SCIENTIFIC ARTICLE

Reconstruction of Segmental Stability of Goat Cervical Spine with Poly (D, L-lactic acid) Cage

Xiao-hui Li, MD¹, Yue-ming Song, MD², Hong Duan, MD²

¹Department of Orthopaedics, Tianjin Hospital, Tianjin and ²Department of Orthopaedics, West China Hospital, Sichuan University, Chengdu, China

Objectives: To compare the characteristics of inter body fusion after using an established poly (D, L-lactic acid) (PDLLA) cage, a titanium cage or autologous tricortical iliac crest graft in a goat cervical spine model *in vivo*.

Methods: Twenty-four goats were assigned to three groups: PDLLA cage group ($n = 8$), titanium alloy cage group ($n = 8$) and autologous iliac bone group ($n = 8$), and underwent C₃₋₄ discectomy and fusion with the allocated procedure. Radiography was performed pre- and post-operatively and after 1, 2, 4, 8 and 12 weeks and disc space height (DSH), intervertebral angle (IVA), and lordosis angle (LA) measured at these time points. After 12 weeks, the goats were killed and fusion sites removed. Biomechanical testing was performed in flexion, extension, axial rotation, and lateral bending to determine the stiffness and range of motion (ROM). All specimens were also assessed histomorphologically.

Results: The IVA of PDLLA cage four weeks postoperatively and DSH eight and twelve weeks postoperatively were significantly greater than that of autologous iliac bone graft ($P < 0.05$). The LA values did not differ significantly between groups. The stiffness of both types of cages for axial rotation and lateral bending, and ROM for every movement, were significantly greater than that of the autologous iliac bone graft group ($P < 0.05$). PDLLA and titanium cages did not differ significantly ($P > 0.05$). Radiographic and histomorphological assessment showed better fusion in the cage than the autologous bone groups.

Conclusion: The PDLLA cage can provide good intervertebral distract ability and enough biomechanical stability for cervical fusion.

Key words: Animal experiment; Biomechanics; Cage; Cervical spine

Introduction

Since Smith and Robinson¹ and Cloward² performed and reported anterior cervical decompression and bone graft fusion in the 1950's, many patients with segmental instability of the cervical spine have benefited. However, classical anterior cervical decompression and fusion has some serious drawbacks, outcomes potentially being compromised by graft absorption, collapse, loosening, shift, prominence or projecting into spinal canal and even failure of fusion or development of pseudoarticulation.

Additionally, transplanting autologous iliac bone can be associated with increased blood loss, hematoma, pain at the donor site, fracture of the ilium, dysesthesias in the femoral region and so on³⁻⁵. In recent years, a series of studies have reported the use of cervical interbody fusion cages⁶⁻¹¹. These cages are mainly composed of titanium alloy, have good biocompatibility and are artifact-free in MR images. However, it is not possible to monitor the growing, joining and fusion of bone trabecula because titanium alloy cages do not allow passage of X-rays¹². In addition, both non-metallic carbon fiber

Address for correspondence Yue-ming Song, MD, Department of Orthopaedics, West China Hospital, Sichuan University, Chengdu, China 610041
Tel: 0086-013820058675; Fax: 0086-28-85422430; Email: chdxiaohuilee@163.com

Disclosure: The authors declare that they have no competing interests.

Received 17 April 2015; accepted 24 June 2015

and polyether-ether-ketone^{8,13,14} are neither degraded nor absorbed and do not induce osteogenesis.

Some studies have shown that poly (D, L-lactic acid) (PDLLA) has excellent biocompatibility and can be degraded and absorbed completely by the body^{15,16}. Additionally, its biomechanical properties are close to those of cortical bone and its small stress shielding creates a good biological and biomechanical environment for bone fusion.

The present study aimed to explore the ability of PDLLA cage to maintain disc space height, create biomechanical stability in phase II and achieve interbody fusion by comparing the postoperative outcomes of PDLLA and titanium alloy cages and tricortical iliac crest implants in C₃₋₄ of living goats. The histological characteristics of the fusion sites were also assessed.

Materials and Methods

Experimental Animals and Groups

Twenty-four 2-year-old female Sichuan Ma goats (body weight: 30.10 ± 1.38 kg) were acquired and allocated randomly to a PDLLA cage ($n = 8$), titanium alloy cage ($n = 8$) or tricortical iliac crest goats ($n = 8$).

Preparation of Fusion Cages

Titanium alloy and PDLLA cages have the same shape. The former is manufactured by a machining method and the latter by a compression moulding method. These cages has of box type, measure 5 mm × 12 mm × 14 mm and contain upper and lower alar plates, each of which is fixed with one screw (Fig. 1).

Operative Procedures and Postoperative Treatment

Two million four hundred thousand U of penicillin was injected i.v. before and during surgery. Combined i.v. anesthesia was administered. Specifically, 10 mg of midazolam and 0.1 mg of fentanyl was injected for induction, and pentobarbital sodium was administered i.v. for maintenance. The skin of the neck and right iliac region was prepared. A right anterolateral longitudi-

nal incision was made in the neck to separate the superficial and deep fascia, thereby exposing the tissues among the musculus longus colli. A Caspar distraction device was used to excise the C₃₋₄ discs. The cartilage endplate was removed with a curette until there was even slow bleeding on the bone surface; the posterior longitudinal ligament was not severed nor the spinal canal exposed. Right iliac bone was granulated and then used to fill the cavities of the PDLLA and titanium alloy cages. After the cages had been implanted, the Caspar distraction device was released and the cages fixed into the upper and lower vertebral bodies by screwing in the upper and lower fixation plates. Autologous iliac bone was grafted using the Robinson technique¹. The incision was then rinsed with saline, sutured layer by layer and bandaged with an aseptic dressing. The animals were observed until they regained consciousness. Eight million units of penicillin were injected i.m bid for 5 consecutive days.

All animals were examined and evaluated by X-ray before and 0, 1, 2, 4, 8 and 12 weeks after the surgery. They were killed by i.v. injection of potassium chloride under pentobarbital sodium anesthesia 12 weeks after surgery, after which their C₃₋₄ segments, peripheral muscles and tissues were removed with preservation of ligaments. Biomechanical testing and histological examination were then performed.

Outcome Indicators

General Observations

Postoperative feeding, activities and wound healing were observed.

Radiological Assessment

Before surgery and 0, 1, 2, 4, 8 and 12 weeks after surgery, X-ray films were taken in China Hospital, Sichuan University. Disc space height (DSH) at C₃₋₄, intervertebral angle (IVA) and lordosis angle (LA) were measured on lateral views. DSH was calculated from the mean of the anterior disc space height, mid disc space height and posterior disc space height, as shown in Fig. 2.

Fig. 1 (A) PDLLA cage. (B) Tricortical iliac crest. (C) Titanium alloy cage.

Fig. 2 Measurement method of DSH (anterior + mid + posterior DSH/3), IVA and LA on the lateral radiographs. aDSH, anterior DSH; mDSH, mid DSH, pDSH, posterior DSH.

Biomechanical Testing

After the animals had been killed, the resected specimens were forward flexed, backward extended, laterally bended and rotated. A non-destructive method was used in which the specimens were given zero periods preloading with maximum torque three times. On the third preloading, creep was allowed for 30 s to decrease the viscoelasticity of the specimens, after which the specimens were remeasured. The torque was increased to 4 Nm from 1 Nm and 2 Nm. The angular displacement ($^{\circ}$) of C_3 relative to C_4 was also measured.

Histological Examination

The bone of all C_{3-4} segmental specimens was examined histologically. The specimens were cut along the median sagittal plane and trimmed to an appropriate size with the fusion zone as the center, then immersed in 80% alcohol for 7 days, after which they were embedded in organic glass. The resulting specimens were trimmed with a Leica SP1600 Trimmer (Newcastle, UK) and cut in the sagittal plane with a Leica SP2600 hard tissue slicer to obtain 5 μ m tissue sections. The sections were stained with hematoxylin and eosin (HE) and Masson and their histomorphology assessed.

Statistical Analysis

SPSS 20.0 software was used for all statistical analysis. The results are expressed as mean $\pm$ standard deviation (SD). $P < 0.05$ was considered statistically significance.

Results

Imaging Findings

General Observations

As assessed on X-ray films, the DSH in the PDLA cage and tricortical iliac crest groups had declined 12 weeks after

surgery, with a greater loss of DSH in the tricortical iliac crest group. Additionally, the fusion segments exhibited different degrees of ossification and the ossification in the PDLA cage group was less pronounced than in the tricortical iliac crest group. Because titanium alloy cages are impervious to X-rays, the pre- and post-operative DSHs in this group could not be compared; however, well defined bone islands were apparent around the cages (Figs. 3 and 4).

Measurements on X-ray Films

Preoperative measurements of LA, IVA and DSH on X-ray films did not differ significantly between the groups ($P > 0.05$), as shown in Table 1.

There were no significant differences in LA among the three groups for any postoperative time point ($P > 0.05$); however, IVAs 4 and 8 weeks after surgery and the mean DSH 8 and 12 weeks after surgery differed significantly between the PDLA cage and tricortical iliac crest groups ($P < 0.05$ for all). Because of the interference of titanium alloy cages with X-rays, no exact postoperative measurements of DSH were obtained in the titanium alloy cage group. Hence, in this study we were unable to quantitate the presence or absence of subsidence. DSH–time curves showed a trend to decrease in DSH showed in all three groups, this being most obvious in the tricortical iliac crest group.

Results of Biomechanical Testing

Comparison of Average Stiffness

Stiffness was calculated by the equation:

$EF = P/DL$, where P is the load and DL the magnitude of displacement.

The average stiffness was calculated according to the equation:

Fig. 3 Postoperative X-ray films. (A) PDLLA cage. (B) Titanium alloy cage. (C) Tricortical iliac crest.

Fig. 4 X-ray films 12 weeks after surgery. (A) PDLLA cage. (B) Titanium alloy cage. (C) Tricortical iliac crest.

Average stiffness = change in torque (Nm)/angular displacement increment ($^{\circ}$). The average stiffness was significantly greater in both cage groups than in the tricortical iliac crest group for lateral bending and rotation ($P < 0.05$ for both); however, the difference between the two cage groups was not significant ($P > 0.05$). No significant differences were found between the groups for flexion and extension ($P > 0.05$), as shown in Table 2 and Fig. 5.

Comparison of Range of Motion ROM

The three-dimensional motion of the spine can be represented by six degrees of freedom, each of which is used to calculate

three motion variables to evaluate the stability of the vertebral body. The neutral zone is the displacement between the vertebral body at the zone load and neutral position, whereas the elastic zone refers to the displacement between the vertebral body at maximum load and zone load. ROM is the sum of the displacement in the neutral and elastic zones. Because a step-wise loading method was used in this study, ROM was calculated based on the change in angular displacement at 4 Nm. ROM did not differ significantly between the PDLLA and titanium cage groups for flexion, extension and rotation (all $P > 0.05$). However, for lateral bending the difference in ROM between the two cage groups was significant ($P < 0.05$). For all

TABLE 1 Measurements on preoperative X-ray films according to surgical procedure (mean $\pm$ SD)

Groups	DSH (mm)*	IVA ($^{\circ}$)*	LA ($^{\circ}$)*
PDLLA group (n = 8)	3.57 $\pm$ 0.37	7.25 $\pm$ 1.39	5.88 $\pm$ 0.99
Titanium alloy cage group (n = 8)	3.71 $\pm$ 0.52	7.87 $\pm$ 1.25	6.63 $\pm$ 1.30
Tricortical iliac crest group (n = 8)	3.77 $\pm$ 0.62	8.00 $\pm$ 1.60	7.13 $\pm$ 1.36

* , No significant inter-group differences ($P > 0.05$).

TABLE 2 Comparison of average stiffness among groups (mean $\pm$ SD)

Groups	Stiffness (Nm/°)			
	Flexion	Extension	Lateral rotation	Lateral bending
PDLLA cage group (n = 8)	1.85 $\pm$ 0.18	1.51 $\pm$ 0.12	3.60 $\pm$ 0.12*	2.10 $\pm$ 0.13*
Titanium alloy cage group (n = 8)	1.85 $\pm$ 0.09	1.59 $\pm$ 0.10	3.54 $\pm$ 0.34*	2.19 $\pm$ 0.09*
Autologous iliac crest group (n = 8)	1.69 $\pm$ 0.45	1.41 $\pm$ 0.29	2.48 $\pm$ 0.25	1.59 $\pm$ 0.20

*, Significant difference compared with autologous iliac crest group ($P < 0.05$).

Fig. 5 Average stiffness 12 weeks postoperatively according to procedure.

motions, ROM in both cage groups was significantly better than in the autogenous iliac crest group ($P < 0.05$), as shown in Table 3 and Fig. 6.

Histomorphology Results

Twelve weeks after surgery, sections from animals that had received PDLLA or titanium cages showed abundant newly formed bone tissues on the interfaces between cages and vertebral endplates. In addition, the grafts and endplate bone trabeculae were continuous; the bone trabeculae in the graft had been absorbed and a wide range of new bone tissues and abundant chondrocytes and connective tissue were observed. The material in the PDLLA cage group had segmented and embedded and was surrounded with a plentiful bone or cartilage tissues. In the sections from the autogenous iliac crest group there were abundant new bone tissues on the interfaces between grafts and vertebral endplates and the autogenous iliac crest had collapsed (Fig. 7).

Discussion

Effect of PDLLA Cages on Maintaining DSH

Imaging is an important means of postoperative follow-up observation. The standard means of performing anterior cervical interbody fusion is with autogenous iliac crest grafts. However, this is associated with a significant incidence of collapse of the graft and loss of DSH, a reliable indicator that subsidence of the graft has occurred^{17,18}. Postoperative X-ray findings provide reference data for evaluating the outcomes of interbody fusion. The greatest advantage of the PDLLA cages assessed in this study is that they are X-ray-permeable, which

TABLE 3 Comparisons of ROM 12 weeks after surgery among groups (mean $\pm$ SD)

Groups	ROM (°)			
	Flexion	Extension	Lateral rotation	Lateral bending
PDLLA cage group	2.55 $\pm$ 0.14*	3.55 $\pm$ 0.14*	1.71 $\pm$ 0.98*	2.61 $\pm$ 0.13*#
Titanium alloy cage group	2.47 $\pm$ 0.17*	3.43 $\pm$ 0.21*	1.60 $\pm$ 0.19*	2.37 $\pm$ 0.13*
Autologous iliac crest group	3.16 $\pm$ 0.53	4.13 $\pm$ 0.46	2.19 $\pm$ 0.13	3.14 $\pm$ 0.23

*, Significant difference compared with the autologous iliac crest group ($P < 0.05$); #, Significant difference compared with titanium alloy cage group ($P < 0.05$).

Fig. 6 ROM 12 weeks postoperatively according to procedure.

facilitates postoperative follow-up. In this study, we measured DSH, LA and IVA in the fusion segments on postoperative X-ray films and found that significant differences in IVA 4 and 8 weeks after surgery between the PDLLA cage and tricortical iliac crest groups, but no significant difference in average DSH

at the eighth weeks and twelfth weeks. The DSH in both groups tended to decrease, this being more obvious in the tricortical iliac crest group. These findings suggest that PDLLA cages have an important function in maintaining DSH and stability of fusion segments. We therefore concluded that PDLLA cage have good prospects for being used more widely.

Effect of PDLLA Cages on Biomechanical Stability in Phase II

The PDLLA that the PDLLA cages used in this experiment were made of can be absorbed by the human body. Because PDLLA has similar biomechanical properties to cortical bone and small stress shielding, it creates a good biomechanical environment for bone fusion. Twelve weeks after surgery, the stiffness of the two types of cages in rotation and lateral bending was significantly greater than that in the tricortical iliac crest group; however, they did not differ significantly for flexion and extension. PDLLA and titanium cages did not differ significantly in stiffness for any movement at the 12th week. These findings indicated that the biomechanical characteristics of fused PDLLA and titanium alloy cages are similar and that are both better than those of tricortical iliac crest grafts. The initial biomechanical stability of cage filled with graft *in vivo* is provided almost exclusively by the cage itself, being conferred by their design and the biomechanical properties of the material. Postoperative stability in phase II depends on new interbody fusion bone. Biomechanical stability in *in vivo* studies is reportedly more pronounced than *in vitro* studies, which may be attributable to better new interbody fusion bone formation¹⁵. Intraoperative preservation of the endplate may minimize concentration of stress,

Fig. 7 Hard tissue sections stained with HE and Masson 12 weeks after surgery. (A, B) PDLLA group. (C, D) Titanium cage group. (E, F) Tricortical iliac crest group. Magnification, $\times 100$.

retain the interface of the stress and thus minimize graft subsidence and loosening. Furthermore, preservation of the endplate not only retains the strength of the cervical spine but also ensures its stiffness and stability.

Promotion of Osteogenesis by PDLLA Cages

According to some studies, PDLLA has excellent biocompatibility and can be degraded and absorbed completely by the body; it can therefore create a good biomechanical environment for bone fusion^{15,16}. Twelve weeks after surgery, histomorphological examination of resected specimens revealed abundant new bone tissues on the interfaces between cages and vertebral endplates, continuity between grafts and endplate bone trabeculae, absorption of trabeculae of grafted bone and a wide range of new bone tissues. The material in the PDLLA cages had fragmented and embedded and was surrounded by plentiful bone and cartilage tissues, indicating that PDLLA cages can result in good interbody fusion. The graft filling the cages was not only protected by the biomechanical stability of fusion, but was also provided with an environment conducive to fusion, resulting in formation of more and more stable interbody bone. In contrast, tricortical iliac crest grafts are subjected to compression stress after being implanted into the cervical spine. Although cages protect grafted bone from compression, they do not prevent the effects of shear force. Thus, more new fusion bone can form in cages.

The ideal biological factors for bone fusion include an adequate transplantation technique, maximum intervertebral gap for the bone graft, and minimal stress shielding¹⁹⁻²¹. To maximize mechanical stability, the volume of cage is minimized,

thus allowing the maximum amount of interbody bone graft. In the present study, the total volume of the two types of cages after being filled with graft was the same; the amounts of new interbody fusion bone were therefore similar. However, PDLLA cages result in better interbody fusion because they provide less stress shielding of the graft than titanium alloy cages.

X-ray films showed that the fusion segments exhibited varying degrees of osteogenesis. Nevertheless, osteogenesis was more evident in the tricortical iliac crest graft than the PDLLA cage group. We attributed this to the process of degradation and absorption of PDLLA. Also, its creeping substitution is slower; thus, less new bone may be formed than with autologous bone. However, it has a similar elastic modulus to human bone, a significant initial stabilizing effect and good biocompatibility, thus supporting osteogenesis. In particular, it has an advantage in maintaining DSH.

Conclusions

Because the biocompatibility of PDLLA is excellent, it can be degraded and absorbed completely by the human body. Additionally, PDLLA cages can be filled with bone graft and their elastic modulus is close to that of human bone. Thus, they provide an appropriate biological environment for bone ingrowth and osteogenesis at bone-implant interfaces. Furthermore, PDLLA cages can maintain DSH and increase the stability of fusion segments to create a good biomechanical environment for eventual bone fusion. Finally, PDLLA cages allow the passage of X-ray, which facilitates postoperative follow-up. In conclusion, PDLLA cages have good prospects for being more widely used.

References

1. Smith GW, Robinson RA. The treatment of certain cervical-spine disorders by anterior removal of the intervertebral disc and interbody fusion. *J Bone Joint Surg Am*, 1958, 40: 607-624.
2. Cloward RB. The anterior approach for removal of ruptured cervical disks. *J Neurosurg*, 1958, 15: 602-617.
3. Korovessis P, Repantis T, Vitsas V, Vardakastanis K. Cervical spondylodiscitis associated with oesophageal perforation: a rare complication after anterior cervical fusion. *Eur J Orthop Surg Traumatol*, 2013, 23 (Suppl. 2): S159-S163.
4. Jamjoom AB, Rawlinson JN, Kirkpatrick JN. Reoperation related to graft complication following anterior cervical fusion. *J Neurol Neurosurg Psychiatry*, 1990, 53: 1017.
5. Kraus DR, Stauffer ES. Spinal cord injury as a complication of elective anterior cervical fusion. *Clin Orthop Relat Res*, 1975, 112: 130-141.
6. Daentzer D, Willbold E, Kalla K, et al. Bioabsorbable interbody magnesium-polymer cage: degradation kinetics, biomechanical stiffness, and histological findings from an ovine cervical spine fusion model. *Spine (Phila Pa 1976)*, 2014, 39: E1220-E1227.
7. Nayak AN, Stein MI, James CR, et al. Biomechanical analysis of an interbody cage with three integrated cancellous lag screws in a two-level cervical spine fusion construct: an *in vitro* study. *Spine J*, 2014, 14: 3002-3010.
8. Hermansen A, Hedlund R, Vavruch L, Peolsson A. A comparison between the carbon fiber cage and the Cloward procedure in cervical spine surgery: a ten- to thirteen-year follow-up of a prospective randomized study. *Spine (Phila Pa 1976)*, 2011, 36: 919-925.
9. Farrokhi MR, Torabinezhad S, Ghajar KA. Pilot study of a new acrylic cage in a dog cervical spine fusion model. *J Spinal Disord Tech*, 2010, 23: 272-277.
10. Lind BI, Zoega B, Rosen H. Autograft versus interbody fusion cage without plate fixation in the cervical spine: a randomized clinical study using radiostereometry. *Eur Spine J*, 2007, 16: 1251-1256.
11. Pitzen T, Kiefer R, Munchen D, Barbier D, Reith W, Steudel WI. Filling a cervical spine cage with local autograft: change of bone density and assessment of bony fusion. *Zentralbl Neurochir*, 2006, 67: 8-13.
12. Kanayama M, Hashimoto T, Shigenobu K, Oha F, Ishida T, Yamane S. Pitfalls of anterior cervical fusion using titanium mesh and local autograft. *J Spinal Disord Tech*, 2003, 16: 513-518.
13. Vavruch L, Hedlund R, Javid D, Leszniewski W, Shalabi A. A prospective randomized comparison between the Cloward procedure and a carbon fiber cage in the cervical spine: a clinical and radiologic study. *Spine (Phila Pa 1976)*, 2002, 27: 1694-1701.
14. Arts MP, Wolfs JF, Corbin TP. The CASCADE trial: effectiveness of ceramic versus PEEK cages for anterior cervical discectomy with interbody fusion; protocol of a blinded randomized controlled trial. *BMC Musculoskelet Disord*, 2013, 14: 244.
15. Pflugmacher R, Schleicher P, Gumnior S, et al. Biomechanical comparison of bioabsorbable cervical spine interbody fusion cages. *Spine (Phila Pa 1976)*, 2004, 29: 1717-1722.
16. Wuisman PI, Krijnen MR, Helder MN, Smit TH. Bioabsorbable interbody cages in a sheep cervical spine fusion model. *Spine (Phila Pa 1976)*, 2005, 30: 2005-2006.
17. Soini J. Lumbar disc space heights after external fixation and anterior interbody fusion: a prospective 2-year follow-up of clinical and radiographic results. *J Spinal Disord*, 1994, 7: 487-494.
18. Dennis S, Watkins R, Landaker S, Dillin W, Springer D. Comparison of disc space heights after anterior lumbar interbody fusion. *Spine (Phila Pa 1976)*, 1989, 14: 876-878.
19. Mashhadinezhad H, Samini F, Zare R. Comparison of outcomes and safety of using hydroxyapatite granules as a substitute for autograft in cervical cages for anterior cervical discectomy and interbody fusion. *Arch Bone Jt Surg*, 2014, 2: 37-42.
20. Dağlı M, Er U, Simşek S, Bıvbe M. Late results of anterior cervical discectomy and fusion with interbody cages. *Asian Spine J*, 2013, 7: 34-38.
21. Abbott A, Halvorsen M, Dederling A. Is there a need for cervical collar usage post anterior cervical decompression and fusion using interbody cages? A randomized controlled pilot trial. *Physiother Theory Pract*, 2013, 29: 290-300.