Factors influencing arthrodesis rates in a rabbit posterolateral spine model with iliac crest autograft

Jason H. Ghodasra · Erika L. Daley ·
Erin L. Hsu · Wellington K. Hsu

Received: 10 April 2013 / Revised: 12 October 2013 / Accepted: 13 October 2013 / Published online: 29 October 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract

Purpose The rabbit posterolateral intertransverse spine arthrodesis model has been widely used to evaluate spinal biologics. However, to date, the validity and reproducibility of performance of iliac crest bone graft, the most common and critical control group, has not been firmly established. We evaluated original research publications that utilized this model, identified which experimental conditions affected fusion rates, and developed an algorithm to predict fusion rates for future study designs.

Methods A MEDLINE search was performed for publications through December, 2011 that utilized this model to evaluate fusion rates elicited by iliac crest autograft. All study parameters were recorded, and logistic regression analyses were performed to determine the effects of these variables on fusion rates as determined by either manual palpation or radiographs.

Results Seventy studies with 959 rabbits in 102 groups met the inclusion criteria. Excluding studies that measured fusion at 4 or fewer weeks or intentionally tried to decrease the fusion rate, the overall fusion rate for autograft was 58.3 ± 16.3 % (mean $\pm$ SD) as determined by manual palpation and 66.4 ± 17.8 % by plain radiographs. Regression analysis demonstrated a difference between these outcome measures with a trend towards significance ($p = 0.09$). Longer time points and larger volumes of

autograft resulted in significantly greater reported fusion rates ($p < 0.0001$ and $p < 0.05$, respectively). Neither strain, age, weight, nor vertebral level significantly affected fusion rates.

Conclusions Although experimental conditions varied across studies, time point evaluation and autograft volume significantly affected fusion rates. Despite some variability demonstrated across certain studies, we demonstrated that when the time point and volume of autograft were controlled for, the iliac crest control group of the rabbit posterolateral spinal arthrodesis model is both reliable and predictably affected by different experimental conditions.

Keywords Rabbit · Spinal fusion · Autograft · Animal model · Pseudarthrosis

Introduction

Preclinical animal models play a critical role in the understanding of human disease processes and the development of new therapeutics. These models often allow insights into biologic processes and mechanisms of action, facilitate experimental manipulation, and are cost-effective relative to clinical studies. In the investigation of spinal fusion, numerous animal models have been utilized. Since the first report of a canine model in 1913 [1], at least nine distinct mammalian models have been established to study spinal fusion [2, 3].

The first successful spinal fusion in rabbits was documented by Ritsilä and Alhopuro [4] in 1975; however, its use was not popularized until after a lumbar intertransverse process technique was first published in 1995, where Boden et al. [5] noted key advantages of this model over others. First, the surgical technique closely approximated

J. H. Ghodasra (✉)
Department of Orthopaedic Surgery, David Geffen School of
Medicine at UCLA, 10833 Le Conte Ave., 76-143 CHS,
Los Angeles, CA 90095, USA
e-mail: jasonghodasra@gmail.com

E. L. Daley · E. L. Hsu · W. K. Hsu
Department of Orthopaedic Surgery, Northwestern University
Feinberg School of Medicine, Chicago, IL 60611, USA

that used in human posterolateral fusions. Second, the intertransverse process healing environment provided a distinct area that improved histologic analysis and provided a reliable method of distinguishing fusion compared to other models. Last, published fusion rates with iliac crest bone graft (ICBG) in rabbits were similar to those observed in humans [5]. Subsequently, the rabbit has been the most widely used preclinical model for lumbar spine arthrodesis.

More recently, bone graft substitute technology has expanded the current clinical methods in spine surgery, often allowing prospects for minimally invasive procedures and obviating the morbidity of iliac crest autograft [6]. Rabbit studies utilizing biologics have routinely compared those fusion rates to that of autogenous ICBG, the historical gold standard. However, sufficiently powered animal control groups generally lead to prohibitive costs and subsequently, underpowered studies. A large, pooled historical control may ultimately reduce the required cohort size of such control groups, thereby improving the power and feasibility of such studies.

In addition to minimizing costs, a pooled control group may prove valuable for resource management in future preclinical studies. A recent movement to limit the unnecessary experimentation of animals has led to the ARRIVE guidelines [7]. With the ultimate goal of replacing, refining, or reducing (the “3 R’s”) the use of animals in research, the guidelines were established to improve the reporting and quality of animal research. The establishment of a historical control in this commonly utilized animal model would be one productive step towards potentially reducing the required sample size of such studies.

Historically, the reported fusion rates for autograft in the rabbit posterolateral spine model have varied significantly. Drespe et al. [2] noted that autograft fusion rates ranged from 42 to 73 %. This wide range could be attributed to a number of variables including volume of graft, surgical technique, and the method of assessment. In spite of this, the potential factors that might affect these rates have not been sufficiently evaluated.

Despite widespread use of the rabbit posterolateral spine arthrodesis model in the biologics field, the validity and reproducibility of performance of ICBG has not been established. Utilizing original research publications, this study aims to (1) evaluate the effects of experimental conditions on fusion rates with ICBG and (2) develop a model to serve as a historical control for future studies in this model.

Methods

Literature search and inclusion/exclusion criteria

A MEDLINE search for publications through December 2011 was performed using Medical Subject Headings (MeSH) and

Table 1 Inclusion/exclusion criteria

Inclusion criteria	Exclusion criteria
Bilateral	Unilateral
Single-level	Multi-level
Lumbar	Cervical or thoracic
Iliac crest autograft	Allograft or muscle pedicle, lamina chip autograft
Attempted fusion between transverse processes	Attempted fusion between interbody, spinous processes, laminae
No instrumentation	Instrumentation
Graft +/- saline	Bone graft substitutes
Written in English	Written in language other than English

keywords for “spine fusion”, “spinal fusion”, “spine arthrodesis”, “lumbar arthrodesis”, or “lumbar fusion” with “rabbit”. Publications written in English that included one or more study groups that underwent a bilateral, single-level, lumbar posterolateral fusion with ICBG between transverse processes were included (Table 1). Exclusion criteria included a unilateral or multilevel technique, allograft, autograft not harvested from iliac crests, instrumentation, or an adjunct product with autograft other than saline. A hand search of reference lists from obtained literature was also performed to identify further relevant articles. Each article was reviewed for inclusion in the study independently by two of the authors. If the two reviewers disagreed, the senior author determined final eligibility of the paper.

Independent variables

Studies that met the inclusion criteria were compiled with associated variables including sample size, strain, age, weight, sex, vertebral level, volume of autograft, and time point evaluation. The university/hospital where the study was performed was also recorded. For those studies with more than one group evaluating different experimental conditions, each study group was recorded separately. Animals that were operated at L3–L4 and L6–L7 came from one study each. Therefore, the fusion rates for rabbits that underwent fusion at only L4–L5 and L5–L6 were compared to avoid bias. Utilizing ratio calculations from three independent studies, when amount of autograft was reported in mass, a conversion ratio of 2 cm³/g was used [8–10]. When the age, weight, or volume of autograft was reported as a range, a mean was used. Two authors independently evaluated each study and recorded each variable. Any inconsistencies between the two were reconciled by the senior author.

Outcome measures

The primary outcome recorded was the fusion rate as determined by manual palpation. The secondary outcome

was the fusion rate as determined by high-resolution anteroposterior plain radiographs. When radiographic fusion was assessed per intertransverse region rather than per animal, the number of unions and non-unions were divided in half in order to report the same n as number of animals in the study. For example, for the purposes of our study, an experiment with 16 animals that reported 20 of 32 regions fused by radiographs was interpreted to have a fusion rate of 10 out of 16 animals, or 62.5 % [11]. Among animal researchers in bone healing, unilateral fusion is thought in general to lead to successfully surgery. However, few studies reported results as no fusion, unilateral fusion, and bilateral fusion, and this method of standardization allowed data from studies that reported fusion rates by animal versus by side to be combined. This method of standardization is further considered in the “Discussion” section.

Statistical analysis

Statistical analysis was performed using Statistical Analysis System v. 9.2 (SAS Institute, Inc., Cary, NC, USA). Random-effects logistic regression modeling with PROC GLIMMIX (SAS v. 9.2) procedure was used to identify experimental conditions that were significantly associated with fusion rates. To evaluate correlated data nested by labs and studies, mixed models were utilized. Age and weight were assessed simultaneously due to the expected strong correlation between the two variables. Inter- and intra-laboratory reproducibility for those with five or more study groups was evaluated with regression analysis. PROC LOGISTICS (SAS v. 9.2) procedure was used to create a logistic equation that could predict fusion rates

based on different variables. Only experimental conditions that significantly affected fusion rates were included in the equation. Statistical significance was accepted with a p value of less than 0.05.

Results

Of 209 studies initially identified, 120 did not meet the inclusion criteria, 17 did not report an extractable fusion rate by manual palpation or plain radiographs, and two contained redundant data (Fig. 1). A total of 70 studies met the inclusion criteria, with 959 rabbits in 102 separate ICBG study groups.

Study population characteristics (Table 2) varied among study groups. Only two strains (New Zealand White and Japanese White) were used in the studies included. The

Fig. 1 Study flow diagram of the studies included in the analysis

Table 2 Study population characteristics

Categorical variables	Rabbits ($n = 959$) (%)	Effect on fusion rate (p value)
Strain		Not significant ($p = 0.92$)
New Zealand White	885 (92.3)	
Japanese White	74 (7.7)	
Sex		Not analyzed
Male	245 (25.5)	
Female	162 (16.9)	
Not reported	552 (57.6)	
Vertebral level		Not significant ($p = 0.68$)
L4–L5	167 (17.4)	
L5–L6	792 (82.6)	
Continuous variables	Mean $\pm$ SD (range)	
Weight (kg)	4.1 $\pm$ 0.7 (2.4–5.0)	Not significant ($p = 0.22$)
Age (months)	10.2 $\pm$ 2.9 (4.8–18.0)	Not significant ($p = 0.22$)
Volume of autograft per animal (cm ³)	4.2 $\pm$ 2.0 (1.4–12.0)	Significant ($p = 0.04$)
Time point evaluation (weeks)	7.3 $\pm$ 3.5 (1.0–24.0)	Significant ($p < 0.0001$)

Table 3 Fusion rates

	Mean $\pm$ SD (%)
Overall fusion rate	
By manual palpation ($n = 827$)	48.2 $\pm$ 26.0
By radiographs ($n = 665$)	56.0 $\pm$ 30.4
Fusion rate excluding studies with time points ≤ 4 weeks and paucity model	
By manual palpation ($n = 632$)	58.3 $\pm$ 16.3
By radiographic assessment ($n = 508$)	66.4 $\pm$ 17.8

Fig. 2 The effect of time point evaluation on fusion rates determined by manual palpation (a) and by radiographs (b). The fusion rates of study groups (*filled diamond*) and those utilizing the paucity model (*open triangle*, decreased autograft volume in order to intentionally lower fusion rates) are shown. The *solid line* represents the predicted fusion rate at different time points with the mean volume of autograft (4.2 cm^3)

majority of study groups were operated at L5–L6 (79 groups) although a number were operated at L4–L5 (23 groups). Notably, more than half of the studies failed to report the sex of rabbits used in the study, and accordingly, sex was not evaluated in the regression analyses. Of the 70 studies that met the inclusion criteria, the average number of study groups was 1.3 ± 0.9 (average $\pm$ SD), and each study group contained an average of 9.4 ± 5.6 rabbits. Average time point evaluation was 7.3 ± 3.5 weeks.

Fig. 3 The effect of volume of autograft on fusion rates determined by manual palpation (a) and by radiographs (b). The fusion rates of study groups (*filled diamond*) and those utilizing the paucity model (*open triangle*, decreased autograft volume in order to intentionally lower fusion rates) are shown. The *solid line* represents the predicted fusion rate with different autograft volumes at the mean time point evaluation (7.3 weeks)**Table 4** Laboratories that published five or more study groups that met the inclusion criteria

University/Hospital	Senior author	Number of study groups
Emory University, Atlanta, GA	Boden	16
Chang Gung University, Taiwan	Chen	8
Tsinghua University, China	Liao/Sun	8
Yale University, New Haven, CT	Grauer	8
Wakayama Medical College, Japan	Minamide	7
Case Western Reserve University, Cleveland, OH	Yoo	6
University of Iowa, Iowa City, IA	Fredericks	5
Pontificia Universidad Catolica de Chile, Chile	Urrutia	5

Successful lumbar fusion rates were assessed in 827 rabbits by manual palpation and 665 rabbits by radiographs (Table 3). Excluding study groups that either evaluated at time points when fusion was not expected) had the same

fusion rates. After controlling for independent variables, fusion rates were slightly higher utilizing plain radiographs compared to manual palpation ($p = 0.09$). Independent variables which affected successful fusion rate included evaluation at a later time point ($p < 0.0001$; Fig. 2) and a larger volume of autograft ($p = 0.04$; Fig. 3). When comparing the fusion rates by manual palpation, the mean for study groups with a time point of 4 weeks or fewer ($17.9 \pm 25.1\%$) was statistically different from the mean for study groups with a time point of 5 weeks or more ($58.3 \pm 16.3\%$, $p < 0.0001$). However, age, strain, weight, and vertebral level had no significant effect on fusion rate ($p = 0.22$, $p = 0.92$, $p = 0.22$, and $p = 0.68$, respectively).

Eight laboratories with five or more study groups were identified (Table 4). There was no significant difference in fusion rate among all eight labs when controlling for time point evaluation and volume of autograft ($p > 0.05$). There were also no significant differences in fusion rates among study groups conducted by the same lab when controlling for independent variables ($p > 0.05$).

Based on the compiled data in the studies that met the inclusion criteria, the following logistic equation was derived to predict fusion rate:

$$p = \frac{e^x}{1 + e^x}$$

where $x = 1.4404 + 0.1350m + 0.1085t + 0.1427v$

Independent variables included m for mode of assessment (0 for manual palpation and 1 for radiographs), t for time (in weeks), and v for volume of autograft (in cm^3). This equation was established with all available data including studies that utilized the paucity model and time points of 4 weeks or fewer (Figs. 2, 3). For any given mode of assessment, time and volume entered into the equation, the resulting expected fusion rate represents an average of all available data.

Discussion

Each year, over 400,000 spine fusions are performed in the United States [12]. Although iliac crest autograft has been the historical gold standard, complications including donor site morbidity and pseudarthrosis can occur. In the past two decades, a number of bone graft substitutes have been developed to avoid such complications. Preclinical research has allowed these experimental technologies to be compared with autograft in animal models. For lumbar fusion, the rabbit model has become an important investigational step along the path towards clinical implementation. Minimizing the sample size of animal control groups

may ultimately improve the efficiency of such studies and limit unnecessary animal experimentation.

Utilizing data from 70 original research publications evaluating rabbit posterolateral lumbar fusion with autograft, time point evaluation and graft volume were found to significantly influence fusion rates while age, strain, weight, and vertebral level did not. Fusion rates determined by plain radiographs were higher than those by manual palpation, with a trend towards significance ($p = 0.09$). Notably, little intra- or inter-laboratory variability was seen. To our knowledge, this study is the first to systematically analyze the effects of experimental conditions and outcome measures on reported fusion rates in the rabbit spine fusion model with ICBG.

Based on the data reviewed in this study, we conclude that the ideal time point for lumbar fusion evaluation in this model is a minimum of 5 weeks postoperatively. When time point evaluation was 4 weeks or fewer, there were significantly lower fusion rates when compared to study groups with time points of 5 or more weeks. Studies investigating the inhibitory effects of adjunct compounds and other less robust bone graft substitutes may benefit from time points even later than 5 weeks. Ultimately, the optimal time point evaluation depends on a number of factors including the osteoinductive capacity of the bone graft substitute, the volume of autograft, and the study goals and hypothesis.

Several studies have shown that the quantity of autograft positively correlates with fusion rates [13, 14]. In fact, Curylo et al. [9] developed a “bone paucity model” in which an “underdose” of autograft was used. The aim of this study was to achieve a fusion rate of less than 40 % and then determine whether autologous bone marrow augmented arthrodesis. Other studies subsequently used this bone paucity model to provide a larger potential for improved fusion rates with a variety of bone graft substitutes [13–22]. While our study found a significant positive relationship between volume of autograft and fusion rates, a tenfold range of volume of autograft ($1.4\text{--}12\text{ cm}^3$) was used in the studies evaluated. Although fusion rates varied significantly among studies using the same volume of autograft, based on the available data, using the most common volume of $4\text{--}5\text{ cm}^3$ (per animal) leads to the most predictable results, while using significantly lower volumes can lead to inferior reliability.

Although there was no definitive association established between age or weight of rabbits and fusion rates, these variables have previously been suggested to affect arthrodesis [15]. Chen et al. [15] argued that the higher fusion rate seen in the younger rabbits utilized in their study might be attributed to a more vigorous healing response relative to older rabbits used in previous studies. Indeed, younger rabbits may have a higher capacity for

spinal fusion since their skeletal systems are relatively immature, and ongoing bone remodeling could accelerate fusion. Likewise, in humans, significantly faster healing rates and lower nonunion rates have been noted in children compared to adults (Lindaman et al. [23]). In this study, the age range of included rabbits was 7.2 (4.8–12.0) months, but no correlation between age and fusion rate was observed. In addition to age, obesity has been shown to be a risk factor for pseudarthrosis and other postoperative complications in humans [24, 25]. However, in this study, where the weight range of included rabbits was 2.5 (2.5–5.0) kg, no correlation was observed between weight and fusion rate.

It has been postulated that fusion rates at L6–L7 should be lower than L5–L6 due to the smaller transverse processes at L7 and its proximity to the sacrum [26]. However, the limited data in our study could not confirm or refute this hypothesis. In humans, the biomechanical properties of adjacent lower lumbar segments vary considerably [27]. For example, L5–S1 is deep-set within the pelvis and adjacent to a number of iliolumbar ligaments, which protect the joint from torsional strain but increase axial compression. On the other hand L4–L5 has a higher degree of axial torsion and, therefore, is a more common site for lumbar instability. While no other study to our knowledge has compared fusion rates in rabbits at different vertebral levels, varying biomechanical properties of adjacent vertebral levels may affect the capacity for fusion across joints.

Although no difference between male and female rabbits was expected, sex was not reported in more than half of the studies that met the inclusion criteria. Therefore, the effect of sex on fusion rates was not evaluated in this study. As such, all future studies that utilize this rabbit posterolateral spine fusion model should explicitly report all potential variables including strain, sex, age, weight, vertebral level, graft volume (per animal), and time point evaluation. Accurate measurement and reporting of these variables would provide stronger analysis and improve our understanding on which variables affect fusion rates.

Previous studies performed in humans have shown that radiographic assessment of fusion correlated poorly with the clinical gold standard of direct surgical exploration in the identification of arthrodesis [28–30]. Furthermore, Boden et al. [5] reported that radiographic assessment was noted to be incorrect in 13 of 24 (54 %) specimens when compared to manual palpation, the analysis that most closely replicates direct surgical exploration in rabbits. These investigators also demonstrated that evaluation of fusion by manual palpation correlated more closely with biomechanical test results than did radiographic evaluation [31]. This concept is also supported by data from other studies

[21, 32–34]. Although some studies have suggested that radiographs underestimate the actual fusion rate [28, 35], our results appear to support the notion that plain radiographic assessment of fusion can be an overestimation of actual fusion rates. Though alternative measures exist in the assessment of successful lumbar fusion, such as biomechanical testing, micro CT, or histology, relatively few studies have reported fusion rates evaluated using these methods. While a number of studies utilized micro CT for a number of purposes including bone volume quantification, only three studies reported an ICBG fusion rate assessed by this imaging technique.

There are a number of animal models used for the investigation of spine fusion. Small animal models, such as rats and rabbits, have the advantages of lower costs, ease of handling and housing, and shorter times to fusion due to their higher metabolic rate [2]. On the other hand, larger animals models tend to be higher phylogenetic species that better mimic human anatomy and allow similar surgical techniques that are used clinically. However, for the study of lumbar spine fusion, the rabbit posterolateral model provides as consistent a ICBG fusion rate as seen with autograft in humans, with the advantages associated with a smaller animal model. Notably, in the corresponding rat model, autograft results in poor fusion rates [36], and low or highly variable autograft fusion rates are also seen in larger animals including dogs (0–100 %), goats (0–45 %), and non-human primates (0–33 %) [2].

There are a number of limitations to this study. First, because experimental conditions were not reliably reported across all studies, missing data can lead to erroneous conclusions. Of the 70 studies that met the inclusion criteria, 4 (5.7 %) did not report volume, 5 (7.1 %) did not report the weight, 41 (58.6 %) did not report sex, and 49 (70 %) did not report age [5, 8, 10, 11, 13–22, 33–35, 37–88]. The lack of reliably reported data across all studies is an inherent limitation of systematic reviews. Second, in some studies, fusion was recorded differently depending on the mode of assessment. With manual palpation, successful fusion was always determined per animal; however, with plain radiographs, fusion was at times reported per side (2 per animal) with no mention of laterality. A study with 16 animals that reported 20 of 32 sides fused by radiographs could have had a per-animal-fusion-rate ranging from 62.5 (10 bilateral fusions and 6 pseudarthroses) to 100 % (12 unilateral and 4 bilateral fusions) [11]. For the purposes of this study, we chose to standardize animal fusion rate by dividing the total sides fused by a factor of 2 to include all relevant data. In addition, there was little risk of bias since none of the authors had a direct conflict of interest with the products tested or study design. Furthermore, no authors were involved in any of the studies reviewed or in any similar future study.

Conclusions

The rabbit posterolateral spine arthrodesis model has been a popular, well-utilized pre-clinical model for the testing of bone graft substitutes. Despite some variability in fusion rates likely due to differences in study conditions, the data in this study suggest that a predictable result within a commonly accepted timeframe (5 weeks) with iliac crest autograft can be expected. As the external pressure mounts to conserve resources and design studies with conservation in mind, we hope that data from our study can help stratify the utilization of historical controls in future studies.

Acknowledgments The authors would like to thank Dr. Shengli Shi for his assistance with statistical analysis. This work was supported by the Department of Orthopaedic Surgery at Northwestern University Feinberg School of Medicine. No animal or human studies were carried out by the authors for this article.

Conflict of interest Jason H. Ghodasra and Erika L. Daley have no disclosures. Wellington K. Hsu and Erin L. Hsu consult for Medtronic Inc., Stryker, Pioneer, Zimmer, Graftys, Lifenet, and AONA.

References

- Albee FH (1913) An experimental study of bone growth and the spinal bone transplant. *JAMA* 60(14):1044–1049
- Drespe IH, Polzhofer GK, Turner AS, Grauer JN (2005) Animal models for spinal fusion. *Spine J* 5(6 Suppl):209S–216S. doi:10.1016/j.spinee.2005.02.013
- Schimandle JH, Boden SD (1994) Spine update. The use of animal models to study spinal fusion. *Spine* 19(17):1998–2006
- Ritsila V, Alhopuro S (1975) Spinal fusion with free periosteal grafts and its effect on vertebral growth in young rabbits. *J Bone Jt Surg Br* 57(4):500–505
- Boden SD, Schimandle JH, Hutton WC (1995) An experimental lumbar intertransverse process spinal fusion model. Radiographic, histologic, and biomechanical healing characteristics. *Spine* 20(4):412–420
- Vaccaro AR, Chiba K, Heller JG, Patel T, Thalgot JS, Truumees E, Fischgrund JS, Craig MR, Berta SC, Wang JC (2002) Bone grafting alternatives in spinal surgery. *Spine J* 2(3):206–215
- Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol* 8(6):e1000412. doi:10.1371/journal.pbio.1000412
- Boden SD, Schimandle JH, Hutton WC (1995) 1995 Volvo award in basic sciences. The use of an osteoinductive growth factor for lumbar spinal fusion. Part II: study of dose, carrier, and species. *Spine* 20(24):2633–2644
- Currylo LJ, Johnstone B, Petersilge CA, Janicki JA, Yoo JU (1999) Augmentation of spinal arthrodesis with autologous bone marrow in a rabbit posterolateral spine fusion model. *Spine* 24(5):434–438 (discussion 438–439)
- Yao G, Qian Y, Chen J, Fan Y, Stoffel K, Yao F, Xu J, Zheng MH (2008) Evaluation of insoluble bone gelatin as a carrier for enhancement of osteogenic protein-1-induced intertransverse process lumbar fusion in a rabbit model. *Spine* 33(18):1935–1942. doi:10.1097/BRS.0b013e31817e1cf1
- Tortolani PJ, Park AE, Louis-Ugbo J, Attallah-Wasef ES, Kraiwattanapong C, Heller JG, Boden SD, Yoon ST (2004) The effects of doxorubicin (adriamycin) on spinal fusion: an experimental model of posterolateral lumbar spinal arthrodesis. *Spine J* 4(6):669–674. doi:10.1016/j.spinee.2004.05.254
- Agency for Healthcare Research and Quality (2013) Healthcare Cost and Utilization Project (HCUP). US Department of Health and Human Services. <http://hcupnet.ahrq.gov/databases.jsp>. Accessed 11 May 2013
- Martin GJ Jr, Boden SD, Titus L, Scarborough NL (1999) New formulations of demineralized bone matrix as a more effective graft alternative in experimental posterolateral lumbar spine arthrodesis. *Spine* 24(7):637–645
- Miller CP, Jegede K, Essig D, Garg H, Bible JE, Biswas D, Whang PG, Grauer JN (2011) The efficacies of two ceramic bone graft extenders for promoting spinal fusion in a rabbit bone paucity model. *Spine* 37(8):642–647. doi:10.1097/BRS.0b013e31822e604e
- Chen WJ, Huang JW, Niu CC, Chen LH, Yuan LJ, Lai PL, Yang CY, Lin SS (2009) Use of fluorescence labeled mesenchymal stem cells in pluronic F127 and porous hydroxyapatite as a bone substitute for posterolateral spinal fusion. *J Orthop Res* 27(12):1631–1636. doi:10.1002/jor.20925
- O'Loughlin PF, Cunningham ME, Bukata SV, Tomin E, Poynton AR, Doty SB, Sama AA, Lane JM (2009) Parathyroid hormone (1–34) augments spinal fusion, fusion mass volume, and fusion mass quality in a rabbit spinal fusion model. *Spine* 34(2):121–130. doi:10.1097/BRS.0b013e318191e687
- Sethi PM, Miranda JJ, Kadiyala S, Patel T, Panjabi M, Troiano N, Friedlaender GE (2008) Evaluation of autologous platelet concentrate for intertransverse process lumbar fusion. *Am J Orthop (Belle Mead, NJ)* 37(4):E84–E90
- Urrutia J, Briceno J, Carmona M, Olavarria F, Hodgson F (2010) Effect of a single dose of pamidronate administered at the time of surgery in a rabbit posterolateral spinal fusion model. *Eur Spine J* 19(6):940–944. doi:10.1007/s00586-010-1288-y
- Urrutia J, Mery P, Martinez R, Pizarro F, Apablaza D, Mardones R (2010) Cultured autologous bone marrow stem cells inhibit bony fusion in a rabbit model of posterolateral lumbar fusion with autologous bone graft. *J Clin Neurosci* 17(4):481–485. doi:10.1016/j.jocn.2009.06.024
- Yee AJ, Bae HW, Friess D, Robbin M, Johnstone B, Yoo JU (2003) Augmentation of rabbit posterolateral spondylodesis using a novel demineralized bone matrix-hyaluronan putty. *Spine* 28(21):2435–2440. doi:10.1097/01.BRS.0000090828.65638.8C
- Yee AJ, Bae HW, Friess D, Robbin M, Johnstone B, Yoo JU (2004) Accuracy and interobserver agreement for determinations of rabbit posterolateral spinal fusion. *Spine* 29(12):1308–1313
- Yee AJ, Bae HW, Friess D, Roth SM, Whyne C, Robbin M, Johnstone B, Yoo JU (2006) The use of simvastatin in rabbit posterolateral lumbar intertransverse process spine fusion. *Spine J* 6(4):391–396. doi:10.1016/j.spinee.2005.10.017
- Lindaman LM (2001) Bone healing in children. *Clin Podiatr Med Surg* 18(1):97–108
- Patel N, Bagan B, Vadera S, Maltenfort MG, Deutsch H, Vaccaro AR, Harrop J, Sharan A, Ratliff JK (2007) Obesity and spine surgery: relation to perioperative complications. *J Neurosurg Spine* 6(4):291–297. doi:10.3171/spi.2007.6.4.1
- Baumgarten KM, Carlson WO, Watson ES (2011) The effect of obesity on orthopaedic conditions. *S D Med* 41–44
- Bransford R, Goergens E, Briody J, Amanat N, Cree A, Little D (2007) Effect of zoledronic acid in an L6–L7 rabbit spine fusion model. *Eur Spine J* 16(4):557–562. doi:10.1007/s00586-006-0212-y
- Okoro T, Sell P (2010) A short report comparing outcomes between L4/L5 and L5/S1 single-level discectomy surgery.

- J Spinal Disord Tech 23(1):40–42. doi:[10.1097/BSD.0b013e3181b38537](https://doi.org/10.1097/BSD.0b013e3181b38537)
28. Blumenthal SL, Gill K (1993) Can lumbar spine radiographs accurately determine fusion in postoperative patients? Correlation of routine radiographs with a second surgical look at lumbar fusions. *Spine* 18(9):1186–1189
29. Brodsky AE, Kovalsky ES, Khalil MA (1991) Correlation of radiologic assessment of lumbar spine fusions with surgical exploration. *Spine* 16(6 Suppl):S261–S265
30. Kant AP, Daum WJ, Dean SM, Uchida T (1995) Evaluation of lumbar spine fusion. Plain radiographs versus direct surgical exploration and observation. *Spine* 20(21):2313–2317
31. Boden SD, Martin GJ Jr, Morone M, Ugbo JL, Titus L, Hutton WC (1999) The use of coralline hydroxyapatite with bone marrow, autogenous bone graft, or osteoinductive bone protein extract for posterolateral lumbar spine fusion. *Spine* 24(4):320–327
32. Boden SD, Schimandle JH, Hutton WC, Chen MI (1995) 1995 Volvo award in basic sciences. The use of an osteoinductive growth factor for lumbar spinal fusion. Part I: biology of spinal fusion. *Spine* 20(24):2626–2632
33. Erulkar JS, Grauer JN, Patel TC, Panjabi MM (2001) Flexibility analysis of posterolateral fusions in a New Zealand white rabbit model. *Spine* 26(10):1125–1130
34. Lehman RA, Kuklo TR, Freedman BA, Cowart JR, Mense MG, Riew KD (2004) The effect of alendronate sodium on spinal fusion: a rabbit model. *Spine J* 4(1):36–43. doi:[10.1016/s1529-9430\(03\)00427-3](https://doi.org/10.1016/s1529-9430(03)00427-3)
35. Jackson RS, Asher MA, Lark RG (2000) Analysis of posterior spinal wiring in a validated rabbit model. *Clin Orthop Relat Res* 373:285–294
36. Wang JC, Alanay A, Mark D, Kanim LE, Campbell PA, Dawson EG, Lieberman JR (2007) A comparison of commercially available demineralized bone matrix for spinal fusion. *Eur Spine J* 16(8):1233–1240. doi:[10.1007/s00586-006-0282-x](https://doi.org/10.1007/s00586-006-0282-x)
37. Babat LB, McLain R, Milks R, Ferrara L, Sohn MJ (2005) The effects of the antiresorptive agents calcitonin and pamidronate on spine fusion in a rabbit model. *Spine J* 5(5):542–547. doi:[10.1016/j.spinee.2005.01.008](https://doi.org/10.1016/j.spinee.2005.01.008)
38. Chen WJ, Lai PL, Chang CH, Lee MS, Chen CH, Tai CL (2002) The effect of hyperbaric oxygen therapy on spinal fusion: using the model of posterolateral intertransverse fusion in rabbits. *J Trauma* 52(2):333–338
39. Choi Y, Oldenburg FP, Sage L, Johnstone B, Yoo JU (2007) A bridging demineralized bone implant facilitates posterolateral lumbar fusion in New Zealand white rabbits. *Spine* 32(1):36–41. doi:[10.1097/01.brs.0000250982.41666.55](https://doi.org/10.1097/01.brs.0000250982.41666.55)
40. Cinotti G, Patti AM, Vulcano A, Della Rocca C, Polveroni G, Giannicola G, Postacchini F (2004) Experimental posterolateral spinal fusion with porous ceramics and mesenchymal stem cells. *J Bone Jt Surg Br* 86(1):135–142
41. Cunningham BW, Orbegoso CM, Dmitriev AE, Hallab NJ, Seftor JC, Asdourian P, McAfee PC (2003) The effect of spinal instrumentation particulate wear debris. An in vivo rabbit model and applied clinical study of retrieved instrumentation cases. *Spine J* 3(1):19–32
42. Cunningham BW, Orbegoso CM, Dmitriev AE, Hallab NJ, Seftor JC, McAfee PC (2002) The effect of titanium particulate on development and maintenance of a posterolateral spinal arthrodesis: an in vivo rabbit model. *Spine* 27(18):1971–1981
43. Feiertag MA, Boden SD, Schimandle JH, Norman JT (1996) A rabbit model for nonunion of lumbar intertransverse process spine arthrodesis. *Spine* 21(1):27–31
44. France JC, Norman TL, Buchanan MM, Scheel M, Veale M, Ackerman ES, Clovis NB, Kish VL, Simon B (2006) Direct current stimulation for spine fusion in a nicotine exposure model. *Spine J* 6(1):7–13. doi:[10.1016/j.spinee.2005.05.380](https://doi.org/10.1016/j.spinee.2005.05.380)
45. Fredericks DC, Smucker J, Petersen EB, Bobst JA, Gan JC, Simon BJ, Glazer P (2007) Effects of direct current electrical stimulation on gene expression of osteopromotive factors in a posterolateral spinal fusion model. *Spine* 32(2):174–181. doi:[10.1097/01.brs.0000251363.77027.49](https://doi.org/10.1097/01.brs.0000251363.77027.49)
46. Fu TS, Chen WJ, Chen LH, Lin SS, Liu SJ, Ueng SW (2009) Enhancement of posterolateral lumbar spine fusion using low-dose rhBMP-2 and cultured marrow stromal cells. *J Orthop Res* 27(3):380–384. doi:[10.1002/jor.20644](https://doi.org/10.1002/jor.20644)
47. Glazer PA, Heilmann MR, Lotz JC, Bradford DS (1997) Use of electromagnetic fields in a spinal fusion. A rabbit model. *Spine* 22(20):2351–2356
48. Glazer PA, Heilmann MR, Lotz JC, Bradford DS (1998) Use of ultrasound in spinal arthrodesis. A rabbit model. *Spine* 23(10):1142–1148
49. Grauer JN, Patel TC, Erulkar JS, Troiano NW, Panjabi MM, Friedlaender GE (2001) 2000 Young investigator research award winner. Evaluation of OP-1 as a graft substitute for intertransverse process lumbar fusion. *Spine* 26(2):127–133
50. Hile DD, Kandziora F, Lewandowski KU, Doherty SA, Kowaleski MP, Trantolo DJ (2006) A poly(propylene glycol-co-fumaric acid) based bone graft extender for lumbar spinal fusion: in vivo assessment in a rabbit model. *Eur Spine J* 15(6):936–943. doi:[10.1007/s00586-005-1001-8](https://doi.org/10.1007/s00586-005-1001-8)
51. Huang JW, Lin SS, Chen LH, Liu SJ, Niu CC, Yuan LJ, Wu CC, Chen WJ (2011) The use of fluorescence-labeled mesenchymal stem cells in poly(lactide-co-glycolide)/hydroxyapatite/collagen hybrid graft as a bone substitute for posterolateral spinal fusion. *J Trauma* 70(6):1495–1502. doi:[10.1097/TA.0b013e318216b9ee](https://doi.org/10.1097/TA.0b013e318216b9ee)
52. Konishi S, Nakamura H, Seki M, Nagayama R, Yamano Y (2002) Hydroxyapatite granule graft combined with recombinant human bone morphogenetic protein-2 for solid lumbar fusion. *J Spinal Disord Tech* 15(3):237–244
53. Lamghari M, Antonietti P, Berland S, Laurent A, Lopez E (2001) Arthrodesis of lumbar spine transverse processes using nacre in rabbit. *J Bone Miner Res* 16(12):2232–2237. doi:[10.1359/jbmr.2001.16.12.2232](https://doi.org/10.1359/jbmr.2001.16.12.2232)
54. Lee T-C, Ueng SW-N, Chen H-H, Lu K, Huang H-Y, Liliang P-C, Su T-M, Yang L-C (2005) The effect of acute smoking on spinal fusion: an experimental study among rabbits. *J Trauma* 59(2):402–408. doi:[10.1097/01.ta.0000174918.38764.00](https://doi.org/10.1097/01.ta.0000174918.38764.00)
55. Lee TC, Wang CJ, Yang YL, Huang YH, Lin WC, Chang SY (2010) Bone morphogenetic protein-2 expression in spinal fusion masses enhanced by extracorporeal shock wave treatment: a rabbit experiment. *Acta Neurochir (Wien)* 152(10):1779–1784. doi:[10.1007/s00701-010-0744-0](https://doi.org/10.1007/s00701-010-0744-0)
56. Liao SS, Guan K, Cui FZ, Shi SS, Sun TS (2003) Lumbar spinal fusion with a mineralized collagen matrix and rhBMP-2 in a rabbit model. *Spine* 28(17):1954–1960. doi:[10.1097/01.BRS.0000083240.13332.F6](https://doi.org/10.1097/01.BRS.0000083240.13332.F6)
57. Long J, Lewis S, Kuklo T, Zhu Y, Riew KD (2002) The effect of cyclooxygenase-2 inhibitors on spinal fusion. *J Bone Jt Surg Am* 84-A(10):1763–1768
58. Magit DP, Maak T, Trioano N, Raphael B, Hamouria Q, Polzhofer G, Drespe I, Albert TJ, Grauer JN (2006) Healos/recombinant human growth and differentiation factor-5 induces posterolateral lumbar fusion in a New Zealand white rabbit model. *Spine* 31(19):2180–2188. doi:[10.1097/01.brs.0000232823.82106.0a](https://doi.org/10.1097/01.brs.0000232823.82106.0a)
59. Martin GJ Jr, Boden SD, Titus L (1999) Recombinant human bone morphogenetic protein-2 overcomes the inhibitory effect of ketorolac, a nonsteroidal anti-inflammatory drug (NSAID), on posterolateral lumbar intertransverse process spine fusion. *Spine* 24(21):2188–2193 (discussion 2193–2184)
60. Miller CP, Simpson AK, Whang PG, Erickson BP, Waked WR, Lawrence JP, Grauer JN (2009) Effects of recombinant human bone morphogenetic protein 2 on surgical infections in a rabbit

- posterolateral lumbar fusion model. *Am J Orthop* (Belle Mead, NJ) 38(11):578–584
61. Minamide A, Kawakami M, Hashizume H, Sakata R, Tamaki T (2001) Evaluation of carriers of bone morphogenetic protein for spinal fusion. *Spine* 26(8):933–939
 62. Minamide A, Kawakami M, Hashizume H, Sakata R, Yoshida M, Tamaki T (2004) Experimental study of carriers of bone morphogenetic protein used for spinal fusion. *J Orthop Sci* 9(2):142–151. doi:[10.1007/s00776-003-0749-0](https://doi.org/10.1007/s00776-003-0749-0)
 63. Minamide A, Tamaki T, Kawakami M, Hashizume H, Yoshida M, Sakata R (1999) Experimental spinal fusion using sintered bovine bone coated with type I collagen and recombinant human bone morphogenetic protein-2. *Spine* 24(18):1863–1870 (discussion 1871–1862)
 64. Minamide A, Yoshida M, Kawakami M, Okada M, Enyo Y, Hashizume H, Boden SD (2007) The effects of bone morphogenetic protein and basic fibroblast growth factor on cultured mesenchymal stem cells for spine fusion. *Spine* 32(10):1067–1071. doi:[10.1097/01.brs.0000261626.32999.8a](https://doi.org/10.1097/01.brs.0000261626.32999.8a)
 65. Minamide A, Yoshida M, Kawakami M, Yamasaki S, Kojima H, Hashizume H, Boden SD (2005) The use of cultured bone marrow cells in type I collagen gel and porous hydroxyapatite for posterolateral lumbar spine fusion. *Spine* 30(10):1134–1138
 66. Morone MA, Boden SD (1998) Experimental posterolateral lumbar spinal fusion with a demineralized bone matrix gel. *Spine* 23(2):159–167
 67. Motomiya M, Ito M, Takahata M, Kadoya K, Irie K, Abumi K, Minami A (2007) Effect of hydroxyapatite porous characteristics on healing outcomes in rabbit posterolateral spinal fusion model. *Eur Spine J* 16(12):2215–2224. doi:[10.1007/s00586-007-0501-0](https://doi.org/10.1007/s00586-007-0501-0)
 68. Nakajima T, Iizuka H, Tsutsumi S, Kayakabe M, Takagishi K (2007) Evaluation of posterolateral spinal fusion using mesenchymal stem cells: differences with or without osteogenic differentiation. *Spine* 32(22):2432–2436. doi:[10.1097/BRS.0b013e3181573924](https://doi.org/10.1097/BRS.0b013e3181573924)
 69. Namikawa T, Terai H, Suzuki E, Hoshino M, Toyoda H, Nakamura H, Miyamoto S, Takahashi N, Ninomiya T, Takaoka K (2005) Experimental spinal fusion with recombinant human bone morphogenetic protein-2 delivered by a synthetic polymer and beta-tricalcium phosphate in a rabbit model. *Spine* 30(15):1717–1722
 70. Riew KD, Long J, Rhee J, Lewis S, Kuklo T, Kim YJ, Yukawa Y, Zhu Y (2003) Time-dependent inhibitory effects of indomethacin on spinal fusion. *J Bone Jt Surg Am* 85-A(4):632–634
 71. Sawin PD, Dickman CA, Crawford NR, Melton MS, Bichard WD, Sonntag VK (2001) The effects of dexamethasone on bone fusion in an experimental model of posterolateral lumbar spinal arthrodesis. *J Neurosurg* 94(1 Suppl):76–81. doi:[10.3171/spi.2001.94.1.0076](https://doi.org/10.3171/spi.2001.94.1.0076)
 72. Schimandle JH, Boden SD, Hutton WC (1995) Experimental spinal fusion with recombinant human bone morphogenetic protein-2. *Spine* 20(12):1326–1337
 73. Silcox DH 3rd, Daftari T, Boden SD, Schimandle JH, Hutton WC, Whitesides TE Jr (1995) The effect of nicotine on spinal fusion. *Spine* 20(14):1549–1553
 74. Smucker JD, Bobst JA, Petersen EB, Nepola JV, Fredericks DC (2008) B2A peptide on ceramic granules enhance posterolateral spinal fusion in rabbits compared with autograft. *Spine* 33(12):1324–1329. doi:[10.1097/BRS.0b013e3181732a74](https://doi.org/10.1097/BRS.0b013e3181732a74)
 75. Sun TS, Guan K, Shi SS, Zhu B, Zheng YJ, Cui FZ, Zhang W, Liao SS (2004) Effect of nano-hydroxyapatite/collagen composite and bone morphogenetic protein-2 on lumbar intertransverse fusion in rabbits. *Chin J Traumatol* 7(1):18–24
 76. Tanaka K, Takemoto M, Fujibayashi S, Neo M, Shikunami Y, Nakamura T (2011) A bioactive and bioresorbable porous cubic composite scaffold loaded with bone marrow aspirate: a potential alternative to autogenous bone grafting. *Spine* 36(6):441–447. doi:[10.1097/BRS.0b013e3181d39067](https://doi.org/10.1097/BRS.0b013e3181d39067)
 77. Tang Y, Ye X, Klineberg EO, Curtiss S, Maitra S, Gupta MC (2011) Temporal and spatial expression of BMPs and BMP antagonists during posterolateral lumbar fusion. *Spine* 36(4):E237–E244. doi:[10.1097/BRS.0b013e3181d73541](https://doi.org/10.1097/BRS.0b013e3181d73541)
 78. Tang ZB, Cao JK, Wen N, Wang HB, Zhang ZW, Liu ZQ, Zhou J, Duan CM, Cui FZ, Wang CY (2011) Posterolateral spinal fusion with nano-hydroxyapatite-collagen/PLA composite and autologous adipose-derived mesenchymal stem cells in a rabbit model. *J Tissue Eng Regen Med* 6(4):325–336. doi:[10.1002/term.445](https://doi.org/10.1002/term.445)
 79. Tay BK, Le AX, Heilman M, Lotz J, Bradford DS (1998) Use of a collagen-hydroxyapatite matrix in spinal fusion. A rabbit model. *Spine* 23(21):2276–2281
 80. Toribatake Y, Hutton WC, Tomita K, Boden SD (1998) Vascularization of the fusion mass in a posterolateral intertransverse process fusion. *Spine* 23(10):1149–1154
 81. Urrutia J, Carmona M, Briceno J (2011) Do corticosteroids affect lumbar spinal fusion? A rabbit model using high-dose methylprednisolone. *J Orthop Sci* 16(4):439–442. doi:[10.1007/s00776-011-0061-3](https://doi.org/10.1007/s00776-011-0061-3)
 82. Urrutia J, Mardones R, Quezada F (2007) The effect of ketoprofen on lumbar spinal fusion healing in a rabbit model. Laboratory investigation. *J Neurosurg Spine* 7(6):631–636. doi:[10.3171/SPI-07/12/631](https://doi.org/10.3171/SPI-07/12/631)
 83. Urrutia J, Thumm N, Apablaza D, Pizarro F, Zylberberg A, Quezada F (2008) Autograft versus allograft with or without demineralized bone matrix in posterolateral lumbar fusion in rabbits. Laboratory investigation. *J Neurosurg Spine* 9(1):84–89. doi:[10.3171/SPI/2008/9/7/084](https://doi.org/10.3171/SPI/2008/9/7/084)
 84. Valdes M, Moore DC, Palumbo M, Lucas PR, Robertson A, Appel J, Ehrlich MG, Keeping HS (2007) rhBMP-6 stimulated osteoprogenitor cells enhance posterolateral spinal fusion in the New Zealand white rabbit. *Spine J* 7(3):318–325. doi:[10.1016/j.spinee.2006.02.005](https://doi.org/10.1016/j.spinee.2006.02.005)
 85. Walsh WR, Oliver RA, Gage G, Yu Y, Bell D, Bellemore J, Adkisson HD (2011) Application of resorbable poly(lactide-co-glycolide) with entangled hyaluronic acid as an autograft extender for posterolateral intertransverse lumbar fusion in rabbits. *Tissue Eng Part A* 17(1–2):213–220. doi:[10.1089/ten.TEA.2010.0008](https://doi.org/10.1089/ten.TEA.2010.0008)
 86. Walsh WR, Vizesi F, Cornwall GB, Bell D, Oliver R, Yu Y (2009) Posterolateral spinal fusion in a rabbit model using a collagen-mineral composite bone graft substitute. *Eur Spine J* 18(11):1610–1620. doi:[10.1007/s00586-009-1034-5](https://doi.org/10.1007/s00586-009-1034-5)
 87. Wing KJ, Fisher CG, O'Connell JX, Wing PC (2000) Stopping nicotine exposure before surgery. The effect on spinal fusion in a rabbit model. *Spine* 25(1):30–34
 88. Yalcin N, Ozturk A, Ozkan Y, Celimli N, Ozocak E, Erdogan A, Sahin N, Ilgezdi S (2011) The effects of zoledronic acid and hyperbaric oxygen on posterior lumbar fusion in a rabbit model. *J Bone Jt Surg Br* 93(6):793–800. doi:[10.1302/0301-620X.93B6.24257](https://doi.org/10.1302/0301-620X.93B6.24257)