Resorbable cages for spinal fusion: an experimental goat model

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Object: A biomechanical cadaveric study and an in vivo monosegmental spinal fusion study were performed to evaluate a novel bioresorbable poly-L-lactic acid (PLLA) cage.

Methods: The yield strength of a spinal segment was chosen as the main design parameter for the resorbable cages to be used in a goat model. In a 3-year in vivo study the authors found fusion to be significantly faster and more complete when using PLLA cages compared to titanium cages with the same dimensions. In the PLLA group, the intervertebral grafting height did not change and bone remodeling within the cage was completed 2 years after implantation. In terms of degradation of the PLLA, similar features were observed in vivo and in vitro.

Conclusions. Degradation was almost completed 3 years after implantation. Tissue reaction was mild during the 3-year period.

Key Words • bioabsorbable implant • fusion • spine

Ex vivo and in vivo animal models are essential to study spinal fusion. The spines are easily available, have uniform and mechanical properties, and have anatomical and biomechanical similarities to human spines. An advantage of using a goat or sheep model is that the lamellar bone growth rate is nearly equivalent to that in humans.

Methods and Materials

Study Design

We report preliminary data from a study exploring the potential benefits of a novel bioresorbable PLLA cage with less stiffness than most of the current cages. We attempted to design a PLLA cage based on in vitro biomechanical analysis of a lumbar spinal segment of a goat and to evaluate the efficacy of two PLLA cages with different stiffnesses versus a titanium cage of similar design for spinal interbody fusion in an in vivo goat model. The goal is a rapid and solid interbody fusion followed by absorption of the cage via natural pathways.

This study summarizes the biomechanical, radiographic, histological, histomorphometric, and biochemical findings based on experiments in 25 lumbar spinal segments used in vitro testing. Thirty-six Dutch milk goats were treated with single-level (L3–4) PLLA spinal fusion cages filled with autograft. Goats were killed at 3, 6, 12, 24, and 36 months. The goat lumbar spine model was chosen because of the biomechanical similarities between the goat and human lumbar spine.

Abbreviations used in this paper: BMC = bone mineral content; CT = computerized tomography; MAR = mineral apposition rate; PBS = phosphate-buffered saline; PLLA = poly-L-lactic acid; RS = radiographic score; VB = vertebral body.

Ex Vivo Study

For the mechanical tests on native spinal segments, 17 lumbar spinal segments from six Dutch milk goats were cut free from all soft tissues, and the ligaments remained intact. For the experiments on the segments with spinal cages, eight lumbar spine segments from three cadaveric goats were used. Three segments were fitted with titanium cages (stiffness 700 kN/mm in axial compression), three with stiff PLLA cages (4 kN/mm), and two with flexible PLLA cages (2 kN/mm). The cages were filled with tightly compacted trabecular bone.

All specimens were kept wet by packing them in soaked cloths. The BMC of the VBs of both series of specimens was measured with DEXA (QRD 2000; Hologic Corporation, Waltham, MA). The free endplates of each segment were made flat and parallel by gently pressing the segment in a lump of bone cement on the table. The intervertebral disc was kept horizontal. The specimens were placed in a Zwick 1455 (Zwick GmbH, Ulm, Germany) material testing device and compressed with a constant speed of 2 mm/minute. From the force–deformation curves, the yield strength and the ultimate strength were determined. The yield strength was defined as the force at which maximum stiffness was found and the ultimate strength as the maximum force until failure of the specimen. The height of each cage was 10 mm.

In Vivo Study

Based on biomechanical data, two types of PLLA interbody cages were studied. The dimensions of both were 10 × 10 × 18 mm. One interbody cage had a flexible cage–axial compression stiffness of 2 kN/mm and a wall thickness of 0.75 mm (15 cases). The other interbody cage had a stiff cage–axial compression stiffness of 4 kN/mm and a wall thickness of 1.5 mm (15 cases). These cages were designed and produced (Stryker-Howmedica-Osteonics, Rutherford, NJ) from an enantiomerically pure PLLA (PURAC Biochem BV, Gorinchem, The Netherlands). The cages were compared with titanium cages (axial compression stiffness 700 kN/mm...
Mineralizing surface (%) was calculated using the double-labeled bone surface, plus one half of the single labeled surface, which was divided by the time between the labeling periods. This was done at different points, divided by the time between the labeling periods.

**Experimental Materials**

Before sacrifice, bones were labeled with tetracycline and calcein to analyze bone remodeling and bone formation within the cages. After sacrifice, the lumbar spine was excised, trimmed of residual musculature, and radiographs were obtained. Subsequently, the operated motion segment was dissected and standardized using a water-cooled bandsaw (EXAKT, Norderstedt, Germany). This created a 3- and a 5-mm-thick parasagittal section.

Radiographs were taken immediately after surgery and at the time of death to estimate interbody fusion within the cages. High-resolution films were used to produce high-resolution anteroposterior and lateral radiographs of the harvested lumbar spine. Three blinded evaluators for fusion and implant status evaluated the resulting radiographs, which were graded according to a validated three-point radiographic score. Subsidence of the operated motion segment was estimated at time of retrieval by computerized planimetry or were stained with Goldner trichrome, hematoxylin and eosin, and toluidine blue for transmitted light microscopy. The 20-μm sections were stained with basic fuchsin and toluidine blue for transmitted light microscopy.

**Histomorphometric Analysis**

Histomorphometric analysis of all consecutive fields within the cage devices (range 174–232 fields) was performed (magnification ×100) using a semiautomatic image analysis system (OsteoMeasure; Zeiss, Kontron, Image Analysis Division, Oberkochen, Germany). Differentiation between the percentage of woven and lamellar bone within the cage was performed by a real analysis using a 10 × 10 eyepiece grid with manual counting. The MAR (μm/day) of the trabecular bone within the cage devices was calculated by the mean distance between the two parallel running labeled lines at three different points, divided by the time between the labeling periods. Mineralizing surface (%) was calculated using the double-labeled surface, plus one half of the single labeled surface, which was divided by total bone surface.

Remnants of the PLLA cage were carefully collected from one sagittal part without disrupting the integrity of the cage, when solid. After macroscopic evaluation, the retrieved PLLA was tested for inherent viscosity. The PLLA cages were placed in sealed glass jars filled with PBS at 37 ± 1°C. The weight/volume ratio of the samples to PBS was 1:20. The pH of the PBS was measured constantly and was replaced when the initial pH of 7.4 decreased by 0.2 U. After 6, 12, 26, 52, and 73 weeks from the start of the incubation time, a jar containing six PLLA cages was removed from the constant temperature incubator. The samples were allowed to equilibrate to room temperature, after which they were dried to constant weight under vacuum. Inherent viscosities of equilibrated polymer solution (0.1 g of PLLA in 100 ml of chloroform) at 25 ± 0.01°C were calculated using a capillary viscometer (Schott, Chicago, IL).

Samples with core volumes of 13 × 6 × 5 mm were sectioned from the fusion area. The samples were placed in a perspex sample holder and scanned through 360° by a high-resolution micro-CT system (Micro CT 20; Scanco Medical, Basersdorf, Switzerland) with a nominal resolution of 13 mm to determine the trabecular architecture and density of the newly formed bone and to quantify the degree of anisotropy.

In addition to histological analysis of the spinal segment, the spinal cord, lymph nodes, and organs were harvested and fixed in 10% neutral buffered formalin. The tissues were embedded in paraffin blocks for sectioning. The block was cut on a rotary microtome to produce sections 6 to 8 mm thick. Hematoxylin and eosin stain was used to evaluate the sectioned tissues. The sections were analyzed by light microscopy by using a high-power lens.

**Results**

All native goat specimens showed an identical force–deformation curve. Differences in strength could be explained by variability in BMC. The average ultimate strength (7.5 kN) was comparable to that of a middle-aged man (6.7 kN). Within each segment, the yield strength was 46,417.8% of the ultimate strength, with an average of 3.5 kN. In some samples, blood was pressed out of the vertebral pores at compression forces higher than the yield strength. All spinal segments failed within the upper VB, leaving the intervertebral discs intact.

In the instrumented segments group, specimens with titanium cages were less stiff and less strong than those with PLLA cages (Fig. 1). However, BMC and yield strength were highly correlated in the instrumented group (r = 0.94), regardless of the material of the cage. The lower strength of the segments with titanium cages was a result of the lower BMC of the segments. The yield strength of the instrumented specimens was considerably lower than that of the native specimens (2.6 kN vs 3.5 kN), although not significantly (p = 0.12). Bone mineral content, however, was significantly higher for the native specimens (p < 0.05). When corrected for BMC, the yield strength was the same for both the native and the instrumented spine segments (yield strength/BMC 5234 ± 51 and 221 ± 34, respectively; p = 0.53). Also in the instrumented segments, blood was pressed out of the VB at forces higher than the yield strength.

**In Vivo Study**

During the 36-month follow-up period, three goats were excluded from analysis: one goat died of pulmonary complications, and two goats had cage-related bacterial infections.

**Radiographic Analysis**

Radiographic evaluation of fusion (Fig. 2) at 3 months showed advanced ingrowth of trabecular bone within PLLA, but no fusion (RS 0). At 6 months, four (80%) of five PLLA specimens showed interbody fusion with bridging trabecular bone (RS 2), one (20%) of five speci-
mens showed advanced ingrowth but no fusion (RS 1). Three titanium specimens showed bone ingrowth but no fusion (RS 1). Subsequent retrievals at 12, 24, and 36 months (Fig. 2) showed fusion (RS 2) in 15 (88%) of 17 PLLA specimens and ingrowth but no fusion (RS 1) in two (12%) of 17. In the same period, only two (66%) of three titanium specimens showed fusion (RS 2), whereas in one (33%) of three a pseudarthrosis (RS 0) had developed. Considering all specimens, there was a significant difference in fusion rate ($p < 0.05$) between PLLA and titanium cages.

Lateral radiographs of the operated motion segments showed maintenance of interbody fusion of the PLLA specimens during 36 months of follow up. At 3 and 6 months, no PLLA specimen showed radiographic evidence of cage collapse. At 24 and 36 months, the PLLA cages had been replaced progressively by trabecular bone. Flexible PLLA cages, however, showed a slight bending deformation at 6-month follow up.

Lateral radiographs showed a sentinel sign in seven (21%) of 33 motion segments. No evident correlation ($r^2 = -0.26$) was shown between the presence of the sentinel sign and the rate of interbody fusion.

All operated motion segments showed a certain amount of subsidence after implantation of the cage devices. No increase in the amount of subsidence was noted after 6-month follow up.

**Histological and Histomorphometric Analysis**

At 3 months, the process of creeping substitution with woven bone formation toward bone graft particles was observed. At 6 and 12 months, an increasing percentage of lamellar bone and bone marrow volume within the cages was present (Fig. 3). Unfused motion segments showed endochondral bone formation in the fusion area. Histological evaluation of the unfused segment at 36 months demonstrated a "locked pseudarthrosis," which is fibrous tissue with collagen fibers in the fusion zone without endochondral bone formation.16

The percentage of newly formed trabecular bone within PLLA cages showed a steady increase during the first 12 months. Thereafter, a steady state was noted.

The percentage of lamellar bone within the PLLA cages increased up to $92 \pm 3\%$ after 24 months of follow up and $94 \pm 3\%$ after 36 months. Bone remodeling was completed 2 years after implantation of the PLLA cages.

The bone formation rate (the mineralizing surface/bone surface $\times$ MAR) increased at 12 months compared to 6 month most likely due to disintegration of the PLLA cages, leading to an advanced loading of trabecular bone within the cage.

A comparison of stiff and flexible PLLA cages showed similar results in terms of rate of interbody fusion, motion segment subsidence, sentinel sign, local tissue response, bone volume, and activity of bone formation. Flexible PLLA specimens showed a significantly higher percentage of lamellar bone compared to stiff PLLA cages ($p = 0.04$).

At 6 months, PLLA specimens showed a statistically significant ($p = 0.04$) higher rate of fusion and more complete fusions than titanium cages. However, more subsidence was reported in the PLLA specimens ($p = 0.04$). At 36 months, however, the amount of subsidence was comparable between titanium and PLLA specimens. A fibrous tissue layer surrounded the PLLA cages as well as the titanium cages. However, inflammatory cells were not present in the titanium cages. At 6 and 36 months, titanium specimens showed a lower but not significant percentage of newly formed bone compared to PLLA specimens ($23 \pm 11\%$ vs $35 \pm 12\%$, and $24 \pm 11\%$ vs $38 \pm 13\%$, respectively). At 36 months, the percentage of lamellar bone in titanium specimens was comparable to that in PLLA specimens ($93 \pm 1\%$ vs $94 \pm 3\%$). Titanium cages had significantly lower values of mineralized surface and
MAR at 36 months (p = 0.01 and p = 0.002, respectively). Therefore, the bone formation rate was decreased within titanium cages, which indicates a stress-shielded environment within the titanium cage devices.

Absorption and Degradation Analysis

At 3 and 6 months, the original geometry of the PLLA cages had been maintained. Microcracks with interposition of quiescent fibrous tissue were observed after 6 months. At 12 months, the cages had been disintegrated into multiple fragments with interposition of quiescent fibrous tissue. Also, absorption of small PLLA fragments was observed. At 24 months, histological evaluation showed an advanced absorption of the totally fragmented PLLA cage and replacement by trabecular bone and fibrous tissue. Thirty-six months after implantation, 50% of PLLA cages were completely absorbed and replaced by trabecular bone and fibrous tissue. In the remaining PLLA specimens, 1 to 10% of the PLLA still existed (Fig. 2).

Inherent viscosity values of both types of PLLA cages showed similar curves during the incubation period (Fig. 4). The flexible PLLA cages showed a 12% reduction in inherent viscosity at 4 weeks, 36% at 12 weeks, and 87% at 73 weeks of incubation in PBS. The stiff PLLA cage showed a similar absorption pattern with a 13% reduction in inherent viscosity at 4 weeks, 44% at 12 weeks, and 90% at 73 weeks of incubation.

Tissue Reaction to PLLA Cages

At 3, 6, and 12 months, the local tissue reactions to the stiff PLLA cages were similar to local tissue reactions to the flexible spinal cages. Three months after surgery, both stiff and flexible PLLA cages were surrounded by a quiescent fibrous tissue layer containing few dispersed foreign body giant cells. At 6 months, a quiescent fibrous tissue layer predominantly surrounded the PLLA cages. However, direct bone contact with the PLLA spinal fusion cages also was observed. In the fibrous tissue layer toward the PLLA cages, few dispersed foreign body giant cells were present. At 12 months, the fibrous tissue layer contained some foreign body giant cells surrounding PLLA fragments and sparsely dispersed lymphocytes and plasma cells. In addition, small PLLA particles were being phagocytized. The fibrous tissue layer thickness that envelops the implants varied significantly among specimens with the same rate of interbody fusion (range 0–0.94 mm). Analysis of the spinal cord, local lymph nodes, and relevant organs revealed no tissue reactions.

Microcomputerized Tomography Analysis

Micro-CT reconstructions of bone specimens from the fusion zone allow for a more detailed and quantitative three-dimensional analysis of the bone structure. Specimens can easily be sectioned to obtain a quick impression of the state of fusion and the structure of the trabecular bone architecture (Fig. 5). After 3 months, the dense compacted bone graft still exists (Fig. 5a). However, after 6 months, a fine trabecular bone architecture can be found throughout the fusion zone within the PLLA cages (Fig. 5b). After 36 months, this architecture is replaced by a coarser structure with less, albeit thicker, trabeculae (Fig. 3).

Discussion

Biomechanical Characteristics

Instrumented and noninstrumented motion segments were tested in uniaxial compression to failure of the specimens because it was important to analyze motion segments and the designed cages on mechanical sufficiency. Uniaxial compression testing demonstrated that the designed PLLA cages filled with bone graft were mechanically sufficient directly after implantation, and implantation of these cages did not negatively influence the compression strength of the motion segments. Both native and instrumented spinal segments demonstrated the same failure mechanisms: segment stiffness declines, implicating trabecular microdamage, leading to compression fractures of the upper VB and along the growth plate. Thus, compression loads above the local Y-direction are not physiological; the Y-direction can be used as a parameter for cage design.

Radiographic Analysis

Radiographic analysis of the spinal segment specimens clearly demonstrated standardized bone formation within the PLLA cages. From this study, it is reasonable to assume that several stages of creeping substitution occur within cages leading to successful arthrodesis. First, all specimens go through an RS of 1 before fusion occurs (RS 2). However, RS 1 profiles should be interpreted as time dependent: in 3-, 6-, and 12-month retrievals, endochondral bone formation was present as an ongoing process in the fusion zone, which eventually may lead to bone fusion.
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of the segment. Creeping substitution progresses slowly in metal or titanium cages because of limited loading in the fusion area. This slow process may result in a pseudarthrosis.

Endplate Perforation

This study and others show that perforation of the vertebral endplates at the site of implantation may enhance creeping substitution of the bone graft within the cages without compromising the strength of the spinal construct.5,18,33 An added advantage is the locking of the cage into the motion segment, thereby reducing cage migration. Another advantage is the adequate transport of PLLA degradation products because the PLLA is in close contact to well-vascularized vertebral trabecular bone.11,34

Sentinel Sign

In the study, a spontaneous sentinel sign was observed in four (15%) of 27 PLLA specimens and three (50%) of six titanium specimens. In contrast with the titanium specimens, the sentinel sign was not observed in the PLLA specimens after 6 months. Developing sentinel signs after surgical (disc) interventions may be a measure for validity of interbody fusion models focusing on bone formation and remodeling within cage devices. It is unclear if spontaneous development of a sentinel sign can be caused by manipulations of the spine.

Fusion and Subsidence

The study showed that interbody fusion involving PLLA cages can be achieved within 6 months postimplantation, which is comparable to in vivo studies with short-term follow-up.8,13,30 All operated motion segments demonstrated subsidence without clinical implications after the predetermined follow-up periods. Irrespective of cage material, this finding has been described in other investigational studies.30 No further subsidence was observed after fusion, which indicates that settling of the cages occurs before fusion of the motion segment is achieved. In our opinion, motion segment subsidence is inevitable with implantation of stand-alone cage devices; implant-bed preparation techniques (mechanical or thermal), implantation of cage devices, and peak forces at the cage–bone interface after implantation may cause trabecular microdamage. Subsidence of operated motion segments may be prevented by supplementary instrumenta-

Bone Histomorphometry

Currently, there are sparse data regarding bone histomorphometry of anterior fusion masses and these data are difficult to compare.13,25 In the study, histomorphometric analysis revealed an increasing bone volume within the cages up to 40% at 12 months; thereafter, a steady state was noted. The bone formation rate and bone remodeling were elevated at 12 months, most likely because of disintegra-
tion of the PLLA cages, leading to an advanced loading of newly formed bone within the cage devices. A steady state was reached 2 years after implantation of the PLLA cages. Histomorphometric analysis of the fusion areas 36 months after implantation demonstrated that bone volume and bone formation rates within titanium cages were significantly decreased compared to PLLA specimens, which supports in vitro biomechanical test results showing that titanium cage devices provide a stress-shielded environment.20

Biocompatibility and Disintegration

A long-term in vivo study was warranted to evaluate the histological characteristics of the degrading PLLA because a local host tissue response will be elicited concomitantly with the implantation of bioabsorbable poly-
ester implants.26,28 Late complications (sinus formation,
osteolytic reactions) have been reported with the use of polyester implants.6,7,28 The amount and intensity of an inflammatory response to polyester implants are influenced by implant- and environment-related factors.4,6,27,36 Generally, the biocompatibility of PLLA is excellent, which can be supported by the study.

In the current study, a mild inflammatory response was observed during the complete absorption of the PLLA cages. In the course of absorption of PLLA fragments, some dispersed lymphocytes and plasma cells were present in the fibrous tissue layer, which is a common feature encountered in degrading PLLA implants.3,15,20 Thirty-six months after implantation, no inflammatory cells were observed in the fibrous tissue of specimens with completely absorbed PLLA cages. Tissue reaction of local and distant tissues could not be observed.

Disintegration of the PLLA cages into multiple fragments and initial absorption of small PLLA fragments was observed 1 year after implantation. Thereafter, PLLA cages had been replaced by trabecular bone and dispersed fibrous tissue progressively.10,24 The in vitro and in vivo degradation results in this study show the same tendency as in previous reports, in which the polymers degraded significantly faster in vivo than in an in vitro environment.10,23,24 The stiff and flexible PLLA cages showed a similar decline in inherent viscosity. The PLLA cages subjected to the in vitro degradation disclosed a slower decline in inherent viscosity as compared to the PLLA cages implanted in the spinal motion segments. Similarly, in vivo loaded PLLA cages degrade faster compared to the in vitro unloaded PLLA cages. Although the stiff and flexible PLLA cages showed an advanced reduction in inherent viscosity (64 and 81%, respectively) 6 months after implantation, radiographic evaluation disclosed no evidence of cage collapse in terms of alteration in cage configuration.

Conversely, macroscopic and microscopic evaluation disclosed that the stiff and flexible PLLA cages had maintained original heights of 10 mm at 3 and 6 months after implantation. At 6 months, the flexible PLLA cages showed a slight bending deformation without sequelae for fusion and clinical outcome. The bending deformation may be attributed to the extent of cage loading and the limited wall thickness of the flexible PLLA cage.

In addition to the difference in increase of crystallinity, the stiff and flexible PLLA cages showed no significant differences in terms of decline of inherent viscosity, fusion results, or local tissue reaction to the implants during the 12-month follow-up period. Between 12 and 36 months, macroscopic and microscopic evaluation demonstrated further disintegration of the PLLA cages while maintaining interbody fusion. Mainly healthy trabecular bone and few dispersed areas of interposition of quiescent fibrous tissue replaced the gap. In line with the present study, similar findings have been reported with the use of bioabsorbable osteosynthesis devices in other high load-bearing regions such as mandibula and femoral neck.14,19,27

Limitations of the Study

This study focused primarily on in vitro biomechanical and in vivo radiographic, histological, histomorphometric, and degradation analyses of PLLA. Biomechanical testing of the operated motion segments was not included because the applied undecalcified histology and bone histomorphometry of the fusion areas may have been changed after biomechanical testing, also in a “nondestructive” mode. However, definitive trends with regard to achieving interbody fusion, complete bone remodeling, and complete PLLA resorption without negative tissue response were observed.

Summary

There is a subtle balance among load, fusion, degradation, and absorption of PLLA cages. Histological and histomorphometric data provided additional information and a better understanding of time-dependent bone formation and bone remodeling within cage devices. Although there are similarities between the spines in humans and animals, there are important differences. The axial compression stress in quadrupeds is higher, which leads to higher bone densities in the vertebrae.6 In addition, the model goat spines did not exhibit gross degenerative changes or spinal deformities that, in conjunction with clinical symptoms, are usually indications for spinal interventions.40 Therefore, there are limitations on the transferability of the results in animals to the human situation.

References

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