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Application of polylactides in spinal cages: Studies in a goat model

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Abstract Spinal cages are currently made of non-resorbable materials, but they only have a temporary function: after fusion, resorption is desirable both from a biological and mechanical point of view. We studied different polylactides in stand-alone condition in a goat model. Cages were made of 100% poly(L-lactic acid) (PLLA) or 70/30 poly(L/DL-Lactic acid) (PLDLLA); titanium served as control.

After six months, all titanium cages showed non-unions comparable to that observed in a clinical retrieval, thus showing validity of the goat model. PLLA cages maintained their mechanical integrity for six months, enough to allow fusion. After that, the material resorbed within 48 months without adverse tissue reactions. Bone formation was faster in PLDLLA cages, but these already failed within three months, thus losing their stabilising function: 50% ended in pseudo-arthrosis. Additional internal fixation provided enough stability for fusion (83%). Biocompatibility of both PLLA and PLDLLA was excellent.

The long-term results show that PLLA cages can be used for stand-alone interbody fusion, and that PLLA is an improvement over titanium in terms of fusion rate. PLDLLA showed enhanced bone formation, but also earlier failure of the implant. Chances for spinal fusion were better with additional internal fixation.

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1 Introduction

In spinal disorders like instability or severe deformation, fusion of two or more vertebrae may be indicated. Devices used for this purpose should restore the normal alignment of vertebrae and stabilize the segment in order to facilitate bony fusion. Traditionally, interbody fusion is performed with autologous cortical bone graft [1], but this has several disadvantages, including morbidity at the donor site, the risk of retropulsion with possible neural damage, and untimely resorption of the graft leading to instability of the segment [2]. Therefore, artificial cages have been developed, which realign the vertebrae into a more anatomical position and provide sufficient stability for spinal fusion [3, 4].

Today, interbody fusion has become a routine procedure with high success rates at short-term follow-up [5–8]. Various cage designs and materials, including steel, titanium, carbon fiber and PEEK, have been evaluated in an effort to improve clinical success [9–12]. Recently, however, an increasing number of failures has been reported [24], mostly related to cage material. Metal devices, for example, considerably exceed the stiffness of vertebral bone, which leads to stress shielding, migration of the cage, pseudo-arthrosis, or a combination of these events [13, 14]. Figure 1 shows a titanium tumor cage retrieved from a 4-year old after two years of implantation [15]. The cage had been functioning well without radiological signs of migration or loosening, suggesting complete fusion of the segment. Nevertheless, histology showed a non-union: there was bone ingrowth from both sides, but a layer of fibrocartilage remained in the center of the cage. At closer examination, the non-fusion zone showed an on-going process of enchondral ossification; this suggests that the implantation of the cage-given enough time-could have resulted in a complete fusion. Although this particular case was not considered a clinical failure, it is a perfect

Fig. 1 Histological cross-section of a titanium tumor cage (black) showing a delayed union. Trabecular bone (purple) grows into the cage from both sides, but between the bone fronts there is a layer of cartilage (pink) in a process of ossification (invisible at this magnification)

illustration of the problems occurring with metal implants: they eclipse the fusion zone; they cause stress shielding over the fusion area resulting in delayed unions; and they are a permanent foreign body, always susceptible to late complications which require second operations for removal.

Orthopedic implants for bone healing and fusion essentially have a temporary function: when the bone parts have grown together, the implant is superfluous and can even be harmful on the longer term. This consideration gave the initial impetus to the development of bioresorbable implants for fusion surgeries. Bioresorbables, however, have their own drawbacks and pitfalls. First, their strength is usually considerably lower than that of metals or non-degradable polymers. Also the brittleness of some frequently used polymers is worrisome. The main concern, though, is the production of waste products like acids and crystals, because too high concentrations may lead to serious tissue responses like inflammation and osteolysis [16, 17]. Degradation is a multi-factorial process involving material properties (polymer type, molecular weight distribution, porosity, permeability), implant design (implant mass, bulkiness), handling (sterilization, mechanical loading), and biochemical environment (pH) [18–20]. This makes it difficult to predict the behavior of a certain implant in a specific environment.

In a series of experiments, we explored two types of polylactides: a poly(L-lactide acid) (PLLA), and a 70/30 poly(L/DL lactic acid) (PLDLLA). We determined their mechanical properties and degradation profiles, and applied them in an *in vivo* goat model for lumbar spinal fusion. The general goal of these studies was to evaluate the suitability of these polylactides for application in an interbody fusion device, and to establish their biocompatibility upon resorption *in vivo*.

2 Materials and methods

Spinal fusion and tissue reactions against degradation products are biological processes that require *in vivo* examinations in an animal model. Since dynamic loading was considered to be a critical condition, we looked for an animal model with comparable spinal loads. Being a quadruped was not an exclusion factor: as in man, the main loading condition of the quadruped spine is axial compression, with minor components of axial torsion and anterior shear [21]. Indeed, the trabecular bone structure in the vertebral body is strikingly similar in man and quadruped [21, 22]. The main difference is the magnitude of loading: human and bovine spines have comparable geometries and dimensions [23], but trabecular bone density in the bovine spine (as in most other quadruped spines) is higher than in humans, indicating also higher loading amplitude [22]. We found that the strength of a lumbar spine segment of a goat is comparable to that of a human lumbar spine [24]; assuming a similar safety factor for fracture in mammals [25], we concluded that the goat would experience similar spinal loads and thus would be a proper animal model for spinal studies on bioresorbable cages.

The mechanical properties of lumbar spinal segments of female Dutch milk goats were determined *in vitro* [24]. The average ultimate strength of 17 lumbar spine segments was about 7.5 kN, which is comparable to the lumbar segment strength in a middle-aged man (6.7 kN). However, the yield strength was found to be about 3.5 kN, indicating that failure of the specimen occurred at lower loading amplitudes; bone marrow was pressed out of the vertebral bodies well below the ultimate strength. Therefore, we assumed that the yield strength would be the maximum spinal load in a goat *in vivo*. With 95% certainty and a safety factor of 40%, we specified the cage strength at 7.0 kN [24].

To determine the external geometry of the cage, we measured the endplate dimensions of the vertebral bodies at level L3-L4, the intended site of implantation. Average width and depth were 26.9 and 18.7 mm, respectively (unpublished

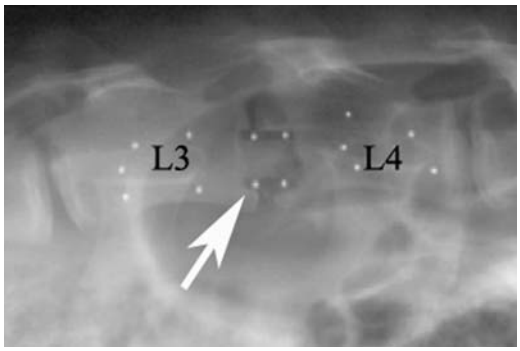


Fig. 2 Post-operative lateral X-ray of the lumbar goat spine showing a square, radiolucent PLDLLA cage (white arrow) in a L3-L4 segment penetrating the endplates of both vertebrae. The white dots in the vertebree and the cage are tantalum markers for RSA analyses

results), allowing external cage dimensions of 18 by 10 mm. The intervertebral discs were wedge-shaped with a height of 4 mm posteriorly, and 6 mm anteriorly. In order to secure vascularization and good access for cells and growth factors from the bone marrow, we chose the height of the cage at 10 mm; the cage device thereby would penetrate both vertebral endplates (Fig. 2). The operation procedure has been described in detail elsewhere [26]. The cages were tested at level L3-L4 in a stand-alone condition, i.e.: without additional fixation.

Two polylactides were evaluated. The first cages were produced from 100% poly(L-lactic acid) (PURAC Biochem BV, Gorinchem, The Netherlands), with a material strength of about 100 MPa. Requiring a cage strength of 7.0 kN, the cage wall thickness was set at 1.5 mm (Fig. 3). The PLLA was injection molded and sterilized by low-temperature plasma. The mean and numerical molecular weight of the stiff cages after production and sterilization were $M_w = 395500$ g/mol and $M_n = 240500$ g/mol, respectively. Initially, inherent viscosity was 2.68 dl/g, and crystallinity 11% [27]. The average initial strength of the stiff PLLA cages was 6.7 kN [27].

The second cage material was a mixture of 70% poly(L-lactic acid) and 30% racemic mixture of L- and D-lactic acid (PLDLLA) (MacroPore, San Diego, USA). Cages with the same design were compression molded and e-beam sterilized. The mean and numerical molecular weight of the material after production and sterilization were $M_w = 172000$ g/mol and $M_n = 90000$ g/mol, respectively. Inherent viscosity was 1.43 dl/g. As an amorphous material, crystallinity of the PLDLLA was negligible. The initial strength of the cages was 6.5 kN.

An overview of the number of animals included in this study is given in Table 1. The follow-up periods were 3, 6, and 12 months for the PLLA and the PLDLLA cages, and 6, 12 and 36 months for the titanium cages. The PLLA cages were subsequently followed until complete degradation after 48 months. As an extension, another group with PLDLLA cages was studied, provided with an additional internal fixation, as explained below.

After sacrifice, a mid-sagittal slice of 5 mm was sectioned from the spinal segments. Contact radiographs were made in order to evaluate radiological fusion. The sections were then placed in fixative (4% phosphate buffered formalin). After one week, the sections were dehydrated using ascending grades of ethanol, and embedded in methyl methacrylate (BDH Laboratory Supplies, Poole, England). The specimens with polylactide cages were cut into 7 μm sections using a Jung-K microtome (R. Jung, Heidelberg, Germany). Specimens with titanium cages were sawn into sections of 20 μm with a diamond-edge saw blade. Sections were either left unstained for examination by fluorescence and polarized light microscopy or they were stained with Goldner's trichrome, hematoxylin and eosin, or toluidine blue for transmitted light

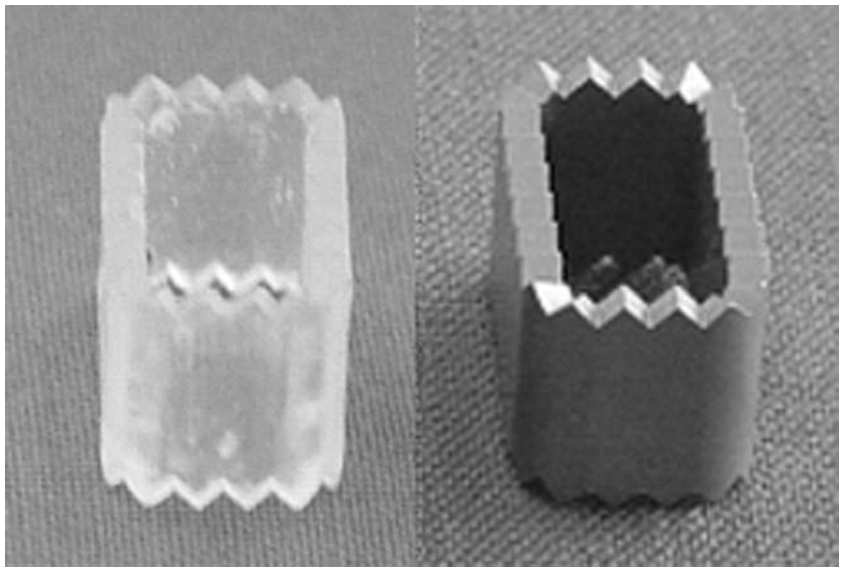


Fig. 3 Design of the resorbable (left) and the titanium cage (right) used for the studies. External dimensions were $18 \times 10 \times 10$ mm. The wall thickness of the regular cage was 1.5 mm

Table 1 Matrix of the number of goats provided with the various cage types and follow-up periods. The radiological score is an indication of clinical success (fusion)

Cage type	*n*	Follow-up (months)	No bone ingrowth (RS0)	Bone ingrowth (RS1)	Fusion (RS2)
PLDLLA	6	3		100%	
PLLA	6	3		100%	
PLDLLA	6	6		67%	33%
PLDLLA + fixator	6	6		17%	83%
PLLA	6	6	17%*		83%
Titanium	3	6		100%	
PLDLLA	8	12	12%	50%	38%
PLLA	6	12	17%*	17%	67%
Titanium	6	12		33%	67%
PLLA	6	24			100%
PLLA	6	36		17%	83%
Titanium	6	36		33%	67%
PLLA	7	48		14%	86%

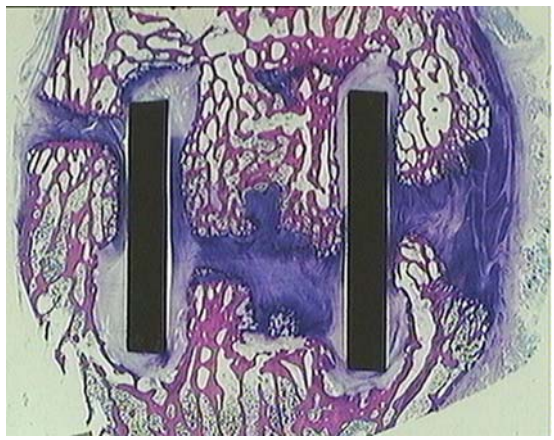
Specimens with * were infected.

microscopy [26]. Other parts of the cage were retrieved for biochemical analysis: inherent viscosity and crystallinity were determined [27]. Mann Whitney *U* tests were used for non-parametric data. Unpaired one-way analysis of variance (ANOVA) and a Tukey-Kramer post hoc multiple comparisons test were used to make specific comparisons between the different groups. An unpaired, two tailed t-test was performed when the data was not suitable for an ANOVA test. Statistical significance was set at a $p < 0.05$.

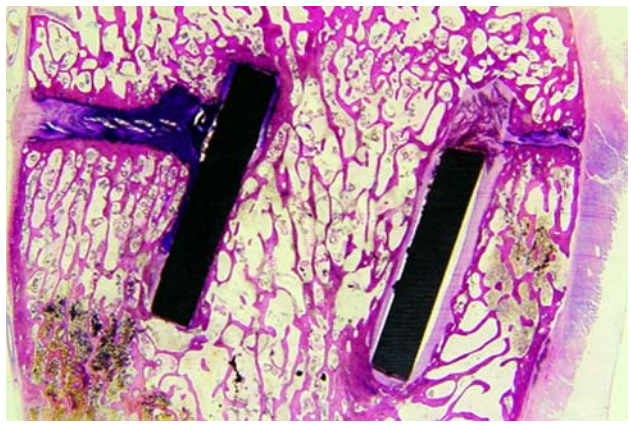
3 Results

The outcome of all animals reviewed in this paper is summarized in Table 1. After six months, all specimens with titanium cages showed a similar histological result as the clinical retrieval cage discussed earlier (compare Figs. 4(a) and 1): there is bone ingrowth from both sides, but there remains a layer of cartilage in a process of enchondral ossification. A layer of fibrous tissue is found around the entire cage. The fusion process is still going on, though, and 66% of the specimens in both the 12- and 36-months group showed fusion. The bone density in the fused cages, however, is lower than the bone density of the vertebral bodies, thus illustrating the stress-shielding effect of metal implants.

After three months, the PLLA cages still contained impacted bone graft (Fig. 5(a)), being replaced by new, woven bone in a process of creeping substitution [24]. In contrast to the titanium cages, fusion in the PLLA cages occurred by direct bone formation. After six months, all bone graft was resorbed, and a bridge of vital, woven bone was formed in all cases (Fig. 5(b)), except in one case of infection. After two years, the newly formed woven bone was replaced by lamellar bone in a trabecular bone structure wellaligned to the local stress trajectories [24, 28]. The PLLA cage disintegrated from 12 months on, and the material was completely

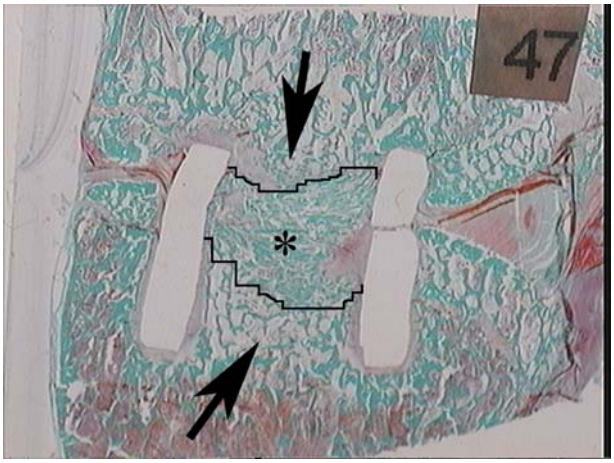


(a)

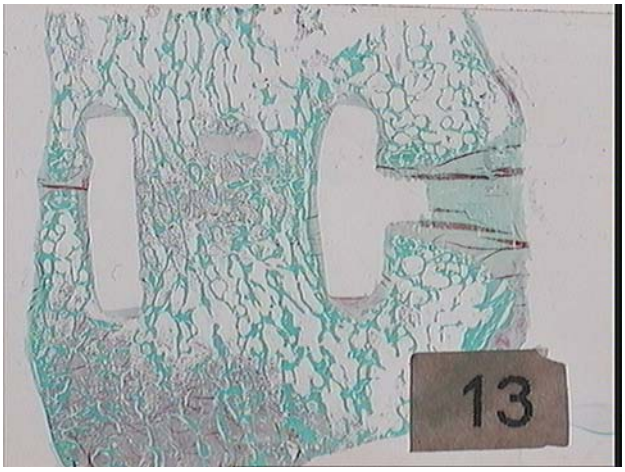


(b)

Fig. 4 Histological overview of specimens with titanium cages with six (a) and 12 months follow-up (b). At six months, bone (purple) grows into the cage from both sides, but in between the bone fronts an area of mineralizing fibro-cartilage (blue) is still present. The cage is surrounded by fibrous tissue (white) indicating the presence of shear stresses at the implant surface. At twelve months, the cage is fully filled with new trabecular bone. Also the amount of fibrous tissue at the implant surface is reduced. The blue areas outside the cage is fibro-cartilage from the intervertebral disc annulus



(a)



(b)

Fig. 5 Histology of specimens with a PLLA cage. (a) At three months, there is bone ingrowth from both sides (arrows), but about half of the cage still contains the densely impacted bone graft (*). (b) At six months, the cages are fully filled with newly formed trabecular bone (fusion)

resorbed after 48 months without adverse tissue reactions (Fig. 6) [24, 29]. None of the fusions failed after resorption of the cage, and the trabecular bone density of the fusion zone was comparable to that of the adjacent vertebral bodies. The volume occupied by the cage at implantation was completely filled with vital trabecular bone of comparable quality (Fig. 6)

The process of creeping substitution was much faster in the PLDLLA cages as compared to the PLLA cages: almost all impacted bone graft was resorbed at three months, and the fusion zone within the cage area was filled for some 80–90% by vital, woven type trabecular bone (Fig. 7(a)). However, we consistently found a small area of fibrous tissue between the closing bone fronts, indicating that there was no (longer a) process of direct bone formation. The cages showed remarkably more cracks than the PLLA cages at three months, indicating that the mechanical strength was not quite suf-

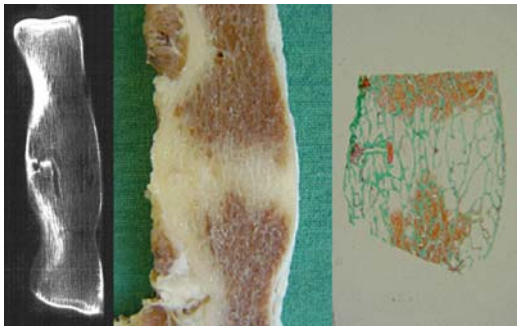
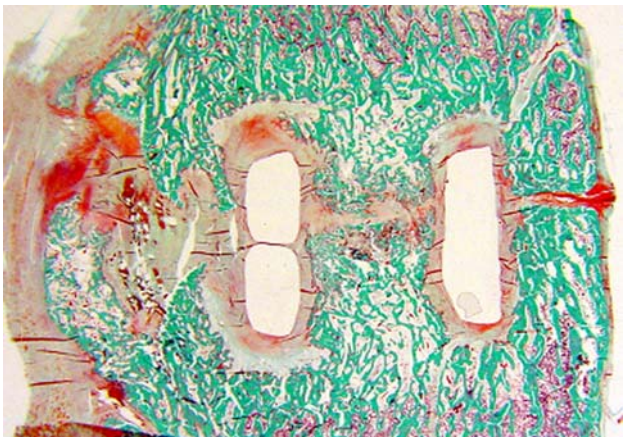
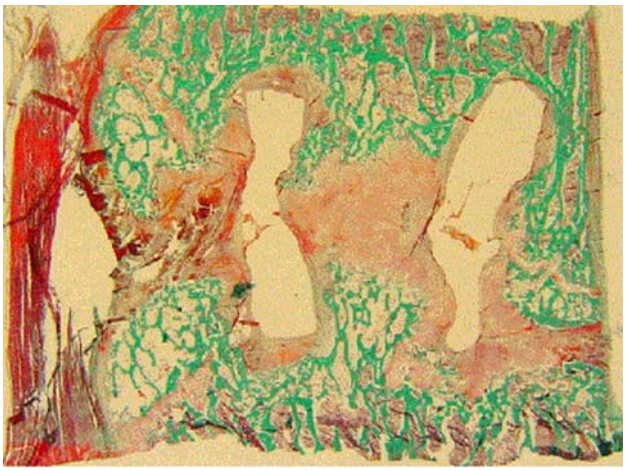


Fig. 6 Specimen with a PLLA cage after four years of follow-up. Left: radiograph showing sound fusion and bone at the site where the cage used to be. Middle: overview of the sawn specimen, no PLLA is found anymore, as confirmed by histology (right)



(a)



(b)

Fig. 7 Histology of specimens with a PLDLLA cage. (a) At three months, the cages no longer contain bone graft (compare with PLLA at thre months, Fig 5 (a)). Instead, newly formed bone (green) almost fills the entire cage, leaving a thin layer of fibrous tissue between the bone fronts (pink). The cage itself is surrounded by a thick layer of fibrous tissue (pink). (b) Three out of six specimens showed more fibro-cartilage and less bone ingrowth at six than at three months, suggesting the development of a pseudo-arthrosis. The other three specimens showed a sound fusion similar to the one shown in Fig. 5

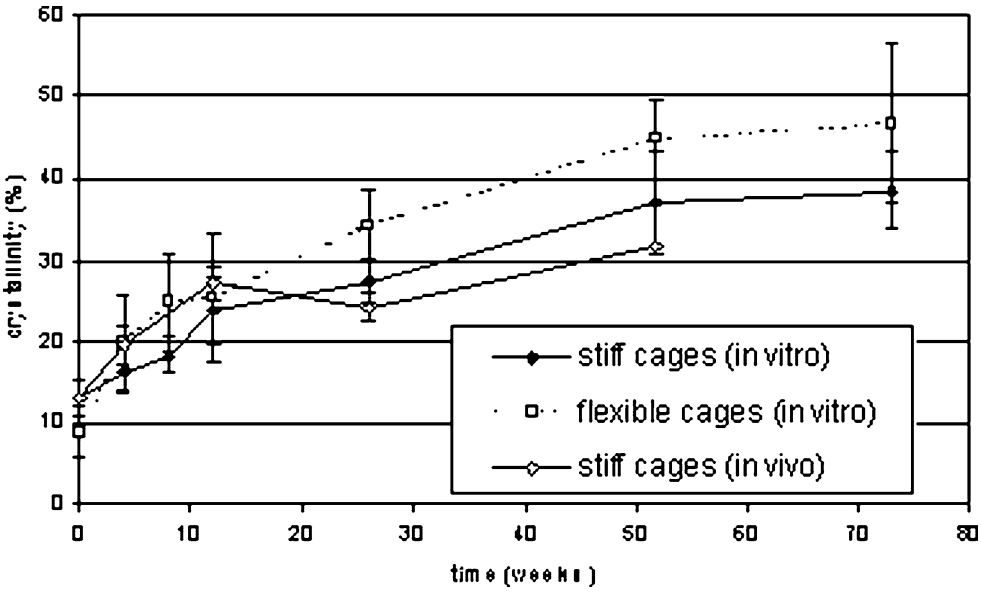


Fig. 8 Crystallinity in the PLLA samples as a function of degradation time. Note that the *in vitro* and *in vivo* crystallinities were comparable

ficient [30]. At six months, only three out of six specimens showed fusion, despite the fact that there was more new bone in the cages after three months. The cages clearly had lost mechanical integrity and strength (Fig. 7(b)). The non-fusions showed more fibro-cartilage than after three months, indicating the formation of a pseudo-arthrosis. After twelve months, only three fusions were found in eight samples, comparable to the results after six months.

In reaction to the premature degradation of the cages, and commensurate to the clinical practice of applying additional internal fixation, we extended the studies with a group of goats provided with PLDLLA cages and additional internal fixation. After six months of follow-up, five out of six ended in a fusion, one in a near fusion (83%). However, chemical analyses of the PLDLLA cages gave no difference in degradation between the stand-alone cages and the cages used in combination with an internal fixator (as yet unpublished results).

The PLLA cages showed some minor cracks after three and six months, but overall maintained their mechanical integrity until fusion occurred. After twelve months, all cages had disintegrated, and their mechanical function was entirely lost and taken over by the trabecular bone bridge. Inherent viscosity of the PLLA material decreased faster than *in vitro* [27], and crystallinity increased from 10% to more than 40%, comparable to the crystallinity found at *in vitro* degradation (Fig. 8). Subsequent resorption evoked mild inflammation in some of the specimens, but there were no severe adverse tissue reactions until complete resorption [29].

The PLDLLA cages already showed numerous microcracks after three months of implantation, and failures with major plastic deformation after six months [30]. However, all physico-chemical parameters (inherent viscosity, glass transition temperature, crystallinity, molecular weight) showed similar degradation profiles as samples tested earlier *in vitro* (preliminary data, as yet unpublished). At twelve months, the PLDLLA cages were fully disintegrated. Magnetic resonance imaging (MRI) performed at these specimens showed the presence of free (unbound) water molecules inside the cage, confirming that water has penetrated throughout the cage material and that integrity was lost; this was not the case with cages scanned after three or six months follow-up [30].

4 Discussion

Intervertebral spinal fusion requires a temporary stabilization of the spinal segment in order to allow new bone formation between two vertebral bodies. Intervertebral cages are efficient implants for this purpose, because they are small and placed in line with the axial compression component of the spinal load. Spinal cages are quite successful on the short term, but late complications are being reported more frequently over the last years. Biodegradable implants could prevent such complications, but should provide sufficient primary stability for spinal fusion to occur. We explored two types of polylactide for this purpose: a pure poly(L-Lactic acid) (PLLA), and a 70/30 mixture of poly(L- and DL-lactic acid) (PLDLLA). Both materials showed sufficient strength (more than 3.5 kN) for at least six months *in vitro*, but *in vivo* PLDLLA appeared to lose mechanical stability too early, thereby causing the formation of pseudo-arthroses. This problem could be overcome by adding internal fixation. Both materials had excellent biocompatibility.

The choice for both materials was well considered: PLLA was chosen because high strength and slow resorption were demanded. High molecular weight and crystallinity are well-known factors that contribute to meet these specifications. Crystallinity, on the other hand, is often held responsible for late adverse tissue reactions [e.g. 31]. In fact, it is commonly given as a reason for using co-polymers with D-lactic acids, which are amorphous and have crystallinity close to zero. The 70/30 mixture of PLLA and racemic (50/50) poly(D/L-lactic acid) used here combined good initial mechanical properties with low crystallinity. However, it must be emphasized, that although the amorphousness of PLDLLA is regularly presented as more biocompatible than pure PLLA, there is no substantial evidence for that in literature: in fact, studies addressing this topic [32, 33] as well as our own studies presented here, indicate that crystallinity is not an important factor in biocompatibility. In the case of our own studies, it might be argued that the cage was implanted in a well-vascularized environment, thereby preventing the accumulation of crystalline particles and reducing the risk of adverse tissue reactions. On the other hand, this could also be interpreted as a recommendation for the proper use of PLLA implants.

The results with the PLLA cages after six months and longer proof the feasibility of the concept of bioresorbable cages: they are able to provide sufficient stability until fusion is complete, and then resorb without adverse tissue reactions. They also show that the fusion process is faster and more consistent than in titanium cages, suggesting that stress shielding is a relevant factor in the healing process indeed. The titanium cages showed a similar histological picture as found in the clinical retrieval, which indicates that the goat model, although a quadruped, is a valuable and maybe even valid model for spinal fusion.

The PLDLLA cages showed faster degradation and therefore failure, but also a faster fusion process. Fast degradation was not expected, because the *in vitro* degradation profiles suggested sufficient strength for at least six months. A possible explanation is that the cages *in vivo* suffered from dynamical loading, whereas the *in vitro* degradation profile was determined at unloaded conditions. Indeed, dynamic loading has been suggested to enhance degradation [19, 29, 34]. However, our chemical analyses on the PLDLLA cages in stand-alone condition and combined with internal fixation, showed no difference in degradation rate, suggesting that dynamic loading does *not* affect cage degradation. Alternatively, the very fact that the material has been used *in vivo* may provide some explanation; phenomena like adsorption of proteins, absorption of lipids, and greater solubility of lactic acid-based oligomers in blood are examples of sources of difference in degradation rate *in vitro* and *in vivo* of the same material [20, 35]. The enhanced PLDLLA degradation rate could also be explained by the way the implants were sterilized. MacroPore's certified and standard way of sterilizing is e-beam: this method is popular because it is quick, safe, and relatively cheap. At the beginning of our studies e-beam was considered not harmful to the polymer. However, we now know that e-beam degrades polymers just by radiation [36]. By contrast, ethylen oxide (EtO) and plasma are much more friendly to polymers, which leaves them with longer polymer chains and thus better mechanical properties [36]. Studies with EtO sterilized PLDLLA cages are now being performed.

Premature degradation of the cage had a profound influence on the spinal fusion process. As mechanical stability was lost, the fusion process was frustrated and ended up in a pseudo-arthrosis in four out of six specimens. This is even more remarkable when considering the situation at three months, where cages were filled with new bone for 80–90%. Whether this is only due to the loss of stability is unclear: the fact that all bone graft had been resorbed at three months in the PLDLLA cages and not in the PLLA cages, suggests that the degradation of the polymer itself may contribute as well. Lactic acids lower the pH of the environment, and osteoclasts-cells that are responsible for bone resorption- become more active at lower pH [37]. Faster graft resorption also allows for faster bone formation, because osteoblasts are no longer impeded by the graft and the osteoclasts. This is a hypothetical explanation for our observations, which needs further investigation in the future. The loss of stability by cage degradation were overcome by adding an additional internal fixation. Despite the fact that a metal rod was placed parallel to the fusion zone, bone growth was quicker and more consistent than in the titanium controls. Considering the clinical practice of adding internal fixation with interbody fusion, PLDLLA still can be considered as a good candidate for bioresorbable cages.

Summarizing, we evaluated two types of polylactides for use in interbody cages for spinal fusion. Although the materials showed similar initial mechanical properties and degradation profiles, actual *in vivo* degradation differed considerably, which affected the fusion process. Nevertheless, short-term and long-term results showed that the concept of bioresorbable cages is feasible. However, if degradation is too fast, the healing process may be frustrated and end up in a pseudo-arthrosis. It may be desirable to add an additional internal fixator in order to prevent such complications.

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