodies were connected by the trabecular bone (bone bridge formation). In addition, complications such as osteolysis, cage subsidence, and screw loosening were investigated using CT.

RESULTS: All clinical results for low back pain, disability, and quality of life in both groups were excellent and showed statistically significant improvements compared with baseline ($p<.0001$). According to the X-ray evaluations, fusion was achieved in 92.31% (204/221) of the patients in the E.BMP-2 group and 82.35% (112/136) of the patients in the control group ($p=.0041$). According to the CT evaluations, the fusion rates were 93.21% (206/221) and 88.24% (120/136) in the E.BMP-2

FDA device/drug status: Not applicable.

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*Corresponding author. Department of Neurosurgery, Spine and Spinal Cord Institute, Severance Hospital, Yonsei University College of Medicine, 50-1, Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea. Tel.: (82)-2-2228-2174; fax: (82)-2-393-9979.

E-mail address: cistern@yuhs.ac (D.A. Shin).

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and control groups (p=.1048), respectively. Except for screw loosening, which had a significantly higher incidence in the control group (p=.0014), the rates of most postoperative complications were not significantly different between the groups.

CONCLUSIONS: This study demonstrated that the adjunctive use of a low dose of E.BMP-2 with HA and β-TCP hydrogel can effectively promote bone fusion, making it a promising option for patients with limited autograft availability or compromised bone quality in PLIF.

Keywords: Beta-tricalcium phosphate; Bone graft; Bone morphogenetic protein-2; Hydroxyapatite; Intervertebral disc degeneration; Lumbar vertebrae; Poloxamer; Spinal fusion; Posterior lumbar interbody fusion

Introduction

Spinal fusion techniques such as posterolateral fusion (PLF) and interbody fusion are used to treat degenerative spinal disorders. Interbody fusion can be classified according to the surgical approach as posterior lumbar interbody fusion (PLIF), transforaminal lumbar interbody fusion (TLIF), anterior lumbar interbody fusion (ALIF), oblique lumbar interbody fusion (OLIF), and lateral lumbar interbody fusion (LLIF). Among these, posterior approaches (PLIF and TLIF) are the most widely used worldwide because they can extensively remove pain generators, have relatively low complication rates, and are associated with good patient prognosis [1,2]. In addition, these posterior approaches can be further stabilized by achieving solid three-column stabilization using pedicle screws.

Bone grafting must be performed inside the cage during interbody fusion to facilitate fusion between the adjacent vertebrae. Autogenous bone grafting is one of the most widely used methods. However, it is important to note that locally harvested laminectomized bone primarily consists of cortical bone, which is difficult to fuse [3]. Additionally, autologous iliac crest transplantation is associated with various risks, including graft-site morbidity, prolonged operative time, increased bleeding, potential for neurovascular injury, extended hospital stay, and potential cosmetic concerns [4−6]. In addition, the patient's iliac crest bone graft supply is limited and may not be sufficient in all cases. Therefore, the need for a bone graft material for effective union without using autogenous bone has emerged.

Allografts, demineralized bone matrix (DBM), ceramics, recombinant human bone morphogenic protein-2 (rhBMP-2), and anorganic bone matrix/15-amino acid peptide fragment (ABM/P-15) have been investigated as substitutes to replace autografts to achieve interbody fusion [7−10]. Several studies have confirmed the effectiveness and safety of rhBMP-2s [11−13]. In some studies on ALIF, the use of rhBMP-2 was associated with postoperative adverse events such as prevertebral swelling, formation of seroma or hematoma, radiculitis, osteolysis, heterotopic ossification, retrograde ejaculation, and increased rates of new malignancy. However, most studies have focused on PLF and ALIF, and few have investigated interbody fusion using posterior approaches. Additionally, studies investigating rhBMP-2 have used rhBMP-2 produced using Chinese Hamster Ovary (CHO) cells. CHO cell-derived rhBMP-2 (C.BMP-2) has been used in various spinal arthrodesis procedures, with high fusion rates at high concentrations [13]. Despite such high fusion rates, C.BMP-2 has been continuously challenged by high production costs, low production yields, and complications associated with high doses [14,15]. Recently, rhBMP-2 has been effectively produced using *Escherichia coli* instead of CHO cells. Preclinical and early clinical studies on *E. coli*-derived rhBMP-2 (E.BMP-2) have shown promising results [16−20]. These studies investigated the efficacy of E.BMP-2 in promoting bone fusion and regeneration. Lee et al. [16] compared different dosages of E.BMP-2 using a hydroxyapatite (HA) carrier and found that higher dosages resulted in a higher fusion rate. Hwang et al. [17] evaluated E.BMP-2 in a mini-pig spinal fusion model and observed improved fusion rates with increasing E.BMP-2 dosage. Kong et al. [18] studied the use of E.BMP-2/HA in posterolateral lumbar fusion in minipigs. Wadhwa et al. [19] investigated bone regeneration in rabbit calvarial defects by using a tooth biomaterial with BMP-2. Cho et al. [20] conducted a multicenter trial and reported the efficacy of E.BMP-2 in posterolateral lumbar fusion. Son et al. [21] evaluated the safety and efficacy of E.BMP-2 in additional lumbar PLF with a minimum of 1-year follow-up. Overall, these studies support the effectiveness of E.BMP-2 for promoting bone fusion and regeneration in animal models as well as clinical situations.

However, studies regarding the effect of E.BMP-2 on PLIF surgery are lacking. This study aimed to determine the effectiveness and safety of E.BMP-2 with HA and β-tricalcium phosphate (β-TCP) poloxamer hydrogels for PLIF.

Materials and methods

Patient selection

This study was approved by the Institutional Review Board (IRB No.4-2022-0184). Data were collected by retrospectively reviewing the medical records of patients who underwent lumbar spine surgery at a single center between 2015 and 2020. The inclusion criteria were adults (≥19 years old) and patients who underwent PLIF with local autogenous bone or E.BMP-2/HA between L1/2 and

L5/S1 because of degenerative disc disorder (spinal stenosis, isthmic or degenerative spondylolisthesis, and spinal instability). Exclusion criteria were previous spinal surgical history, trauma, infection, and malignancy. Additionally, participants lost to follow-up or those without available imaging data were excluded from the study. We identified 633 patients who underwent PLIF at our hospital between 2015 and 2020. Among these 254 patients (357 levels) met the study criteria and were included in the analysis. Of the 254 patients included in the radiological evaluation, clinical outcomes were collected from 136 patients (E.BMP-2 group, 89 patients [118 levels]; control group, 47 patients [69 levels]). All patients had a history of axial or radicular symptoms refractory to conservative treatment for at least 12 weeks, and were observed clinically and radiologically for at least 1 year. A single surgeon performed the operations using the same protocol for all patients, and data were collected and analyzed by other researchers who did not participate in the operation.

Surgery

A standard posterior approach was used in all patients. The patient was placed in the prone position on a Wilson frame. A midline skin incision was made and subperiosteal dissection was performed. The lamina and mammillary processes were exposed. After subtotal laminectomy, a wide facetectomy was performed to expose the intervertebral disc. Bilateral annulotomy was performed using disc shavers and curettes, and the nucleus and cartilaginous endplates were removed. The shavers were gently manipulated to avoid damage to the bony endplates. The cages (LumFix cage, CGBio Co., Ltd., Seoul, Korea) used in the E.BMP-2 group were filled with laminectomized autologous bone, E. BMP-2 with HA granules (Novosis, CGBio Co., Ltd., Seoul, Korea) and β-TCP poloxamer hydrogel (Excelos Inject, CGBio Co., Ltd., Seoul, Korea), while the cages in the control group were filled with laminectomized autologous bone only. In this study, we used a total of 0.5 mg of E.BMP-2 for single-level fusion and 1 mg for 2 to 3 levels of fusion in the E.BMP-2 group (E.BMP-2 dose maximum, 0.5 mg/level). The cage was inserted tightly into the disc space using a root retractor while protecting the nerve. To prevent the leakage of E.BMP-2, the annulotomy sites were sealed with gel foam and fibrin glue. After cage insertion, the Wilson frame was maximally released to restore normal lordosis, and pedicle screw fixation was performed. After hemostasis was achieved, the wound was closed layer-by-layer. The patients wore lumbosacral orthosis (LSO) braces for 1 month postoperatively.

Radiological evaluation

We assessed the fusion rate with an angular difference using dynamic radiography and bony bridging using CT 12 months postoperatively (Figure). At routine postoperative intervals, dynamic lateral lumbar spine radiographs were obtained, with the patient lying on a table. We measured the Cobb angle of the upper and lower endplates of the index level on dynamic X-rays using the PACS software (GE Healthcare, Mi, WI, USA). Solid fusion was defined as a difference was less than 5°. On CT (GE Healthcare, MI, USA), solid fusion was defined as the connection of the upper and lower vertebral bodies by the trabecular bone [22]. Additionally, complications, such as osteolysis, cage subsidence, and screw loosening, were investigated using imaging of each segment. Subsidence was confirmed through dual verification using both X-ray and CT imaging. CT imaging allows for meticulous observation of

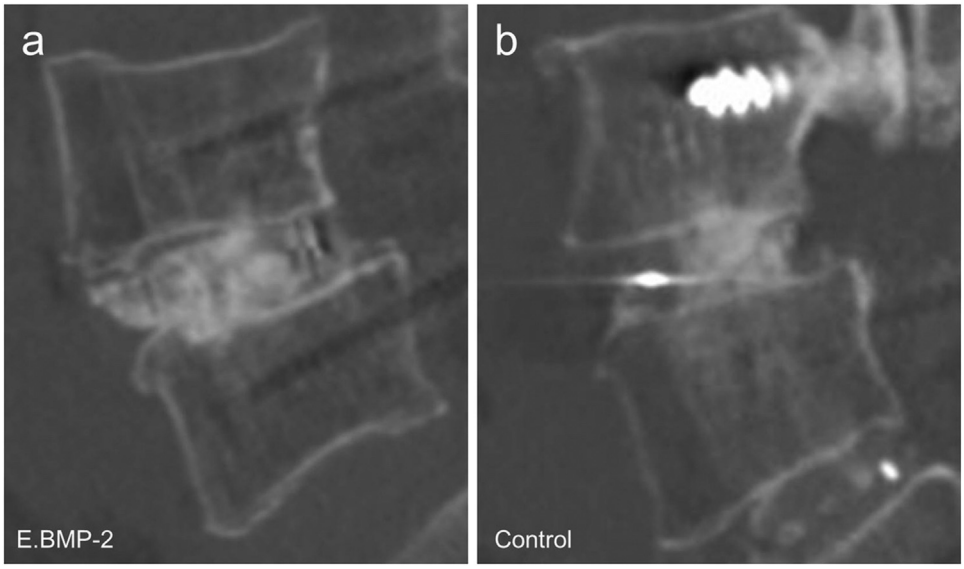

Figure. One-year postoperative lumbar CT scans showing solid fusion at the index level. Bony bridging was more intense in the E.BMP-2 group (A) than the control group (B).

Table 1
Patient demographics

	E.BMP-2 group (n=160)	Control group (n=94)	p-value*
Age (years)	64.20±9.68	63.99±11.33	.5413(w)
Gender, n (%)			
Female	115 (71.88)	54 (57.45)	**.0186(c)**
Male	45 (28.13)	40 (42.55)	
Body mass index (kg/m^2)	24.59±3.01	25.15±3.37	.1067(w)
Bone mineral density (T-score)	−1.46±1.22	−1.40±1.28	.4939(w)
No. of fused levels, n	1.38±0.58	1.45±0.58	.2900(w)
No. of fused levels (patients), n (%)			
1 level	107 (66.88)	56 (59.57)	.4079(c)
2 levels	45 (28.13)	34 (36.17)	
3 levels	8 (5.00)	4 (4.26)	
Fused level, n (%)			
Total	221 (100.00)	136 (100.00)	
L1/L2	0 (0.00)	1 (0.74)	.2118(c)
L2/L3	6 (2.71)	8 (5.88)	
L3/L4	57 (25.79)	30 (22.06)	
L4/L5	135 (61.09)	77 (56.62)	
L5/S1	23 (10.41)	20 (14.71)	
Follow-up (days)	368.57±20.61	370.54±17.21	.2871(w)

* (w) Wilcoxon's rank-sum test or (c) chi-square test.

subsidence by comprehensively analyzing axial, sagittal, and coronal views.

Clinical evaluation

The clinical outcomes were evaluated in 136 patients (E.BMP-2 group, 89 patients [118 levels]; control group, 47 patients [69 levels]) who completed the questionnaire. Outcomes were assessed using a visual analog scale (VAS) for lower back pain, Oswestry Disability Index (ODI) for disability, and Physical Component Summary (PCS) and Mental Component Summary (MCS) scales for quality of life (QoL). All assessments were performed preoperatively and 12 months postoperatively by a pain specialist nurse, which was unrelated to this study.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation and categorical variables were expressed as the number of patients and corresponding proportion. Two-sample *t* tests, Wilcoxon's rank-sum test for continuous variables, and chi-square test or Fisher's exact test for categorical variables were performed according to the distributional normality of the test results. Changes in clinical outcomes from the baseline were analyzed using paired *t* tests or Wilcoxon signed-rank tests. All statistical analyses were performed using SPSS Statistics for Windows, version 25.0 (IBM Corp. Armon, NY, USA). The threshold for statistical significance was set at p<.05.

Results

Patient demographics

A total of 254 patients (357 levels) who met the inclusion criteria were included in this retrospective study:160 patients (221 levels) in the E.BMP-2 group and 94 patients (136 levels) in the control group. The patients' demographic data are summarized in Table 1. The proportion of female patients was 71.88% (115/160) in the E.BMP-2 group and 57.45% (54/94) in the control group (p=.0186). There were no statistically significant differences between the groups in mean age, body mass index, bone mineral density, follow-up period, distribution of sites, or number of fused levels.

Fusion rates

Radiological outcomes were assessed using the segmental fusion rate (Table 2). When an angle difference of <5° on X-ray was regarded as solid fusion, the fusion rate of the E.BMP-2 group was 92.31% (204/221), which was significantly higher than the rate of 82.35% (112/136) in the control group (p=.0042). When bone bridge formation on CT was regarded as solid fusion, 93.21% (206/221) of the E.BMP-2 group and 88.24% (120/136) of the control group achieved bone union. There was no significant difference in fusion rates on CT between the two groups (p=.1048).

The fusion rate was sub-analyzed according to the number of fused levels and sites (Table 3). In the E.BMP-2 group, 107 patients received 1-level fusion, 45 patients received 2-level fusion (90 levels), and eight patients received 3-level fusion (24 levels). In the control group, 56 patients received 1-level fusion, 34 patients received 2-level fusion (68 levels), and four patients received 3-level fusion (12 levels). Based on X-ray, the 1-level fusion rate of the E.BMP-2 group was 92.52% (99/107), which was significantly higher than the 1-level fusion rate of the control group (78.57% (44/56) (p=.0099). The 2-level and 3-level fusion rates of the E.BMP-2 group were 91.11% (82/90)

Table 2
Segmental fusion rate in all patients

	E.BMP-2 group (n=221)	Control group (n=136)	p-value*
X-ray angular difference < 5°, n (%)			
Yes	204 (92.31)	112 (82.35)	**.0042**
No	17 (7.69)	24 (17.65)	
Bony bridge on CT, n (%)			
Yes	206 (93.21)	120 (88.24)	.1048
No	15 (6.79)	16 (11.76)	

* [c] Chi-square test.

and 95.83% (23/24), respectively, compared with 83.82% (57/68) and 91.67% (11/12) in the control group (p=.1632). Regarding the fusion sites, L4/L5 was the most common in both groups (E.BMP-2 group, 135 levels; control group, 77 levels). Overall, the fusion rates for all sites were higher in the E.BMP-2 group, but the difference was statistically significant only for the association with L4/L5 (p=.0139). In the E.BMP-2 group, solid fusion was achieved in 91.11% (123/135) of the L4/L5 region, whereas the fusion rate in the control group was 79.22% (61/77). The CT-based results were generally similar to the X-ray-based results, with no significant differences between them.

Segmental fusion rates were further analyzed in patients for whom the clinical questionnaire data were collected (Supplementary Table 1). Of the 357 levels overall, 187 (E.BMP-2 group, 118 levels; control group, 69 levels) were included in further analysis. Similar to the analysis results for all patients (Tables 2, 3), the fusion rate on X-ray and CT images was higher in the E.BMP-2 group than in the control group. In particular, the X-ray results showed a significant difference between the groups.

Clinical outcomes

Clinical outcomes were analyzed retrospectively only for patients for whom the questionnaire data were collected (Table 4). In both groups, compared with baseline, all clinical outcomes were significantly improved (p<.0001), and there was no significant difference between the groups.

Complications

No neurological compromise, surgical infection, or postoperative radiculitis was observed. The known complications of BMP-2, such as osteolysis, cage subsidence, and screw loosening, were investigated per segment using CT scans (Table 5). Among the 221 levels in the E.BMP-2 group, osteolysis was observed in 14 levels (6.33%), subsidence in 18 levels (8.14%), and screw loosening in eight levels (3.62%). Among the 136 levels in the control group, osteolysis was observed in 13 levels (9.56%), subsidence in nine levels (6.62%), and screw loosening in 17 levels (12.50%). There was significantly more screw loosening in the control group (p=.0014), but there were no statistically significant differences between the groups in terms of osteolysis and subsidence.

Table 3
Segmental fusion rate according to the number of fused levels and fused level (segments)

	E.BMP-2 group		Control group		p-value*
	n/total	(%)	n/total	(%)	
Angular difference < 5° on X-ray					
No. of fused levels					
1 level	99/107	(92.52)	44/56	(78.57)	**.0099**
2 levels	82/90	(91.11)	57/68	(83.82)	.2172
3 levels	23/24	(95.83)	11/12	(91.67)	>.99
Fused level (segments)					
L1/L2	0/0	(0.00)	1/1	(100.00)	*NA*
L2/L3	6/6	(100.00)	7/8	(87.50)	>.99
L3/L4	54/57	(94.74)	28/30	(93.33)	>.99
L4/L5	123/135	(91.11)	61/77	(79.22)	**.0139**
L5/S1	21/23	(91.30)	15/20	(75.00)	.2221
Bony bridge on CT.					
No. of fused levels					
1 level	99/107	(92.52)	51/56	(91.07)	.7663
2 levels	85/90	(94.44)	58/68	(85.29)	.0520
3 levels	22/24	(91.67)	11/12	(91.67)	>.99
Fused level (segments)					
L1/L2	0/0	(0.00)	1/1	(100.00)	*NA*
L2/L3	6/6	(100.00)	6/8	(75.00)	.4725
L3/L4	54/57	(94.74)	28/30	(93.33)	>.99
L4/L5	126/135	(93.33)	68/77	(88.31)	.2071
L5/S1	20/23	(86.96)	17/20	(85.00)	>.99

* [c] Chi-square test or [f] Fisher's exact test.

Table 4
Clinical outcomes

	E.BMP-2 group	Control group	p-value*
VAS	(n=89)	(n =45)	
Preoperative	7.78±1.72	7.42±1.90	.4629
Postoperative	2.53±2.32	3.20±3.09	.4198
Postoperative − Preoperative	−5.25±2.60	−4.22±3.34	.1216
p-value†	**<.0001**(w)	**<.0001**(w)	
ODI	(n=87)	(n=47)	
Pre	49.37±18.55	47.71±18.45	.6194
Post	29.03±18.63	32.05±18.94	.3999
Post − Pre	−20.34±21.03	−15.66±18.84	.1877
p-value†	**<.0001**(t)	**<.0001**(w)	
P.C.S.	(n=86)	(n=47)	
Pre	31.77±15.39	29.99±14.50	.5079
Post	52.80±20.71	48.98±19.01	.2707
Post − Pre	21.02±21.41	19.00±19.61	.5826
p-value†	**<.0001**(t)	**<.0001**(t)	
M.C.S.	(n=87)	(n=46)	
Preoperative	46.99±20.65	46.54±16.82	.8909
Postoperative	61.50±20.39	57.91±19.25	.3136
Postoperative − Preoperative	14.51±22.98	11.37±17.60	.3829
p-value†	**<.0001**(t)	**<.0001**(t)	

VAS, visual analog scale; ODI, Oswestry Disability Index; PCS, Physical Component Scores; MCS, Mental Component Scores.
* (t)Two-sample t-test or (w) Wilcoxon's rank-sum test.
† (t)Paired t-test or (w) Wilcoxon's signed-rank test.

Table 5
Segmental adverse events on CT scan

	E.BMP-2 group (n=221)	Control group (n=136)	p-value*
Osteolysis	14 (6.33)	13 (9.56)	.2632
Subsidence	18 (8.14)	9 (6.62)	.5961
Screw loosening	8 (3.62)	17 (12.50)	**.0014**

* (c) Chi-square test

The incidence of complications was further analyzed in some patients for whom the clinical questionnaire data were collected (Supplementary Table 2). Among the 118 levels in the E.BMP-2 group, osteolysis was observed in 9 levels (7.63%), subsidence in 8 levels (6.78%), and screw loosening in 2 levels (1.69%). Among the 69 levels in the control group, osteolysis was observed in 4 levels (5.80%), subsidence in 5 levels (7.25%), and screw loosening in 6 levels (8.70%). No significant differences were observed between groups in terms of complications.

Discussion

This study was conducted as a retrospective clinical trial to compare the efficacy and safety of using E.BMP-2, a carrier containing HA granules and β-TCP poloxamer hydrogel, mixed with local autograft, versus using only local autograft, for PLIF surgery to treat degenerative lumbar disorder. The aim of this study was to evaluate the effectiveness and safety of these approaches in achieving spinal fusion at the one-year follow-up.

We analyzed the bone fusion rates of the two groups using radiography and CT at 1-year postoperative follow-up. X-ray analysis showed that the E.BMP-2 group exhibited a significantly higher fusion rate (92.31%) than that of the control group (82.35%). However, in the CT analysis of bone fusion, the E.BMP-2 group showed a fusion rate of 93.21%, whereas the control group showed a rate of 88.24%; this difference was not statistically significant. In terms of evaluating bone fusion, X-rays determine the stability of the fused area in a dynamic environment, whereas CT assesses the pattern of newly formed bone in a static environment. Therefore, it is important to consider whether fusion is observed in both X-ray and CT evaluations rather than relying on only one method to draw conclusions about bone fusion. Because the fusion rate in CT evaluation was higher than that in X-ray analysis for both groups, patients deemed fused in both conditions could be considered fused based on the X-ray fusion rate. These results imply that the autogenous bone group may appear fused on CT, but lacks sufficient dynamic stability. These findings suggest that the E.BMP-2 group is advantageous in terms of bone fusion compared with the autogenous bone group.

In spinal fusion surgery, the success rate of bone fusion decreases as the number of operated segments increases, and achieving fusion at the L5/S1 level is challenging [23−25]. Based on the analysis of fusion rates according to the number of operated segments and surgical levels, the E.BMP-2 group showed similar or higher fusion rates compared with the control group in cases of three-level surgery and L5/S1 level surgery. However, these differences were not statistically significant, indicating that fusion rates were comparable. These findings suggest that although the E.BMP-2 group exhibited favorable fusion outcomes, the lack of statistical significance may be attributed to the small sample size in the analysis. Further studies with larger sample sizes are required to validate these results.

We observed significant improvements in all clinical outcomes evaluated by VAS, ODI, and SF-36 compared with baseline in both the E.BMP-2 and control groups following surgery. These findings suggest that the type of bone graft material used in spinal fusion surgery does not have a major impact on clinical functional recovery, consistent with the findings of previous studies [26,27].

Complications related to rhBMP-2 in lumbar spinal fusion surgery include heterotopic ossification, osteolysis, epidural cyst formation, seroma, subsidence, and myositis [24,25,28,29]. In our study, we analyzed osteolysis, subsidence, and screw loosening by using CT imaging. The results showed a lower incidence of all three complications in the E.BMP-2 group than in the control group, with screw loosening occurring four times less frequently in the E.BMP-2 group compared with the control group. Screw loosening is associated with stability provided by solid fusion at

the surgical site, and these findings indicate that the use of E.BMP-2 can contribute to achieving solid fusion and stability.

The differences between C.BMP-2 and E.BMP-2 can be explained by variances in the production of recombinant proteins and the choice of carrier. Recombinant proteins can be produced via gene editing in eukaryotic and prokaryotic cells. C.BMP-2, produced in eukaryotic cells undergoes glycosylation, a posttranslational modification, as part of the process, resulting in the secretion of active BMP-2 outside the cells. This process mimicked the natural production of BMP-2 in the body, allowing it to exhibit its activity without additional processing. On the other hand, E.BMP-2, produced in prokaryotic cells, is not secreted outside the cells but is obtained through the destruction and purification of inclusion bodies. As it is produced without glycosylation, an additional process called refolding is employed to create a structure similar to that of the native protein present in the body [30,31]. It has been argued that the presence or absence of glycosylation can impact the activity of rhBMP-2. However, it has been established that glycosylation only affects the extracellular secretion process and does not influence the activity of the protein [32]. In vitro and in vivo studies using E.BMP-2 and C.BMP-2 confirmed similar levels of osteoinductive ability [33,34].

Another difference lies in the choice of carriers, with C.BMP-2 utilizing a collagen sponge, whereas E.BMP-2 uses inorganic substances, such as HA or a combination of HA and β-TCP. Collagen and HA are key components of the bone structure in the human body. However, from the perspective of bone regeneration, collagen sponges lack osteoconductivity whereas HA and β-TCP exhibit osteoconductive properties [35,36].

Furthermore, the collagen sponge absorbs the rhBMP-2 solution without any binding affinity, which can lead to the burst release of rhBMP-2 in the early phase after application. The burst release of rhBMP-2 has been considered a reason for various early complications that can occur when using rhBMP-2 [37]. Therefore, controlling the release of rhBMP-2 is important for its safety and effectiveness of rhBMP-2.

In this study, a new putty-type carrier, which is a mixture of β-TCP poloxamer hydrogel and HA granules, was used to improve E.BMP-2 release control and durability. The in vitro release kinetics of E.BMP-2 using the new putty-type carrier were compared with the release pattern of E.BMP-2 from a collagen sponge for up to 28 days. The results showed that The biological half-life of E.BMP-2 was 3.8 hours in the collagen sponge and 6.2 hours in the putty-type carrier. This indicates that a putty-type carrier is more appropriate than a collagen sponge for the sustained release of E.BMP-2 [38]. The efficacy and safety of E.BMP-2 with a new putty-type carrier have been confirmed in various animal models, including rat PLF, rat tail interbody fusion, and mini-pig OLIF models [38−40]. In a rat spinal interbody fusion model, the HA/β-TCP hydrogel carrier enabled superior bone induction with low-dose BMP-2, and decreased the incidence of complications caused by high-dose BMP-2 the collagen carrier.

In spinal fusion surgery, the BMP-2 dosage used for bone fusion is known to directly affect its efficacy and safety. Several studies have reported that higher BMP-2 doses can increase the success rate of bone fusion. However, as the dose increased, the incidence of complications also increased [25,41]. Therefore, the use of the lowest effective dose for BMP-2 is recommended to ensure both efficacy and safety.

In studies using C.BMP-2 in PLF, 4.2 to 42 mg/level of C.BMP-2 was used, and the fusion rate was reported to be 62.4 to 97.4%. In clinical trials using E.BMP-2 and HA granules, 6 mg/level (3 mg/side) of E.BMP-2 was used in PLF patients, and bone fusion was achieved in all patients in both the autologous iliac bone graft and E.BMP-2 groups at 6 months to demonstrate noninferiority to autologous iliac bone [20]. A follow-up report of more than 2 years for the same patient cohort reported that treatment-related serious adverse events and neoplasms did not occur [42].

In a study investigating the efficacy of BMP-2 in patients with PLF, E.BMP-2 (2.5 mg) was administered for additional unilateral PLF after lumbar interbody fusion. All patients showed complete bone fusion at six months, and the mean time required for bone union was 4.5 months [43]. Another article reported that 1 mg of E.BMP-2 was mixed with an HA carrier and morselized local bone graft for a unilateral additional PLF after lumbar interbody fusion for 1 (1 mg/level) or 2 levels (0.5 mg/level). The fusion rates at 6 and 12 months after surgery were 79.7% and 94.2% in the AIBG group, and 82.7% and 100% in the E.BMP-2 group, respectively. The results showed that 0.5 mg E.BMP-2 is the adequate minimal dose for bone fusion in PLF without any complications related to E.BMP-2 [21].

In addition, interbody fusion is applied with bone graft materials inside or outside the cage that can withstand physical loads, unlike PLF techniques, which apply bone graft materials around the vertebrae. Therefore, it is important to use a carrier in the formulation that can be applied inside a cage. The HA granule, which was used as a carrier of BMP-2 in PLF, had a limitation in that when applied inside the cage, the granules could escape without being fixed in place. To overcome this limitation, when E.BMP-2 was used for interbody fusion, an injection-type carrier in which β-TCP microbeads were mixed with a poloxamer hydrogel was introduced.

Many studies have reported the safety and efficacy of BMP-2 for ALIF. In studies using C.BMP-2 in ALIF, a total of 2.1 to 12 mg/level of C.BMP-2 was used, and the fusion rate was reported as 88% to 100% [13]. In the ALIF study using E.BMP-2 for patients with adult spinal disorder (ASD), a total of 3 mg/level E.BMP-2 mixed with β-TCP microbead poloxamer hydrogel was used on the L5−S1 lumbosacral region. They achieved fusion rates of 68.4% and 100% at 6 and 12 months after surgery, respectively.

No adverse events were associated with E.BMP-2 [44]. The results indicated that 3 mg/level of E.BMP-2 can induce appropriate fusion for interbody fusion surgery.

Unlike PLF, there have been many reports on the use of low doses of BMP-2, because the use of BMP-2 in interbody fusion surgery has a high risk of complications, including neurological deficits. Studies with C.BMP-2 in PLIF or TLIF used a lower dose of 1.4 to 12 mg/level than that used in ALIF, showing a bone fusion rate of 82.6% to 100% [13].

In this study, based on the results reported in previous studies using E.BMP-2 in PLF and ALIF, the appropriate dose to apply E.BMP-2 in PLIF did not exceed 1 mg (0.3 −0.5 mg/level). We used local autologous bone and 1 mg of E.BMP-2, HA, and β-TCP hydrogel in PLIF and showed 92.31%, and 93.21% fusion rates on simple radiography and CT, respectively, at 12 months after surgery. The results mean that despite using a low dose of 1 mg, it can exhibit a sufficient bone fusion effect.

This study has several limitations. First, since this study retrospectively analyzed patients' medical records, the patient's basic information (comorbidity, etc.) was not controlled. Therefore, the incidence of osteoporosis in the subjects analyzed in this study was higher in females than in males. However, there were no significant differences between the two groups in the average BMD values and the proportion of osteoporosis patients according to gender in each group (data not shown). Second, it was not sufficient to analyze the results of a relatively short observation period (12 months) to evaluate bone fusion after spinal fusion. To obtain a more definitive conclusion, a follow-up study is needed to evaluate the efficacy and safety of bone union after a long period of more than 2 years for the subjects of this study. Third, a comprehensive analysis of the cost-effectiveness of utilizing E.BMP-2 was not performed. To analyze cost-effectiveness, it is crucial to consider not only the expenses associated with the product and surgery, but also factors such as hospitalization duration, potential reoperation costs, and the time required for societal reintegration. The retrospective nature of this clinical trial imposed limitations on the acquisition of essential information for the analysis. However, the introduction of prospective clinical trials in the future holds the promise of facilitating cost-effectiveness analyses, thereby empowering patients and medical professionals to make informed decisions regarding suitable treatment methods.

Conclusion

In this study, we demonstrated the successful bone fusion using low dose of E.BMP-2 (total 1mg) and local bone graft after a one-year follow-up period. These results indicate that the adjunctive use of E.BMP-2 with HA and β-TCP hydrogel can facilitate successful bone fusion in patients who may not have sufficient amounts of local autograft or have poor bone quality due to factors such as inadequate local autograft availability, advanced age, or osteoporosis.

Declarations of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.spinee.2023.07.017.

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