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ORIGINAL ARTICLE

Single-center, consecutive series study of the use of a novel platelet-rich fibrin matrix (PRFM) and beta-tricalcium phosphate in posterolateral lumbar fusion

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Abstract

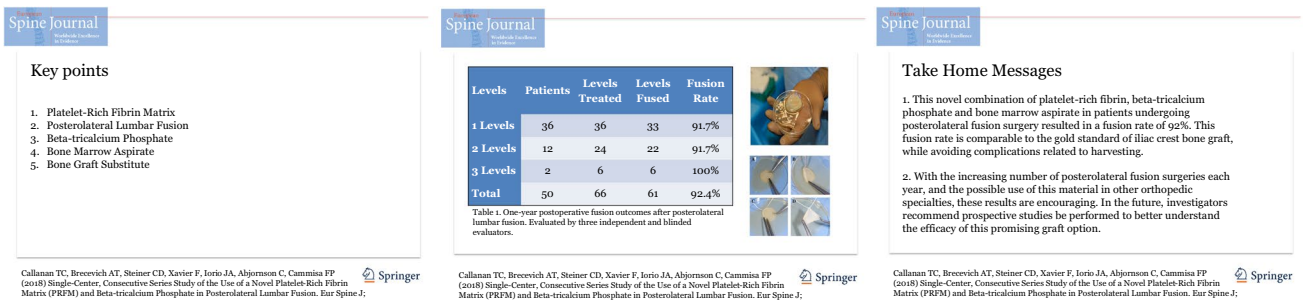
Purpose To evaluate the radiographic and clinical outcomes of the combination of platelet-rich fibrin matrix (PRFM) with beta-tricalcium phosphate (β-TCP) and bone marrow aspirate (BMA) as a graft alternative in posterolateral lumbar fusion procedures.

Methods Researchers evaluated 50 consecutive patients undergoing one-level to three-level posterolateral lumbar fusion procedures, resulting in a total of 66 operated levels. The primary outcome was evidence of radiographic fusion at 1-year follow-up, assessed by three independent evaluators using the Lenke scoring system. Secondary outcomes included back and leg VAS scores, incidence of reoperations and complications, return-to-work status, and opioid use.

Results At 1-year follow-up, radiographic fusion was observed in 92.4% (61/66) of operated levels. There was significant improvement in VAS scores for both back and leg pain ($p < 0.05$). Compared to baseline figures, the number of patients using opioid analgesics at 12-months decreased by 38%. The majority (31/50) of patients were retired, yet 68% of employed patients ($n = 19$) were able to return to work. No surgical site infections were noted, and no revision surgery at the operated level was required.

Conclusions This is the first report to analyze the combination of PRFM with β-TCP and BMA for PLF procedures. Our results indicate a rate of fusion similar to those reported using iliac crest bone graft (ICBG), while avoiding donor site morbidity related to ICBG harvesting such as hematoma, pain, and infection.

Graphical abstract These slides can be retrieved under Electronic Supplementary Material.



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Extended author information available on the last page of the article

Keywords Platelet-rich fibrin matrix · Posterolateral lumbar fusion · Beta-tricalcium phosphate · Bone marrow aspirate · Bone graft substitute

Introduction

It is estimated that more than 400,000 lumbar spine fusion procedures are performed each year in the USA for patients with degenerative conditions of the spine [1]. The gold standard grafting material for these posterolateral lumbar fusion (PLF) procedures remains iliac crest autograft (ICBG). However, donor site morbidity for posterior ICBG harvest can be significant, ranging from 6 to 39% with complications including hematoma, infection, paresthesias, and persistent pain [2, 3]. The associated morbidity with ICBG harvest has driven the development of bone graft substitutes and extenders which seek to mimic the osteoconductive, osteoinductive, and osteogenic effects of autograft to yield a solid arthrodesis [4, 5].

Many materials have been developed for use in arthrodesis procedures, with literature demonstrating equivalent or improved fusion rates for a number of substitutes compared with ICBG [5, 6]. It has been estimated that more than 500,000 bone graft procedures are performed in the USA annually and 2.2 million worldwide, resulting in a cost of approximately 2.5 billion per year [7, 8].

In this study, we focused on combining three elements: platelet-rich fibrin matrix (PRFM) (osteoinductive growth factors), beta-tricalcium phosphate (β-TCP) (osteoconductive scaffold), and bone marrow aspirate (BMA) (osteogenic cells) in order to mimic ICBG in PLF procedures. Researchers hypothesized that this combination would result in a rate of fusion similar to those reported using ICBG while avoiding complications related to ICBG harvesting.

Methods and materials

A retrospective study was undertaken following the appropriate institutional review board approval from the participating institution. Analysis was conducted on a consecutive series of fifty (50) patients from a single center who underwent posterolateral lumbar fusion (PLF) surgery by a single senior surgeon. Each procedure used Integra® Mozaik™ (Integra Lifesciences, Plainsboro, NJ), a beta-tricalcium phosphate (β-TCP), in concert with platelet-rich fibrin matrix (PRFM) (Fibrinet®, Vertical Spine®, Wall, NJ) along with local bone and bone marrow aspirate (BMA). All patients undergoing PLF surgery with this novel combination between August 2012 and December 2014 were identified. Procedures included single and multilevel cases, with or without pedicle screw fixation or interbody fusions. Oncologic, traumatic, and infectious cases were not included. Patients without 1-year postoperative radiographs were excluded.

All posterior procedures were performed by the senior author in the traditional open manner utilizing a midline incision. Bone graft material included locally harvested autograft, BMA, PRFM, and β-TCP. The PRFM membranes were prepared according to manufacturer's specification. For each membrane, an 18 mL aliquot of the patient's peripheral blood was collected and placed into a separation tube. The sample was centrifuged at 1100*g* for 6 min to generate platelet-rich plasma (PRP). The PRP was then transferred into a second "membrane vial" containing calcium-chloride, where it was subjected to a second centrifugation for 25 min at 4500*g*. This process yielded PRFM, a translucent yellowish membrane or disk (Fig. 1). The membrane can then be easily removed from the vial and placed atop the bone graft in the posterolateral gutters. The mechanical properties, cellular content, and growth factor concentration for this PRFM preparation have been previously described in detail by Lucarelli [9].

Fusion was assessed on radiographs at 1-year follow-up. The radiographic fusion grading system described by Lenke was used to radiologically assess the fusion mass at all operated levels on AP and lateral radiographs (Fig. 2). Lenke's classification system separates fusion patterns into one of four grades [10]:

Fig. 1 Platelet-rich fibrin matrix membrane

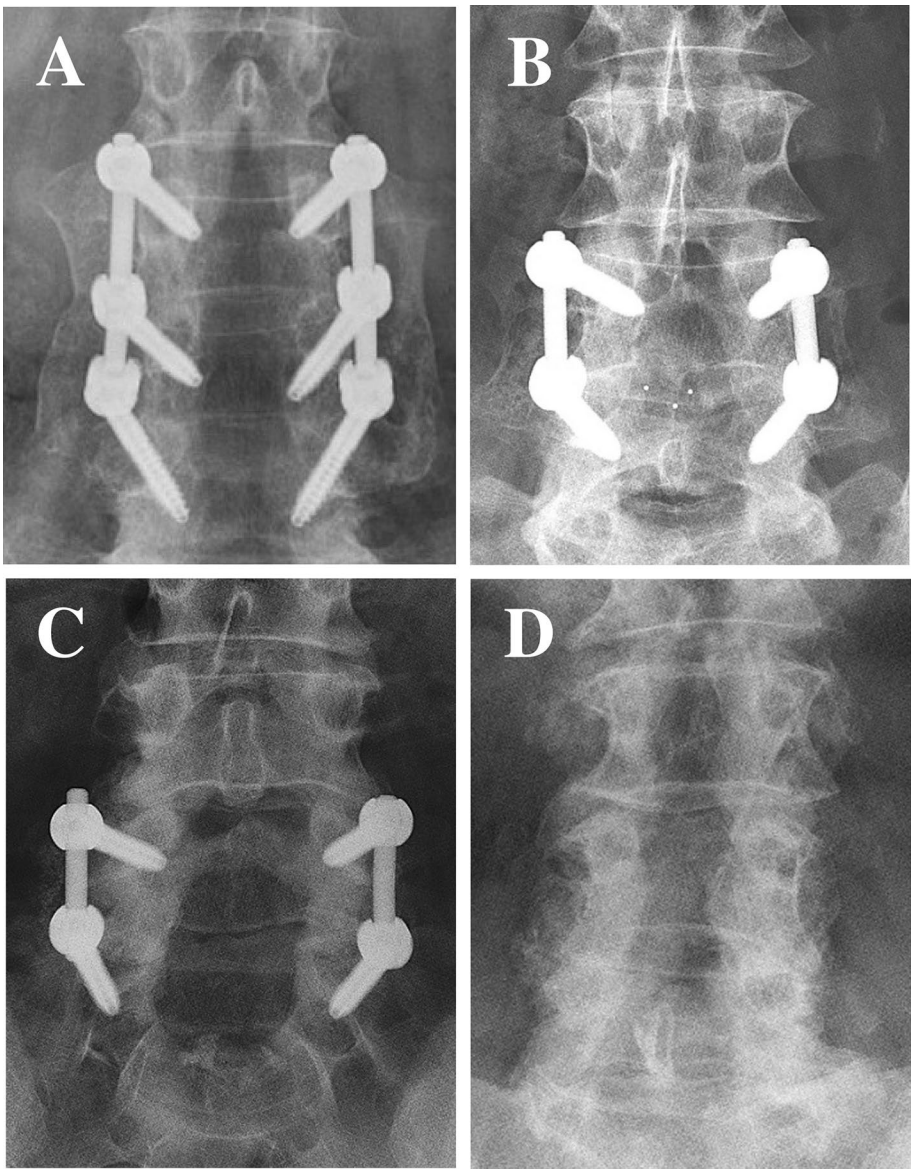

Fig. 2 Representative patients from the series corresponding to the Lenke classification of posterolateral fusion success (**a**–**d**)

(A) Solid, big trabeculated fusions bilaterally (definitely solid)
(B) Solid, big fusion mass unilaterally with a small fusion mass on the contralateral aspect (possibly solid)
(C) Small, thin fusion masses bilaterally with apparent crack (probably not solid)
(D) Graft resorption bilaterally or fusion mass with an obvious bilateral pseudoarthrosis (definitely not solid)

The classification system was used to give each patient a grade based on each vertebral level individually, as well as for their total construct. For example, a patient with a 3-level fusion would receive four grades—one for each individual level as well as a fourth grade for the total construct.

All measurements and fusion assessments were performed by three evaluators: two fellowship trained orthopedic spine surgeons and one spine research fellow. Cobb angles and translation were assessed for all levels on flexion/extension radiographs at 1 year.

In order to be considered fused, patients had to meet a number of criteria. Patients were required to have a Grade A or B via the Lenke criteria, a Cobb angle difference of $< 5°$, translation of < 3 mm on flexion/extension, and no evidence of hardware malfunction including breakage, screw haloing, or pullout. Any criterion indicating pseudoarthrosis superseded any parameter suggesting fusion.

Demographic data included patient age, sex, medical comorbidities, past surgical history, medications, preoperative diagnosis, and number of involved levels. Intraoperative complications were recorded and patients were followed up clinically 1 year after surgery. Preoperative back and leg visual analog scale (VAS) scores, as well as postoperative

back and leg VAS scores were recorded for an assessment of pain.

Statistical Methods

Descriptive statistics were used for continuous variables such as patient demographics and continuous data. A paired sample *t* test (α - 0.05) was used to compare the patients preoperative and postop VAS scores. Interrater reliability between the three evaluators in regard to fusion grades (A-D) was calculated using a weighted kappa statistic. For each patient, the median grade they received between the three evaluators was used as their final assessment grade. All statistical analyses were performed using SPSS statistical software (version 18.0, SPSS/IBM, Chicago, IL).

Results

A total of 50 patients met inclusion criteria, with a 1:1 male-to-female ratio. Posterolateral fusion was performed in these 50 patients for a total of 66 levels. Mean time of 1 year postoperative radiographs was 13 months (±3.7 months) with a minimum of 10 months. Instrumented procedures constituted 45 of 50 cases (90%) and 13 cases (26%) involved the use of an interbody cage. Revisions of prior surgery were performed in eight cases (16%).

Fusion rates were determined both for patients and independent levels, as detailed in the methods section, by three independent and blinded evaluators (Table 1). In total, our results demonstrated that 92.0% of patients and 92.4% of levels met our criteria for fusion.

Table 1 One-year postoperative fusion outcomes after posterolateral lumbar fusion

Levels	Patients	Levels treated	Levels fused	Fusion rate (%)
1 levels	36	36	33	91.7
2 levels	12	24	22	91.7
3 levels	2	6	6	100
Total	50	66	61	92.4

Evaluated by three independent and blinded evaluators

Interrater reliability between each evaluator comparing the total construct grade that was measured using a weighted kappa statistic. The range of the kappa values here range from 0.204 to 0.473, which, according to Landis and Koch's interpretation, is considered slight to moderate agreement. The interrater reliability comparing each individual level grade resulted in a range of 0.263 to 0.447, or fair to moderate agreement. The specific breakdown of median grade between the three independent evaluators is provided in Table 2.

The clinical outcomes at 1-year follow-up are detailed in Table 3. There was significant improvement in VAS scores for both back and leg pain postoperatively in both 1-level and 2-level procedures ($p < .05$). There was insufficient follow-up data to include clinical outcome analysis for three-level procedures. Compared to baseline figures, the number of patients using opioid analgesics at their 1-year follow-up decreased by 38%. With a mean age was 63.1 years, the majority (31/50) of patients were retired, yet 68% of employed patients ($n = 19$) were able to return to work.

No surgical site infections were noted and no revision surgery at the index levels was required. One postoperative complication was reported after removal of the drain, and a diagnostic MRI revealed a lumbar fluid collection. The patient reported pain and spasms; however, no evidence of

Table 3 Preoperative and postoperative VAS scores for patients who underwent posterolateral fusion with β-TCP/PRFM

Clinical outcomes 1 year postoperatively				
	Preoperation	Postoperation	Improvement	P-value
One level				
VAS back	6.96 ($n=25$)	1.82 ($n=22$)	5.39	<.05
VAS leg	7.09 ($n=22$)	1.47 ($n=17$)	6.03	<.05
Two levels				
VAS back	5.94 ($n=9$)	3.42 ($n=5$)	4.45	<.05
VAS leg	6.50 ($n=6$)	2.08 ($n=6$)	4.00	<.05
*TOTAL (±Std. Dev)**				
VAS back	6.69±2.41	2.11±2.04	5.04±2.61	<.05
VAS leg	6.97±2.35	1.63±2.08	5.57±3.04	<.05

*The two patients that underwent three-level fusions did not provide follow-up VAS scores and thus are not included in this table

Table 2 Lenke grades of patients at 1-year follow-up who underwent posterolateral fusion. Grades stratified by individual level and overall construct. Per three independent and blinded evaluators

Lenke grade	A (definitely Solid)	B (Possibly Solid)	C (probably not solid)	D (probably not solid)	Total
By construct (one per patient) *total number and percentage*	25 (50%)	21 (42%)	3 (6%)	1 (2%)	50
By individual level *total number and percentage*	30 (46%)	31 (47%)	4 (6%)	1 (2%)	66

infection was determined. The patient underwent an additional procedure 8 days later to drain the collection. This event was not thought or shown to be correlated to the use of PRFM or β-TCP.

One-year follow-up revealed that 8% (4/50) patients presented with a radiographic nonunion. Although these patients displayed radiographic evidence of nonunion, none required a revision surgery. The demographics of the patients that were determined "Unfused" by our criteria were compared with those determined as "Fused" (Table 4). These results indicate that the majority of the patients that were unfused were of the female sex, were over the age of 69, were previous smokers, and/or were previously diagnosed with osteopenia.

Discussion

Posterolateral lumbar fusion (PLF) surgery is one of the most widely accepted methods in obtaining segmental stability of the lumbar spine and is associated with beneficial outcomes over other non-operative treatments. PLF procedures rely heavily on the three foundational elements of bone regeneration: First, an osteoconductive scaffold that supports bone ingrowth and vascularization. Second, osteoinductive factors (growth factors) that recruit mesenchymal stem cells from the host and induce osteoblastic differentiation of pluripotential stem cells. Third, it requires osteogenic cells to synthesize new bone. ICBG is considered to be the gold standard in bone grafting for spinal fusion due to its possession of all three properties inherently. Extensive efforts have been made to find a graft alternative that is comparable to iliac crest autograft (ICBG), without the drawbacks and complications associated with ICBG harvesting [4, 5]. In this study, we focused on combining these three elements: β-TCP (osteoconductive scaffold), PRFM (osteoinductive growth factors) and BMA (osteogenic cells) in order to mimic ICBG. Our results demonstrate that this novel combination achieved a notable fusion rate (92%) with low complications.

Table 4 Patient demographics for patients who underwent posterolateral fusion. Overall and by fusion outcome

Measure	Population value ($n=50$)	Fused ($n=46$)	Unfused ($n=4$)
Age (years)	63.10	63.07	63.44
Male/female	25:25	24:22	1:3
BMI	29.05	29.05	28.85
Comorbidities and Increased Age (by % of subject population)			
Previous smoker	20%	15%	75%
Age > 69 years	46%	42%	50%
Diabetes	18%	20%	0%
Osteopenia	14%	9%	75%
Revision	16%	13%	50%

This study is unique as it is the first to report the use of PRFM in PLF procedures. Platelet-rich concentrates, such as platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) have emerged in orthopedics as they are derived from the patient's own blood, making them a safe and cost-effective alternative to expensive recombinant factors [11]. These concentrates have been shown to release a number of bioactive factors that have a role in tissue healing and regeneration [11, 12]. However, the clinical use of PRP for lumbar spine fusion remains controversial and has produced mixed results [13]. Most of the controversy surrounding PRP is based on its non-standard methods of preparation, its liquid formulation, and short-term effect of growth factors [9, 11].

Platelet-rich fibrin (PRF) is a second generation alternative to PRP. PRF is similar to PRP as it uses the patient's own blood, however, does not require bovine thrombin making it a simpler and more cost-effective alternative [14]. The success of PRFM in promoting fusion demonstrated in this study can theoretically be attributed to its cellular architecture, its extended release of growth factors, and its mechanical properties. Although this study did not conduct histological or laboratory analysis, there is an abundance of previous basic science research to support the theoretical benefits of PRFM.

The preparation of PRFM produces a solid material with a dense fibrin matrix. This matrix functions as a high molecular weight linear protein, providing hemostasis and trapping platelets resulting in what has been termed a "growth factor reservoir" [15, 16]. It is hypothesized that due to this trapping effect, growth factors are released in physiological proportions more slowly into the wound than other platelet concentrates. Multiple studies have demonstrated that PRFs allow the release of factors (PDGF, VEGF, TGFβ1, bFGF, EGF, etc.) over an extended period of time (7 days in vitro) [9, 17, 18]. Additionally, this fibrin matrix is able to act as a bioactive scaffold and allow host cells to effectively migrate into the area, proliferate and function as needed in the healing cascade [9, 16]. A study in 2010 demonstrated that PRFM had increased cell proliferation over 7 days compared to natural fibrin clots of similar volume [19]. While wound repair occurs typically without incident in normal healthy tissue, it may be inhibited in tissues with limited vascularity, as is in the case of the posterolateral gutters in PLF procedures [19]. Therefore, PRFM's previously described ability to elicit increased levels of proliferation for longer amounts of time in vitro may have contributed to the success of healing in PLF procedures seen in this study.

Finally, due to PRFM's dense fibrin matrix, it can be handled like a solid material, withstand tension (Fig. 3b) and even be held in place with sutures (Fig. 3c, d). A

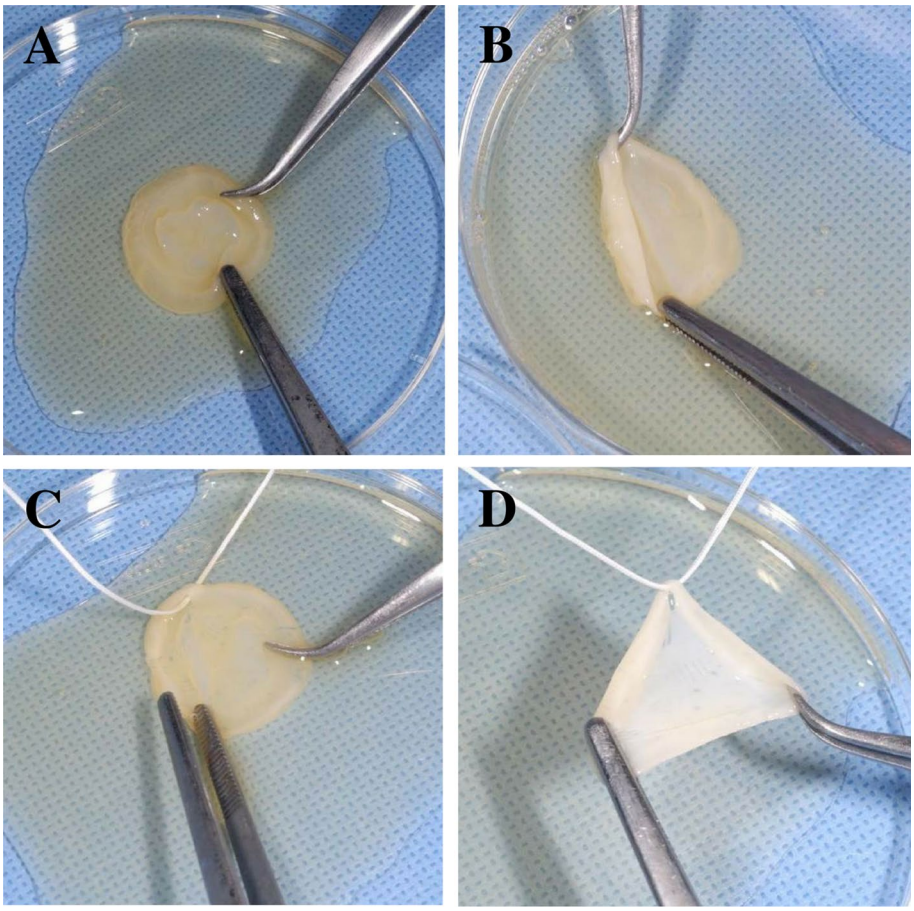

Fig. 3 Intraoperative image of PRFM demonstrating its ability to handle mechanical stress

biomechanical study estimated the tear elastic modulus (expression of stiffness) of PRFM to be 937.3 kPA [9]. This stiffness is an estimated 600 × greater than PRP liquids and gels [9]. These properties are beneficial in the clinical setting, as it allows the surgeon to place the membrane in the correct location during PLF procedures, and reduces the chance of the material shifting or being washed out prior to closing.

Although clinical data on the use of PRF are limited, success has been demonstrated in periodontics [15]. In the current spine literature, there is only one published article (case report, 2008) which describes its use in cervical spinal fusion [20]. To our knowledge, this is the first study to report the use of PRF in PLF procedures.

In this study, plain radiographs were used to assess fusion incorporation, with images confirming a 92% fusion rate. With an average age of 63 years and a high amount of comorbidities such as diabetes, previous smoking, osteopenia, and revision procedures, this fusion rate is notable. Compared to the current literature, this fusion rate is well within the established reports on ICBG in PLF (range: 76–92%) and is comparable to other widely used grafts [21, 22]. Importantly, patients expressed a significant decrease in both leg and back pain ($p < .05$), decreased their opioid intake by 38%, and 68% of working patients returned to work within 1 year. Although 8% of patients failed to achieve solid fusion based on radiographs, their clinical outcomes did not warrant revision surgery.

There are several limitations of this study. First, due to its retrospective design, possible selection bias, and lack of a comparative group, the generalizability of the results are limited. Second, this study did not include histological or laboratory data. Though this study focused on radiographic and clinical outcomes, there is an abundance of previous basic science research to support the theoretical benefits of PRFM. We believe that one of the strengths of this study is how it marries the previously reported basic science (in vitro) to clinical results (in vivo). [9, 11, 12, 15–18]. Third, radiological assessment of fusion is often considered to be subjective, and CT scans have been demonstrated to be more sensitive with a higher degree of intero and intra-observer agreement compared to plain radiographs for assessing instrumented lumbar fusion [23]. Although plain radiographic analysis is not as sensitive, this study used strict criteria in the assessment of fusion by three independent and blinded evaluators. Many studies in the literature rely on only one evaluator [21, 24, 25]. Lastly, the interrater reliability between each evaluator was calculated as "fair" to

"moderate." Due to the kappa statistic not reaching "substantial" agreement, this can be considered a limitation of the study that should be kept in mind when interpreting the results. Despite the limitations, this study is the largest of its kind and it provides valuable information and insight on a promising bone graft option for posterolateral spinal fusion.

Conclusion

This novel combination of platelet-rich fibrin, beta-tricalcium phosphate and bone marrow aspirate in patients undergoing posterolateral fusion surgery resulted in a fusion rate of 92%. This fusion rate is comparable to the gold standard of iliac crest bone graft while avoiding complications related to harvesting. With the increasing number of posterolateral fusion surgeries each year, and the possible use of this material in other orthopedic specialties, these results are encouraging. In the future, investigators recommend prospective studies be performed to better understand the efficacy of this promising graft option.

Compliance with ethical standards

Conflict of interest Dr. Antonio Brecevich discloses consulting and stock options from Vertical Spine. Dr. Justin Iorio discloses consulting and royalties from Medicrea. Dr. Frank Cammisa discloses stock options from Vertical Spine and research support from Integra Life Sciences. Dr. Celeste Abjornson discloses research support from Integra Life Sciences. For the remaining authors, no potential conflict of interests were declared. This study was approved by the Hospital for Special Surgery's Institutional Review Board (IRB).

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study, formal consent was not required.

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