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DISSERTATION

The Dose-Dependent Effect of Bone Morphogenetic Protein 2 on Spinal Fusion Status and Adverse Effects in Right-Lateral Lumbar Interbody Fusion in the Sheep Model

A Comparative CT-Morphological Evaluation

zur Erlangung des akademischen Grades

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Contents

Zusammenfassung

Diese Studie entstand im Anschluss an eine klinische Studie, in der rhBMP-2 "off-label" für posteriore lumbale Wirbelkörperfusion (PLIF) angewendet wurde. In den Computertomographie-Bildern 3 Monate postoperativ zeigte sich Knochenresorption an den Wirbelkörperdeckplatten. Es wurde vermutet, dass die Knochenresorption aufgrund einer gekoppelten Aktivität von Osteoklasten und Osteoblasten, getriggert durch rhBMP-2, auftrat. Außerdem wurde die Hypothese aufgestellt, dass die Knochenresorption zu anderen nachteiligen Auswirkungen führen könnte. Das Ziel dieser Studie war es daher systematisch den Dosis-/Konzentrations-abhängigen Einfluss von rhBMP-2 auf das Fusionsergebnis sowie mögliche nachteilige Auswirkungen nach rechts-lateraler lumbaler Wirbelkörperfusion auf Feinschicht-CT-Bildern zu analysieren.

36 Merino-Schafe wurden einer rechts-lateralen lumbalen Wirbelkörperfusion in Höhe L1-L2 und L3-L4 unterzogen. Sowohl im Kontroll- als auch im Versuchssegment wurde ein PEEK Cage implantiert. In den Versuchssegmenten wurde der Kollagenschwamm mit einer von vier Konzentrationen/Dosen von rhBMP-2 (Gruppe 1: 0.5 mg/ml, 0.5 mg; Gruppe 2: 1.0 mg/ml, 1.0 mg; Gruppe 3: 2.0 mg/ml, 2.0 mg; Pilotstudie: 4.0 mg/ml, 4 mg) gefüllt. Im Kontrollsegment blieb dieser entweder leer oder wurde mit einem Kollagenschwamm gefüllt. Ein dorsales Schrauben-Stab-System wurde in allen Operationssegmenten implantiert. Feinschicht-CT-Bilder wurden direkt postoperativ sowie 3 Monate, 6 Monate und 12 Monate nach dem Eingriff angefertigt, um den Fusionsstatus und mögliche nachteilige Auswirkungen von rhBMP-2 zu beurteilen.

Im Vergleich zur Kontrollgruppe zeigte sich in den Versuchssegmenten eine höhere Anzahl CT-morphologischer Fusionen, jedoch einhergehend mit einer höheren Inzidenz von nachteiligen Auswirkungen. In jeweils zwei Segmenten der Gruppe 1 und Gruppe 3 wurden erhebliche Knochenresorption an den Deckplatten und ektope Knochenbildung, vor allem an der rechts-lateralen Seite des Wirbelkörpers (operativer Zugang) beobachtet. Es zeigte sich, dass die Cage-Form ungünstig war, denn viele Cages wanderten, selbst in der Kontrollgruppe.

Insgesamt zeigten sich in den rhBMP-2 Gruppen bessere Fusionsergebnisse verglichen mit der Kontrollgruppe. Jedoch zeigten sich in dieser Gruppe auch teils schwerwiegende nachteilige Auswirkungen. Mit der niedrigsten Dosierung von 0.5 mg/ml konnten gute

Fusionsergebnisse bei reduzierter Anzahl von nachteiligen Auswirkungen erreicht werden. Zusammenfassend konnten durch keine der verwendeten Konzentrationen/ Dosierungen von rhBMP-2 nachteilige Auswirkungen gänzlich vermieden werden.

Abstract

This thesis was initiated subsequent to a previous clinical study in which rhBMP-2 was used "off-label" for posterior lumbar interbody fusion. In the present study, bone resorption at the endplates was observed 3 months after surgery and was associated with a coupled action of osteoblasts and osteoclasts triggered by the growth factor. It was suggested that the resorptive defects risk other adverse effects. It was therefore the aim of this thesis to suggest a protocol to systematically analyze computed tomography images for the fusion status and adverse effects after right-lateral lumbar interbody fusion in a sheep model. Additionally, the dose-/concentration-dependent effect of rhBMP-2 on fusion status and incidence of adverse side effects compared to intraindividual controls was analyzed.

Thirty-six Merino sheep underwent right-lateral lumbar interbody fusion at L1-L2 and L3-L4 either with the addition of a PEEK (polyetheretherketone) cage which was filled either with one of four different concentrations/doses of rhBMP-2 in the intervention groups (group 1: 0.5 mg/mL, 0.5 mg; group 2: 1.0 mg/mL, 1.0 mg; group 3: 2.0 mg/ml, 2.0 mg; pilot study group: 4.0 mg/mL, 4 mg) or with an ACS (absorbable collagen sponge) only or left empty in the control group. A pedicle-screw system was attached at all surgical levels. Thin-cut CT images were taken directly postoperatively, after 3 months, after 6 months and after 12 months to assess fusion status and adverse effects.

In comparison with the control group, rhBMP-2 groups showed a higher CT-morphological fusion rate but also a higher incidence of adverse effects. Four cases of severe bone resorption at the endplates were observed in the intervention groups. Overzealous bone growth was frequently present mainly at the right-lateral side (site of surgical access) of the vertebral body. The cage design was probably unfavorable because many cages migrated, even in the control group.

In conclusion, in this animal model the application of rhBMP-2 in four different concentrations/doses showed superior fusion results in comparison to the control group but was also associated with a higher incidence of partly tremendous complications. None of the doses/concentrations used was found to completely eliminate the risk for adverse effects.

Abbreviations

3D	three-dimensional
ACS	absorbable collagen sponge
ALIF	anterior lumbar interbody fusion
DBM	demineralized bone matrix
DDD	degenerative disc disease
EMA	European Medicines Agency
FDA	Food and Drug Administration
ICBG	iliac crest bone graft
IPD	individual participant data
IVD	intervertebral disc
MPR	multiplanar reconstruction
NIS	American Nationwide Inpatient Service
OxPEKK	OPM's branded polyetherketoneketone
PEEK	polyetheretherketone
PLIF	posterior lumbar interbody fusion
rhBMP-2	recombinant human bone morphogenetic protein 2
TLIF	transforaminal lumbar interbody fusion
XLIF	extreme lateral lumbar interbody fusion

List of Figures and Tables

1   Introduction

1.1   Epidemiology of Spondylodesis in the Caucasian Population

Spinal fusion (spondylodesis) is the *ultima ratio* and surgical state of the art to treat several disorders of the spine:

- Degenerative disc disease (DDD)
- Discogenic pain
- Vertebral disc herniation
- Vertebral fracture
- Spondylolisthesis
- Spinal instability
- Spinal canal stenosis

Degeneration of the vertebral discs is a natural process of aging associated with dehydration and desiccation of the disc material. DDD is most pronounced in the moving sections of the spine, the cervical and lumbar levels, because these segments are especially susceptible to "wear and tear." In the aging process the annulus of the disc (outer part) can be damaged by small tears and the intervertebral discs wear away and shrink (1). The resulting loss of disc height and unphysiological motion can lead to neurological deficits and severe pain by compression of exiting spinal nerve roots. If a patient's symptoms persist despite conservative therapy (e.g. physiotherapy and pain management) for more than six months, a surgical intervention can be considered. Consequently, spondylodesis is commonly performed on the lumbar vertebral segments.

During spondylodesis two or more vertebral bodies (vertebrae) are fused and the mobile disc segment is immobilized to eliminate unphysiological movements. For the clinical outcome it is decisive to restore the disc space height as well as the spinal alignment. Each vertebral body is composed of dense cancellous endplates on the outside and less dense sintered cancellous bone on the inside (cf. Figure 1) (2). The intervertebral disc is attached to these endplates and keeps space between the vertebrae, allows movement and acts as a "shock absorber" for mechanical forces during physical activities (3). A broad spectrum of surgical techniques for spondylodesis exists.
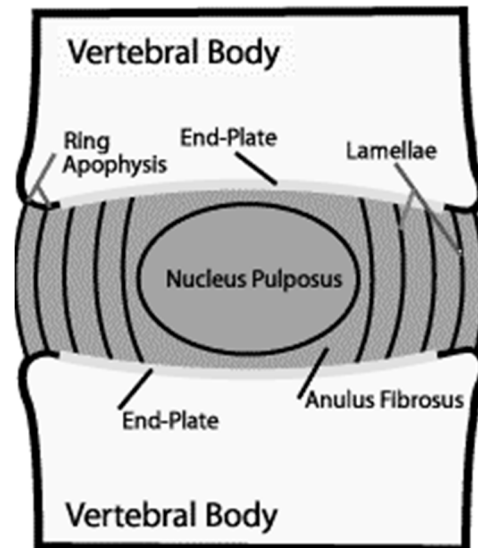
Figure 1. Sagittal view of the motion segment. Adapted from RAD for JIEUN
http://radforjieun.blogspot.de/2012/03/disc-anatomy-chirogeek.html (Accessed August 1, 2014).

For posterolateral fusion the transverse processes (cf. Figure 2) of two or more vertebrae are fused. Typically, each vertebral body has a transverse process at each lateral side to which muscles and ligaments of the spine are attached. Transverse processes of adjacent vertebrae are connected by intertransverse ligaments (4).

Another commonly performed technique for spondylodesis is spinal interbody fusion. The long-term aim of this procedure is to stabilize the spine by a bone column fusing the endplates of adjacent vertebrae. Intraoperatively, the intervertebral disc (nucleus pulposus and annulus fibrosus) is removed and it takes 6 to 12 months until a solid bone bridge is formed (5). An implant (cage) is usually placed between adjacent endplates to serve as a spacer and to help maintain spinal alignment. Many different cage designs (e.g. materials and shapes) are commercially available.

For interbody fusion the spine can be accessed via various operative approaches: anterior (ALIF), posterior (PLIF), transforaminal (TLIF), or transpsoas/extreme lateral (XLIF).
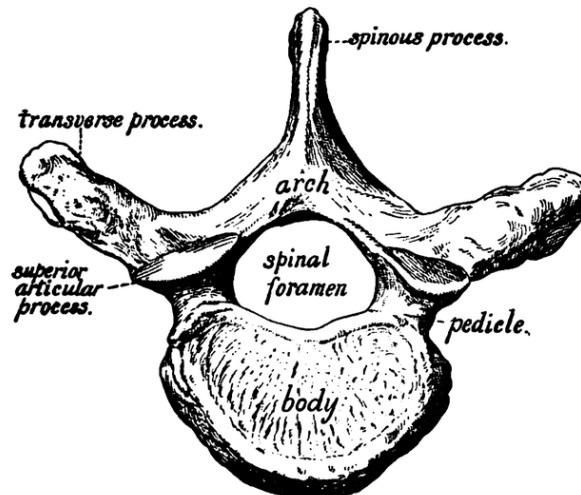
FIG. 1.—A vertebra seen from above.

Figure 2. A vertebra seen from above. From: Sobotta, J. (1909) Sobotta Atlas and Text-book of Human Anatomy (Sobotta 1909).

Dorsal fixation of the spine by a pedicle screw and rod system provides additional postoperative spinal stabilization (7). The pedicle screws in two or more consecutive spine segments are linked by bilateral rods to act as firm anchor points. The pedicles are bilateral tubular bony structures which connect the vertebral body to the vertebral arch on each side (cf. Figure 2). They protect the spinal cord and nerve roots and they are the surgical entry point in posterior spinal fusion procedures. Dorsal fixation improves immediate postsurgical stability until a solid (interbody) fusion has formed.

Although there are many variations of spinal fusion surgery the procedure commonly involves the following processes:

- Adding osteoinductive material (e.g. bone grafts, biologicals)
- Promote/trigger a biological/metabolic response that causes osteogenesis
- Create a fused bone bridge that replaces the mobile intervertebral disc (symphysis) and therefore eliminates unphysiological and painful motion

It is the gold standard for spinal fusion to use bone tissue, either from the patient (autograft, e.g. locally harvested bone or an iliac crest bone graft) or a donor (allograft) to promote new bone growth (8). Unfortunately, in unfavorable settings – e.g. smokers, diabetics, revision surgery, patients suffering from osteoporosis – a relatively high non-fusion rate is observed (9).

When a minimally invasive surgical approach to the spine is applied, the amount of locally harvested bone is minimal. Additionally, new fusion materials are developed to improve the fusion rate. Biologicals (e.g. rhBMP-2) are an attractive osteoinductive material in these situations. RhBMP-2 is commercially available for certain interbody fusion surgeries since 2002 (10).

1.2 RhBMP-2 in Lumbar Interbody Spinal Fusion – From Discovery to Widespread Clinical (Off-Label) Application

The name bone morphogenetic protein was coined by Marshall R. Urist in 1971 (11). He discovered in 1965 that several proteins constitute the active components responsible for bone regeneration. Urist obtained these proteins by purifying demineralized bone matrix (DBM) and thus made the key discovery for the use of BMP-2 as an osteoinductive agent. His work was a milestone for regenerative medicine (11). However, the amount of BMP-2 which could be isolated from native bone extracts yielded only a BMP dose of 1-2 µg/kg. For its clinical application larger quantities were needed. Therefore, another milestone was reached in the 80s and early 90s when BMP genes were cloned and biologically potent recombinant BMPs could be produced in large amounts (12).

Further research discovered that BMPs are a group of cytokines which belong to the TGFβ superfamily. They are involved in manifold signal cascades in the human body. Most – but not all – BMPs play a role in bone growth. Chen et al. (13) report from findings in animals and humans with genetic mutations in BMPs and related genes as well as studies on transgenic and knockout mice that BMP signaling plays critical roles in heart, neural and cartilage development. Thus, Reddi (14) proposed to rename BMPs as "body morphogenetic proteins." Further research is needed to determine the exact mechanism of action of BMPs and to discover even more critical roles of BMPs.

In bone, BMPs are produced by osteoprogenitor cells, osteoblasts, chondrocytes and platelets (15). The effect of BMPs depends on many factors, e.g. the target cell type, the local concentration of BMPs, interactions with other secreted cytokines, and the carrier material. Along with other cytokines and matrix components BMPs induce a cascade of cellular events necessary for bone repair and osteogenesis: chemotaxis, migration, proliferation, and differentiation of mesenchymal stem cells (12). It is known that BMPs

are involved in chondrogenesis, osteogenesis, angiogenesis and the controlled synthesis of extracellular matrix (16).

In 2002 rhBMP-2 was approved as an adjunct for spinal interbody fusion by the Food and Drug Association (FDA) in the USA as "InFuse™ Bone Graft" in combination with the "LT Cage™ Lumbar Tapered Fusion Device" (Medtronic Sofamor Danek, Inc., Memphis, TN, USA; cf. Figure 3). The approval was granted for "[…] spinal fusion procedures in skeletally mature patients with degenerative disc disease (DDD) at one level from L4-S1. [And it] is to be implanted via an anterior open or an anterior laparoscopic approach" (17).
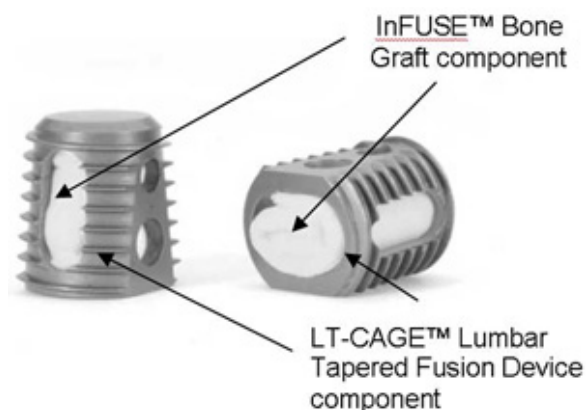
Figure 3. InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device, FDA - Recently Approved Devices (17).

In the European Union, rhBMP-2 is commercially available and approved for single-level anterior lumbar spinal fusion in adults by the European Medicines Agency (EMA) as InductOs® since 2005 (18).

Cited advantages of rhBMP-2 as a bone substitute were (19):

- Elimination of bone graft harvesting pain and complications
- Significantly higher fusion rates
- Shorter surgical time
- Less blood loss
- Shorter hospital stay with earlier mobilization
- Lower reoperation rates (less nonunions)
- Lower median time to normal activities including return to work

Initially published prospective multicenter clinical studies reported superior clinical outcomes with rhBMP-2 compared to the use of an iliac crest bone graft (ICBG). Additionally, unlike autologous bone grafts, rhBMP-2 is available in unlimited quantities and avoids donor site morbidity and longer operation time due to bone harvesting. Hecht et al. (20) report iliac crest bone harvest as the gold standard and cite an associated complication rate of 8-25% (e.g. pain, vessel injury, nerve injury, peritoneal perforation). Furthermore, they criticize that in many cases the amount of bone graft available is insufficient. RhBMP-2 promised to decrease the number of nonunions compared to autologous or allogenous bone grafts. Nonunions (sometimes called "pseudarthrosis") are feared by spine surgeons because they are difficult to treat and regularly require revision surgery. Sandhu (21) summarizes that rhBMP-2 is attractive as a bone substitute because it was shown to lead to "Higher fusion rates, shorter operative times, and shorter hospital stays."

Economic considerations are another important factor regarding the use of rhBMP-2. Although the commercially available Infuse® small and medium kits cost around $1,500 to $3,000, some studies suggest that the use of rhBMP-2 for spinal fusion might be more cost effective than ICBG. The upfront price is reported to be offset "[…] by reduced productivity loss due to faster return-to-work time for patients treated with rhBMP-2" (22).

In conclusion, the initial reports on outstanding results after rhBMP-2 application encouraged its widespread (off-label) use for spinal fusion in only a decade. Many articles published shortly after the FDA-approval supported the effectiveness and safety of Infuse®. In 2004 additional FDA approval was received for a trauma indication, the treatment of acute, open tibial shaft fracture stabilized with intermedullary nail fixation after appropriate wound management in skeletally mature patients (23). In 2007 Infuse® was also approved as an autogenous bone graft alternative in sinus augmentations and for localized alveolar ridge augmentations of defects associated with extraction sockets (24).

1.3   Infuse® in a Decade – From FDA-Approval to Reports of Severe Complications

Initially available industry-sponsored trial publications concluded that rhBMP-2 reliably enhanced spinal fusion while reporting no (intervention-related) complications (25,26).

On the other hand, the alternative procedure using an autologous iliac crest bone graft as osteoinductive material was reported to cause severe donor site morbidity in these studies. However, these reports neglected to mention that in many cases locally available autologous bone could be harvested intraoperatively without causing any additional morbidity (27–29). The published data encouraged further – often off-label – application of Infuse® (27,28,30–35). By 2007 Infuse® was used for more than 50% of primary ALIF, 43% of PLIF/TLIF, and 30% of PLF in the USA (36,37).

Since 2006, non-sponsored studies reported adverse events related to the use of rhBMP-2 in spinal fusion surgery (38–40). In view of these findings, the FDA issued a Public Health Notification on potentially life-threatening complications (swelling of the neck and throat) associated with the (off-label) use of Infuse® in cervical spinal fusion in 2008 (41). In the same year EMA warned that rhBMP-2 leads to a higher incidence of local infections compared to the standard of care if used in reamed nail fixation in acute open tibia fractures (18). In 2009, a publication on rhBMP-2 was retracted from a peer-reviewed journal because of research misconduct, possible fraud and financial ties to Medtronic of the author (42,43). A subsequent review of publicly available data conducted by Carragee et al. (25) suggested an increased risk for complications and adverse events in patients receiving rhBMP-2 compared to the control group (usually iliac crest bone graft). Finally, in 2011 the Yale University Open Data Access (YODA) project was initiated (44) in an agreement with Medtronic. It was established to independently report on the safety and efficacy of Infuse®. Access to full individual participant data and to internal reports of all Medtronic studies on rhBMP-2/Infuse® in spinal fusion surgery was granted for an independent reanalysis.

It became obvious that early journal publications misrepresented the effectiveness and harm through selective reporting, duplicate publication, and underreporting (26). Furthermore, it was revealed that the authors of leading clinical studies had lucrative financial ties with Medtronic and were likely biased (44).

In the clinical off-label use of InductOs® for posterior lumbar interbody fusion (PLIF), Meisel et al. (45) observed zones of bone resorption on the follow-up CT scans. Transient bone resorption was already associated with the use of BMPs in the late 1990s by several authors (46,47). This phenomenon seems to be best explained by the role of BMPs in bone turnover via coupled osteoclastic (bone resorbing) and osteoblastic (bone forming)

activity (cf. Figure 4) (48). Transient bone resorption in spondylodesis risks adverse side effects, e.g. cage migration and cage subsidence.
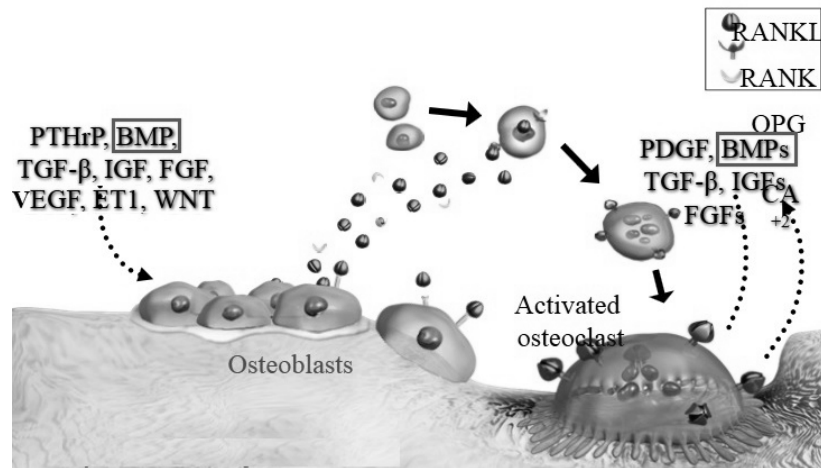
Figure 4. Coupled action of osteoclasts and osteoblasts influenced by BMPs. Adapted from Pecherstorfer, M. "Wie entstehen Knochenmetastasen." August 1, 2014. Available at http://slideplayer.de/slide/867519.

Although rhBMP-2 has been approved by the FDA in 2002 for application in spinal fusion, uncertainty about its clinical safety and effectiveness currently exists. Fu et al. (26) even conclude in their recent review that "[…] rhBMP-2 has no proven clinical advantage over bone graft and may be associated with important harms, making it difficult to identify clear indications for rhBMP-2."

Unfortunately, the present study cannot answer all questions regarding safety and effectiveness of Infuse® remaining after the publication of the YODA project results (26,49). Still, it contributes to the existing data by suggesting a protocol to systematically evaluate adverse effects associated with the application of rhBMP-2 on CT images in the follow-up and therefore adds important insight.

1.4 Dose-Dependent Effect of RhBMP-2

After first reports on adverse side effects associated with the application of rhBMP-2 for spinal interbody fusion, it was suggested that the effect of the protein is dose-dependent. As Sethi et al. (50) point out "The biological response to BMP-2 is probably related to dose; however, the optimal dose is not known." Knox et al. (51) argue that the coupled

osteoclastic bone absorption and bone formation by osteoblasts shows a dose-related behavior. However, due to a lack of data it is not known whether the adverse side effects could be avoided by the application of lower rhBMP-2 doses. Vaidya et al. (52) conclude that endplate resorption occurs – as part of the natural process of osteogenesis – in all patients who undergo rhBMP-2-assisted interbody fusion. Toth et al. (53) found in a study on ovine cancellous bone of the femur "[…] that increasing the local rhBMP-2 concentration by overfilling the defect with rhBMP-2/ACS or hyper-concentrating the rhBMP-2 solution on the absorbable collagen sponge led to a concentration-dependent increase in peri-implant cancellous bone resorption at 1 week." This finding is consistent with the publication of Poynton and Lane (54) who argue that bone resorption by osteoclasts occurs before bone formation by osteoblasts – like in fracture healing – and that "[…] large doses of BMP may lead to localized areas of resorption."

Although Toth et al. (53) report that a concentration of 0.43 mg/mL of rhBMP-2 on ACS is considered the "normal concentration" in the sheep, they observed transient bone resorption in their ovine corticocancellous defect model even at this dose. They state in response to a letter to the editor of SPINE that "[Their model of cancellous femur bone] might simulate the worst-case scenario in the spine in which significant exposure to a cancellous bone environment occurs due to extensive decortication of the endplates" (53). Therefore, the concentration of rhBMP-2 might not be the only factor influencing the amount of bone resorption.

Fu et al. (26) clearly summarize in their review that the available data is insufficient to evaluate the effect of the dose on the effectiveness and harm of Infuse®. In conclusion, it is important to better understand the mechanism of action and dose-dependent effect of rhBMP-2 in spinal fusion.

1.5   Computed Tomography as an Evaluation Tool

Computed tomography (CT) scan imaging is commonly used in the clinical setting to assess bone structures. The present study was designed to analyze the dose-dependent effect of rhBMP-2 in lumbar spinal fusion at several time points in vivo.
Although many preclinical studies concentrate on the fusion status, no study routinely used CT scan imaging for a systematic assessment of adverse side effects (e.g. bone resorption, heterotopic/overzealous bone growth, cage displacement, cage subsidence,

and disc space height maintenance). Fu et al. (26) conclude in their systematic review that adverse events were generally not actively elicited in studies on rhBMP-2.

Overall, thin-cut CT images provide many clinical advantages compared to radiographs:
- Exquisite bone detail and high resolution
- High-quality reformatted images in the coronal and sagittal planes
- Rapidity

Therefore, CT imaging was chosen as the preferred method to assess the fusion status and possible adverse side effects in vivo.

1.6 Aim of the Study

The present thesis is part of a major project which was initiated in 2006 subsequent to a clinical study on InductOs® for PLIF in 17 patients with lumbar DDD (45). Follow-up computed tomography images showed bone resorption ("transient decrease in bone density") at the vertebral endplates of the interventional levels in all patients 3 months after surgery. These zones of bone resorption resulted in cage subsidence. Fortunately, the patients did not suffer from any neurological deficits. Nevertheless, transient bone resorption may lead to transient instability of the fused spine segment and adverse side effects.

Thus, it was the aim of the present study to analyze the dose-dependent effect of rhBMP-2 on lumbar spinal interbody fusion status and frequency of associated adverse side effects and to compare the results to a control group in the sheep model (BMBF: PtJ-Bio, 0315883). We hypothesized that the application of rhBMP-2 in lumbar interbody fusion leads to a higher incidence of adverse side effects, e.g. PEEK cage subsidence, cage displacement, or overzealous bone growth, compared to the control group due to (transient) vertebral bone resorption.

Another focus of the present study was to systematically look for and analyze potential risks associated with the application of rhBMP-2 in spinal lumbar interbody fusion on reformatted thin-cut CT images.

In conclusion, our study is the only prospective controlled trial available at this time which focuses on adverse side effects associated with the application of various doses of

rhBMP-2 in lumbar spinal interbody fusion. It is important to determine whether the clinical observation of bone resorption (45) is reproducible in a controlled animal study and to find CT-morphological criteria to evaluate the overall quality of lumbar interbody fusion when rhBMP-2 is applied.

2   Materials and Methods

2.1   Study Design

In the present study a two-level (L1-2 and L3-4) right-lateral lumbar interbody fusion (ALIF) using polyetheretherketone (PEEK medical grade, Instrumentmakerij FMT, sectie ontwikkeling, VU medisch centrum) interbody spacers with additional dorsal fixation (Xia® 4.5 Spinal System, Stryker Spine) was performed. All experiments were conducted in collaboration with the Translational Centre for Regenerative Medicine (TRM) Leipzig, University of Leipzig (project number 1026AB).

In the intervention group, the PEEK cage was filled with a rhBMP-2-soaked collagen sponge (InductOs®, Medtronic Sofamor Danek, Inc. Minneapolis, MN). In the ovine model, the application of rhBMP-2 in a concentration of 0.43 mg/mL has been reported by several authors (55). The concentration and total dose of rhBMP-2 used in the present study in the sheep model was determined based on these reports and clinical experience. RhBMP-2 was applied to the ACS in one of four different concentrations and the animals were randomly allotted to receive a resulting total dose of either 0.5 mg (0.5 mg/mL), 1.0 mg (1.0 mg/mL), 2.0 mg (2.0 mg/mL) or 4.0 mg (4.0 mg/mL). The highest dosing (4.0 mg/mL) was used in a pilot study group comprising only 3 sheep.

In the internal control segment the PEEK cage was either filled with the ACS only (n=17) or left empty (n=17). No osteoinductive material was added in this group.

The interventional and control level were assigned following a randomization protocol (cf. Figure 5) to exclude bias resulting from a difference in physical workload of the different spinal segments. Studies have proposed that interbody fusion increases mechanical stress on the spine and accelerates degeneration at adjacent levels (56–58). The L2-3 motion segment between the treated segments was thus left intact to reduce this effect. The animals were randomly assigned to three different postoperative survival periods: 3 months, 6 months and 24 months. At each of these time points three to four animals of each dosing group were sacrificed for histomorphological analysis (results discussed elsewhere). Therefore, the number of animals and number of levels to be assessed by CT decreased over time (drop-outs). The results of the histomorphological analyses will be discussed elsewhere.

Computed tomography was performed in vivo under general anesthesia directly postoperatively, after 3 months, after 6 months, after 12 months, and after 24 months. Analyses were conducted by a single person blinded to the interventional and control levels.
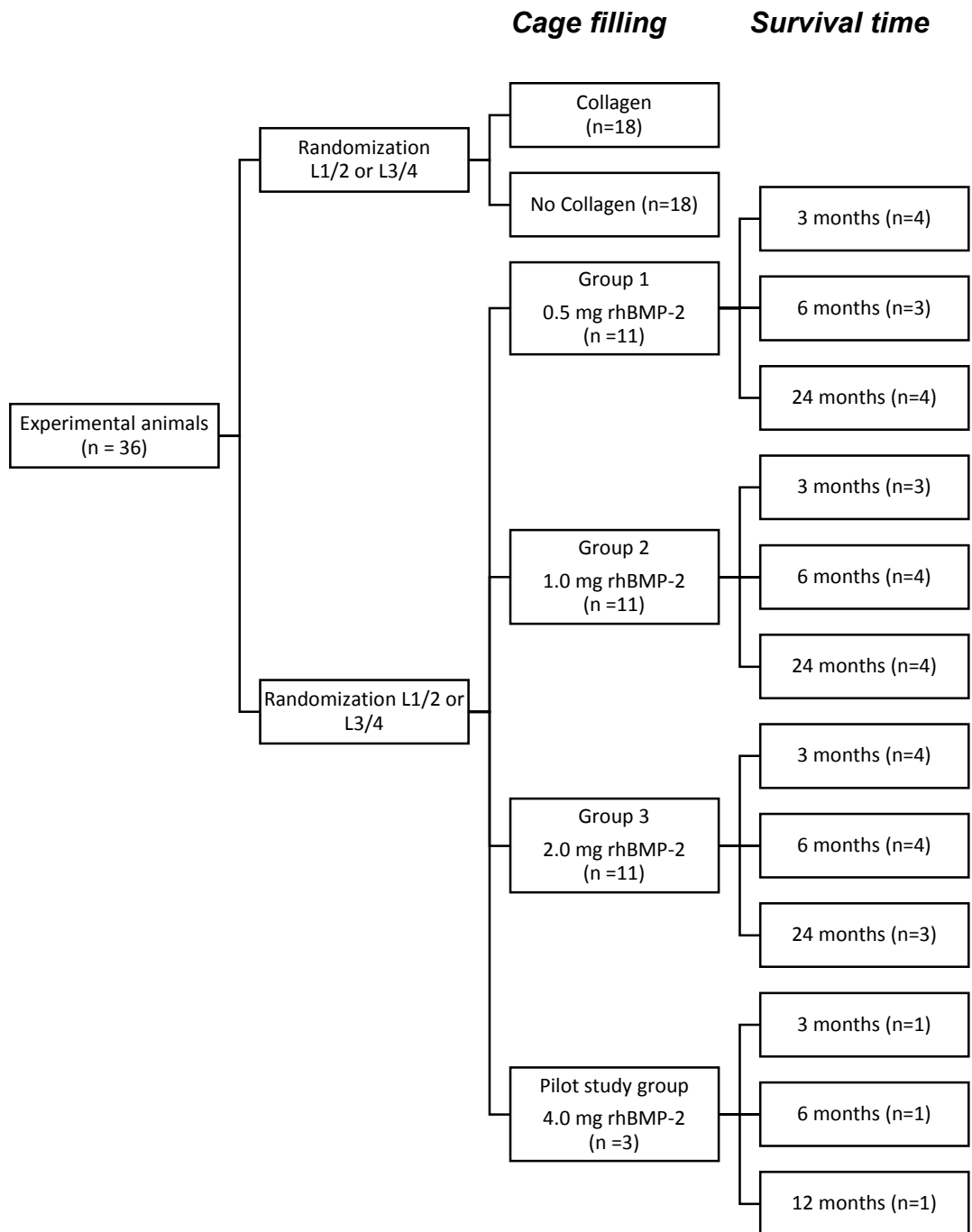
Figure 5. Study protocol showing the randomization protocol and allocation to different groups according to the rhBMP-2 dose applied and time of follow-up.

2.2 Experimental Animals

The experiments were performed on thirty-six 2-year-old female Merino sheep with an average weight of 57 ± 5.2 kg. The sheep were purchased from a local sheep farm and initially examined by a veterinarian to ensure good health. The animals were fed standard chow and tap water ad libitum.

The surgical intervention and follow-up examinations were performed at the Large Animal Surgical Clinic of the University Faculty of Veterinary Medicine Leipzig. The experiments were carried out in accordance with the Animal Welfare Act (§ 8 Abs. 1 Tierschutzgesetz (TierSchG)). The design of the surgery was critically reviewed and approved by the local legal representative (Landesdirektion Leipzig, Sachsen, Germany; TVV 34/10).

2.3 Cage Design and Material

A rectangular radiolucent polyetheretherketone (PEEK) cage (width x length x height): external measurements 12 mm x 10 mm x 4 mm; internal measurements 8 mm x 6 mm x 4 mm; 2 mm wall thickness; internal volume: 0.19 ml) is inserted into each interventional spine segment.
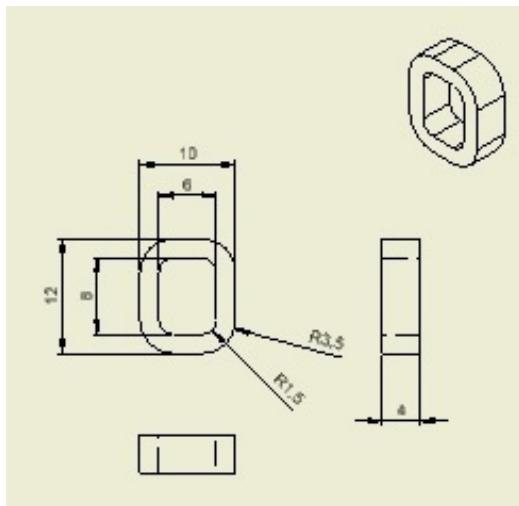
Figure 6. Cage design

The device has flat contact faces on the upper and lower side and a large central opening to allow ingrowth of a solid bone bridge. Instrumented insertion of the cage in the disc space is facilitated by a screwable end cap. The cage is specifically designed and

adjusted to fit the smaller lumbar disc space dimensions of the sheep (PEEK medical grade, Instrumentmakerij FMT, sectie ontwikkeling, VU medisch centrum) and to insert it via a narrow right-lateral approach to the intervertebral disc space. It is fabricated, packaged, and sterilized applying qualified processes identical to those used for commercially available implants. In the present study only one cage per lumbar segment is implanted. Metallic markers to help visualize the radiolucent PEEK cages on CT scans and X-rays were not integrated.

At three levels of the intervention group and four specimens of the control group, a cage made of OxPEKK was used.

2.4 Preparation of InductOs®

InductOs® is a commercially available product containing rhBMP-2 as a bone grafting substitute. It was obtained from Wyeth Europa Ltd (Wyeth Pharmaceuticals, Hants, United Kingdom) as an EMA-approved implant kit (18). The protein is provided in a freeze-dried form and has to be reconstituted with a buffer solution at the time of surgery.

In the present study the rhBMP-2 is reconstituted to three final concentrations (0.5 mg/mL, 1.0 mg/mL, and 2.0 mg/mL) resulting in three different total doses (group 1: 0.5 mg; group 2: 1.0 mg; and group 3: 2.0 mg). All steps are performed using aseptic techniques according to the producer's advice. The appropriate amount of rhBMP-2 is withdrawn from the vial provided and transferred to a sterile vial to obtain the intended concentration. Then, 1.0 ml of the solvent provided is slowly injected into the vial containing the lyophilized rhBMP-2. The vial is gently swirled to aid reconstitution. The absorbable collagen sponge (type I bovine collagen) stripe provided is aseptically transferred from the storage container to a watch glass. In the sterile field the sponge is cut into 1 cm x 5 cm stripes and stored under aseptic conditions. 1.0 ml of the reconstituted rhBMP-2 solution is then applied onto the ACS in a meandering pattern and allowed to bind for at least 15 minutes. Previous studies have demonstrated that 95% of the rhBMP-2 is incorporated into the ACS following a 15-minute soak time (59).

The wetted collagen sponge is rolled using forceps and placed inside the PEEK cage pore. During handling excessive squeezing of the wetted sponge is avoided to prevent oozing of rhBMP-2. Then, the composite is implanted in the prepared disc space.

No additional sponge stripes are placed outside the PEEK cage. The disc space is not sealed with fibrin glue or other sealants.

2.5 Surgery and Animal Sacrifice

2.5.1 Anesthesia and Preparation

The sheep are fasted for 12 hours before surgery and allowed water ad libidum. The animals are sedated by IM administration of xylazine (Rompun® 2%, Bayer Vital GmbH) in a dose of 0.1 mg/kg accompanied by butorphanol (Torbugesic®, Fort Dodge Veterinär GmbH) in a dose of 0.1 mg/kg. As soon as the animal lies down in a lateral position a central venous line is placed into the external jugular vein using a 20 gauge catheter after shearing of the overlying skin and then fixed to the skin with a nylon suture. General anesthesia is induced by an initial intravenous bolus of 4 mg/kg propofol (Propofol 10 mg/mL, Narcofol®, CP-Pharma). The dorsal and lateral lumbar area is sheared. Then, the animals are transferred to the operating room and the surgical field is disinfected and covered with sterile drapes. Supplementary boluses of one-half the initial dose of propofol is given during the procedure if required. The animals are placed on the operating table in a prone position. Supplemental propofol is administered until the swallowing reflex is lost (usually 2-4 mg/kg are necessary). After endotracheal intubation (10 ID), 5% isofluorane in oxygen is insufflated until sufficient depth of anesthesia is reached. During the intervention anesthesia is maintained with isoflurane (2% end-tidal). Analgesic agents are administered via perfusion pump with continuous intravenous injection of 20 µg/kg/h ketamine (Ketavet®, 100 mg/mL, Pharmacia GmbH) and 50 µg/kg/h lidocaine (Lidocainhydrochlorid®, 20 mg/mL, Bela-Pharm GmbH & Co. KG) during surgery.

2.5.2 Surgical Procedure

Right-Lateral Retroperitoneal Approach and Implantation of the Cage

Under general anesthesia the animal is placed on the operating table in the left lateral position. Disc spaces L1-2 and L3-4 are identified under fluoroscopic guidance (mobile C-arm, Philips). Then, the surgical site is shaved and aseptically prepped. A longitudinal incision (length approximately 10 cm) is made on the low back over the spinal levels of

interest. A self-retaining retractor helps to retract the skin edges. The transverse processes are palpated to identify the appropriate spinal levels. Then, the muscles (external and internal oblique muscles) are identified followed by blunt preparation through the fascia and sharp dissection through the oblique muscles. The adequate spinal level is verified by fluoroscopy and disc space L1/2 is identified. Next, sharp dissection of the thoracodorsal fascia from its origin at the transverse processes is performed to access the retroperitoneal space. A soft tissue retractor is used to identify psoas muscle which is dissected from its origin at L1 and L2 vertebral bodies. Hohmann retractors are inserted to protect the soft tissues from injury. Caspar pins are probed in the body of vertebrae L1 and L2. The Caspar retractor is used to achieve sufficient distraction of the disc space.

The annulus fibrosus is incised sharply at both vertebral endplates. Then, the disc material is removed through an incision in the annulus fibrosus using a rongeur. The endplates of vertebrae L1 and L2 are visualized. The remaining disc material and the cartilaginous endplates are carefully curetted. Then, the operating site is carefully irrigated. The intervertebral disc space is sequentially distracted until correct spine alignment is achieved and the PEEK cage filled with the appropriate material is implanted. To avoid a reduced potential of BMP-2 no local antibiotics are used. The distraction is carefully released. A firm cage placement is verified and the Caspar retractor and pins are removed. Then, the incisions are closed with a routine multilayer closure. First, a subcutaneous suture is run. Then, the skin is closed with surgical staples. Lastly, a spray dressing is applied. The identical procedure is repeated on level L3-4.

<u>Dorsal Approach for Implantation of the Pedicle Screw Fixation System (Xia 4.5, Stryker GmbH & Co. KG)</u>

A dorsal fixation system is used to stabilize the fused segment. The animal is carefully transferred into a prone position with pillows placed under the thorax and pelvis. The disc levels L1-2 and L3-4 are identified with fluoroscopy. The surgical field is shaved and aseptically prepped. A longitudinal median incision is made over the spinous processes. Then, the self-retaining retractor is inserted to retract the skin edges. The intervertebral level L1-2 is localized in the anterior-posterior view via fluoroscopy. The thoracodorsal fascia is incised bilaterally at the spinous processes and the paravertebral musculature is cautiously detached. The pedicles of vertebrae L1 and L2 are visualized in the anterior-

posterior view. An awl is used to prepare the screw path in the pedicle of vertebra L1. Kirschner wires, guided by fluoroscopy, are advanced in the pedicle to the appropriate depth. The Kirschner wires are removed and a pedicle probe is used to develop a path for the screw through the cancellous bone of the pedicle into the vertebral body L1. The pedicle screw (Xia® 4.5 Spinal System, 4 x 20 mm, Stryker Spine) is inserted. An identical procedure is repeated for vertebral body L2. The rod is cut to the appropriate length and inserted into the grooves of the screws. Then, the closure screws are inserted followed by final tightening of the fixation system under compression. An identical procedure is repeated for the left side. A final fluoroscopy control in the anterior-posterior and lateral views verifies correct placement of the dorsal fixation system. An identical procedure is performed at level L3-4.

Then, the operating site is carefully irrigated and the muscle fascia is closed with simple interrupted stitches. A running subcutaneous suture follows and the skin is closed with staples. Finally, a spray dressing is applied and the anesthetized sheep is transferred to the CT scanner to take the postoperative CT image.

2.5.3   Postoperative Care

The first CT scan of the lumbar spine is taken immediately after surgery, with the animal still under general anesthesia. The correct placement of the cage and the stable fixation of the pedicle screw system are checked.

The sheep receives an IM injection of 0.2 mg/kg butorphanol (10 mg/mL, Torbugesic®, Fort Dodge Veterinär GmbH) as postoperative analgesia. Another injection is administered after 4 hours simultaneously with the subcutaneous injection of 4 mg/kg carprofen (50 mg/mL, Rimadyl®, Pfizer GmbH). Carprofen is continued in this posology for five days. Prophylactic antibiotic intramuscular 560 mg amoxicillin combined with 140 mg clavulanic acid (140/35 mg/mL, Synulox® RTU, Pfizer GmbH) is administered. This treatment is continued for 10 days. The sheep remains under clinical observation in groups of several animals until the wound healing is completed and the skin staples can be removed. Then, the sheep is transferred to open pastures where they are allowed to roam between the follow-up examinations and until sacrifice. Good health of the sheep is regularly verified.

2.5.4 Animal Sacrifice

According to the group allocation (cf. Figure 5) three to four animals were euthanized at each follow-up time point after 3 months, 6 months or 24 months (termination of the study) according to the initial allocation (cf. "2.1 Study Design"). The sheep are fasted for 12 hours and are allowed water ad libidum. The animals are sedated by IM administration of xylazine (Rompun 2%, Bayer Vital GmbH) in a dose of 0.1 mg/kg accompanied by butorphanol (Torbugesic®, Fort Dodge Veterinär GmbH) in a dose of 0.1 mg/kg. As soon as the animal lies down in a lateral position a central venous line is placed into the external jugular vein using a 20 gauge catheter after shearing of the overlying skin and then fixed to the skin with a nylon suture. General anesthesia is induced by an initial intravenous bolus of 4 mg/kg of propofol (Propofol 10 mg/mL, Narcofol®, CP-Pharma). Then, the sheep is euthanized by an overdose of pentobarbital sodium (900 mg/10 kg IV, Release®, WDT eG). The exact dose of pentobarbital sodium appropriate for small ruminants is not known. Therefore, the dose recommendation for cattle (450-900 mg/10 kg) served as a guideline. Since smaller organisms tend to have a higher metabolic rate the upper dose limit recommended for cattle was used in the present study. Samples for histological assessment are prepared (results will be discussed elsewhere).

2.6 Radiological Assessment

2.6.1 Technical Data of the Computed Tomography Scanner

CT examinations are performed in vivo on a multi-detector-row helical CT unit (Philips Medical Systems MX8000 IDT 16, Hamburg, Germany) at the Large Animal Clinic for Surgery at the University of Leipzig. The sedated sheep are placed in sternal recumbency. Neck and head are extended and kept straight in order to avoid spinal rotation. Tapes are used to secure positioning. Technique settings used for CT scanning are a tube voltage of 120 kV (peak), with effective tube current-time product of 200 mAs, scan speed of 0.75 s/rotation, pitch factor of 0.438, a 512 x 512 matrix and a 350 mm display field of view. Axial scans are obtained with a collimation of 1.0 mm and 1.0 mm reconstruction intervals from the cephalic aspect of Th12 to the caudal aspect of the L5 using a high-frequency (bone) image reconstruction algorithm. Images are viewed at 500 HU window level and 2000 window width. Multi-planar reconstruction of the axial

images are used to obtain images in the coronal and sagittal plane (coronal: reconstruction parallel to the cranial epiphysis of vertebral body L2; sagittal: reconstruction parallel to the sagittal axis of vertebral body L2). The images are digitized and stored on a Picture Archiving Communication System (CuraSmartClient curasystems GmbH, Ettlingen, Germany).

2.6.2 Procedure

For CT scan imaging a central venous line is placed into the external jugular vein using a 20 gauge catheter for safe and effective drug administration. The catheter is attached to the skin with a nylon suture. The sheep is sedated using propofol IV (10 mg/mL Narcofol®, CP-Pharma). This procedure is performed in the standing sheep. Slow administration of 4 mg/kg propofol until the animal lies down. Anesthesia is maintained with a perfusion pump of 24 mg/kg/h propofol. Since propofol may cause respiratory arrest in rare cases, emergency intubation equipment is ready to hand. The sheep is placed in a prone position in the CT scanner. After successful CT imaging the sedation is terminated by turning off the perfusion pump. The animals are usually back to standing position after seven minutes and show full responsiveness 15 minutes after terminating drug administration. The animal is then transferred to the sheep farm to fully recover.

2.6.3 CT Interpretation

2.6.3.1 Software

Imaging data is analyzed on an open-source ClearCanvas Workstation 2.0 SP1 (ClearCanvas Inc., http://www.clearcanvas.ca). This software allows measurements with a precision of 0.1 mm but is approved for experimental use only.

Follow-up images are directly compared to post-operative images using the dual-screen mode.

2.6.3.2 Criteria

Williams et al. (60) suggest a CT protocol to periodically monitor the progress of interbody arthrodesis. Although many important evaluation criteria are mentioned, the protocol does not provide sufficient details for its reliable execution.

2.6.3.2.1 New Bone Formation within the PEEK Cage

Thin-cut (1 mm) computed tomography scans are used to evaluate patterns of new bone growth at 3 months, 6 months and 12 months after surgery. The follow-up images are directly compared to the initial postoperative CT scan.

New bone formation and the fusion status within the PEEK cage pore are assessed on axial CT scans complemented by sagittal and coronal reconstructions. The synchronization mode of the workstation allows to reliably study bone growth in all three planes simultaneously. If the fusion status differed in the three planes the least progressed stage observed was chosen as overall score. If the type of tissue – i.e. bone, cartilage, fibrous tissue or fat – is in doubt the density in Hounsfield Unit (HU) is measured in the region of interest using the "probe" tool provided by the software. Values above +100 HU are considered to be trabecular bone.

Spinal interbody fusion defined as a bony bridge between adjacent vertebrae is the ultimate goal of the procedures. According to Vaidya et al. (52) the fusion status is semi-qualitatively scored using a 4-level classification (cf. Table 1). "Probable fusion" is defined as the presence of a continuous bone bridging from the cranial to the caudal endplate and increased density in the cage (61) in absence of radiolucent lines (identifiable radiographic clefts) (62). If a radiolucent line is visible in the fusing bone the specimen is classified as "possible fusion." At these early time points it would have been presumptuous to classify lucencies as "nonunion." Thus, this category does not exist in the scoring system.

Scoring System

Table 1. Scoring system for computed tomographic assessment of fusion in the cage pore.

Grade	Description
No new bone	No new bone formation visible
Visible new bone	New bone formation visible but no continuous bone bridge connecting the adjacent vertebrae
Possible fusion	Continuous bridging new bone with visible lucency
Probable fusion	Continuous bridging new bone formation
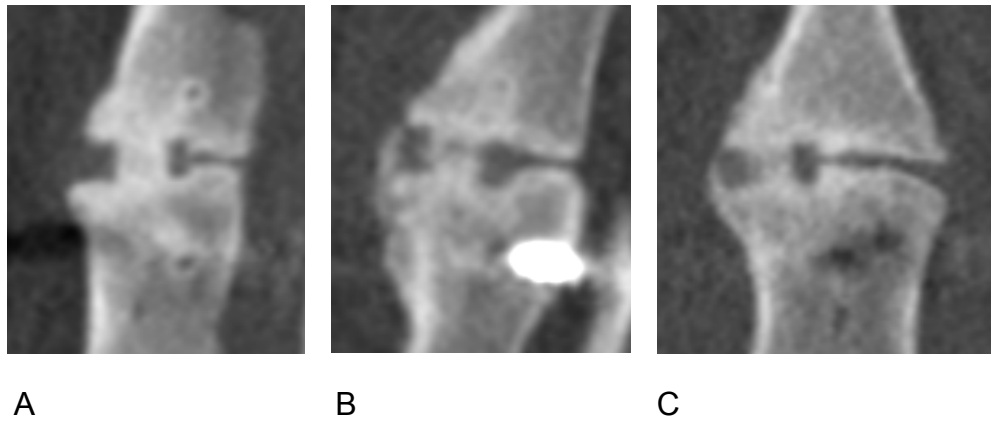
Figure 7. CT scans showing the difference in two specimens between scores "probable fusion" in the sagittal plane (A) and "possible fusion" in the sagittal (B) as well as in the coronal (C) plane after 3 months.
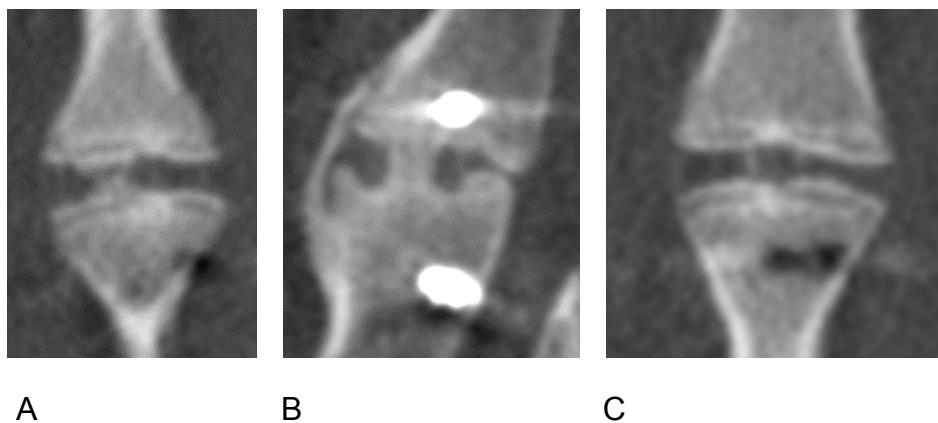
Figure 8. CT scans showing the difference in two specimens between scores "visible new bone" in the coronal plane (A) as well as in the sagittal plane (B) and "no new bone" in the coronal plane (C) after 3 months.

Adverse Side Effects

The main focus of the present study is to analyze the dose-dependent influence of rhBMP-2 on adverse side effects in lumbar spinal interbody fusion.

2.6.3.2.2 Change in Device Position (Migration)

The change in device position is assessed on axial CT images. Since the anatomy of the interbody disc space and vertebrae potentially changes remarkably in the follow-up the pedicle screws (dorsal fixation system) are used as landmarks for measurements.

In each case intact screws and stable screw position are verified by directly comparing the post-operative image to the follow-up images. Then, a line connecting the most external points of the bilateral rods is drawn. The (approximately) mid-sagittal line is based on the first line and the distance between the mid-sagittal line and a prominent edge of the radiolucent PEEK cage is measured in [cm] and compared in the follow-up. Sufficient accuracy of this technique is verified by measuring and matching a stable distance on each image. A discrepancy resulted in an adequate adjustment of the measurements. In the present study intraindividual comparison of the values in the follow-up was more important than interindividual comparison.

The PEEK cage is radiolucent to avoid artefacts on CT scans. Therefore, the exact circumference of the cage can be difficult to define. In intricate cases it is also noted if the cage is rotated to help appreciate any cage displacement.

A   B
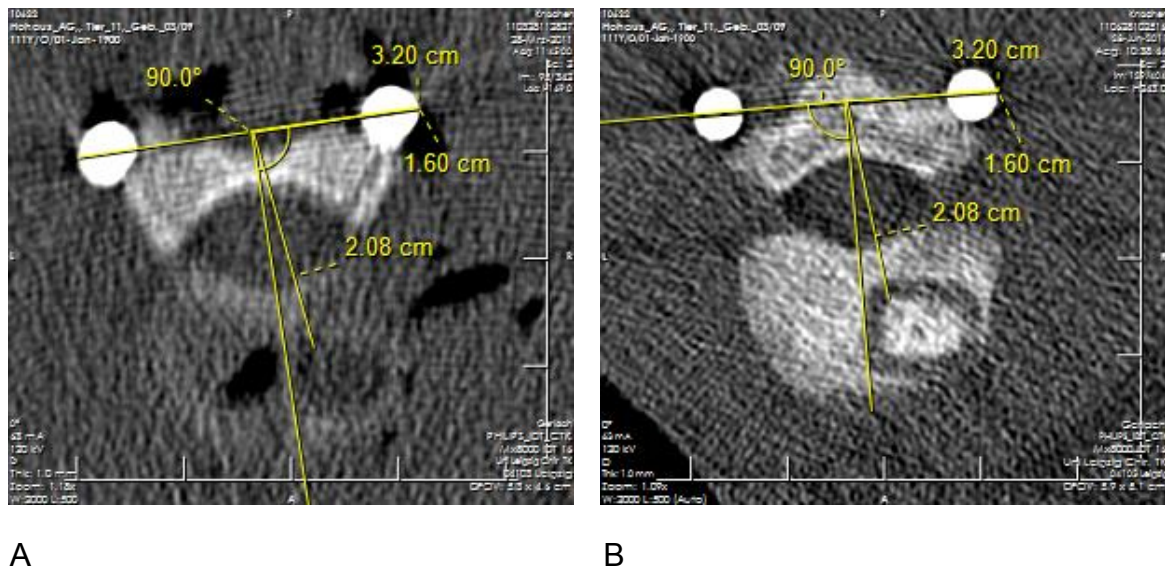
Figure 9. Axial CT scan images of the same specimen postoperatively (A) and 3 months after surgery (B) demonstrating the technique applied to measure cage displacement. No evidence for cage migration exists in this case.

2.6.3.2.3 Maintenance of the Disc Space Height

With great care, comparable anatomical landmarks are chosen for measurement. Since the ovine vertebral endplates are concave maintenance of the disc space height is best measured on sagittal reconstruction images. The same sagittal section has to be chosen to determine exact values of the measurements. "Reference Lines" on multiplanar reconstruction (MPR) images help to identify the same sagittal sections on the follow-up images. Distances between defined anatomical structures on the follow-up images are measured three times and compared to verify an identical location at the vertebral bodies. If these measurements differ significantly, an adequate adjustment is made. It is not possible to use the same landmarks for all individuals since the effect of rhBMP-2 and the cage position vary markedly.

After the right-lateral access to the disc space, most cages in the present study are located mainly on the right half of the vertebra. The effect of a loss of disc space height is therefore most pronounced on the left lateral side. Thus, a left lateral section at a considerable distance from the cage is chosen to measure the disc space height in the follow-up. This measurement gives excellent information on disc space height maintenance.

A   B
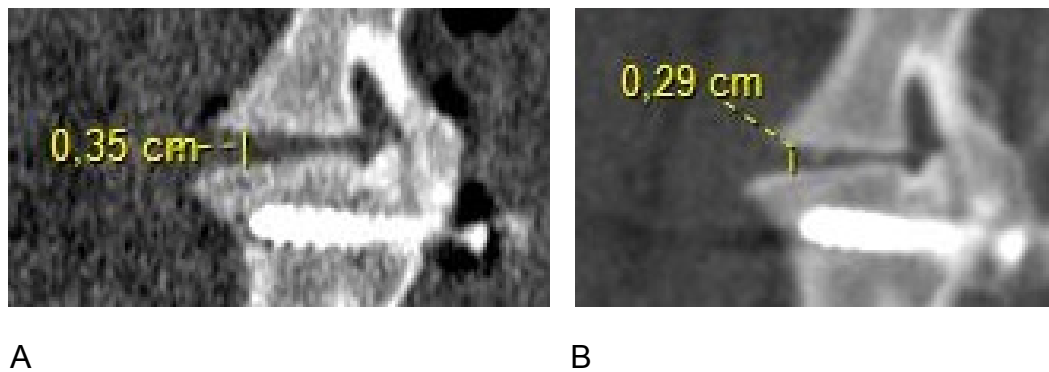
Figure 10. Sagittal CT scan images of the same specimen postoperatively (A) and 3 months after surgery (B) demonstrating the technique applied to compare and measure the disc space height.

2.6.3.2.4 Cage Subsidence in the Endplates

According to Williams et al. (60) subsidence is defined as "[...] a fusion device sinking into one or both of the adjacent vertebral bodies [...]."

Subsidence is assessed by comparing the follow-up CT image to the image taken immediately after surgery. Because of the concave vertebral endplates of the sheep it is decisive to measure the distance at the same location in the follow-up and it is difficult to verify corresponding points on the follow-up images. The distance of the vertebral endplate to a defined landmark is consistent on all CT images of the specimen in the follow-up (preferably Caspar pin holes, central vertebral vein) on coronal images measured. Cage subsidence is measured at the location where it is most pronounced in each specimen. Values obtained for subsidence into the cranial and caudal endplate are added to a summarized value of subsidence. Additionally, the extent of cage subsidence is categorized in "mild" (-0.1 mm to -1.0 mm), "moderate" (-1.1 mm to -2.0 mm) and "severe" (more than -2.1 mm).

Bone growth at the circumference of the cage can mimic cage subsidence and therefore needs to be identified and appreciated correctly. Therefore, it is essential to compare follow-up CT images to images taken immediately after surgery.

If the measurements in the follow-up are almost the same and macroscopically no change is observed (cf. Figure 11) the specimen is categorized as "no subsidence."

A   B
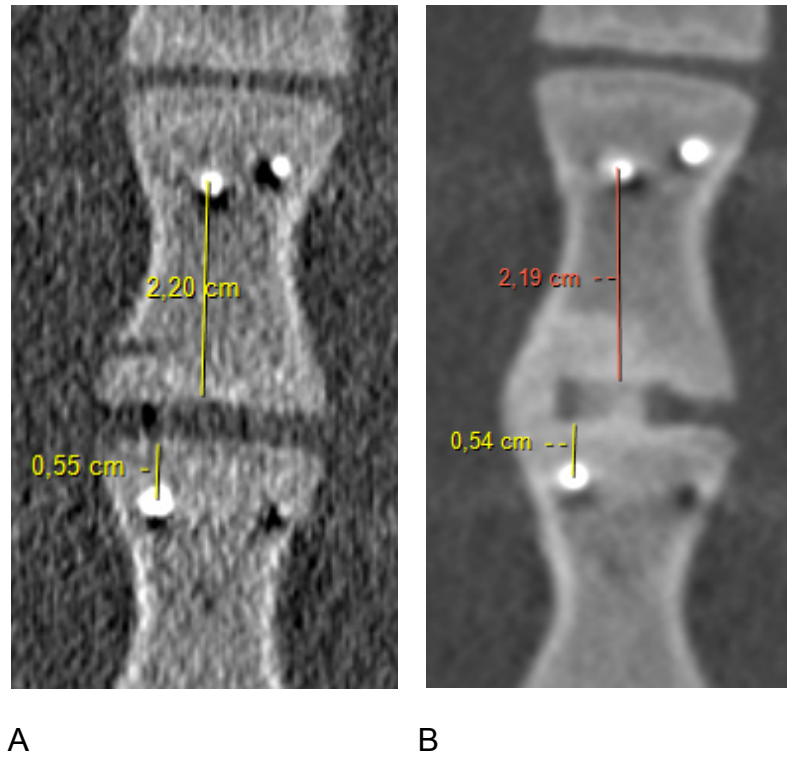
Figure 11. Coronal CT scan images of the same postoperative specimen (A) and 3 months after surgery (B) demonstrating the technique applied to measure cage subsidence.

2.6.3.2.5 Bone Resorption and Overzealous Bone Formation

All CT images are assessed for bone resorption and overzealous bone growth is recorded according to its location (anterior, posterior, left/right of the vertebral body; spinous process) and its cranial and/or caudal expansion.

3 Results

3.1 Pilot Study

Initially, 4.0 mg/mL rhBMP-2 (total dose 4.0 mg per segment) was applied at the interventional levels in three sheep. In the clinical (human) setting of PLIF the maximal total dose of 12.0 mg rhBMP-2 per disc space led to partial bone resorption at the endplates (45). Thus, to reproduce this study in the sheep model, a relatively high concentration was chosen. In the sheep model rhBMP-2 was used in a concentration of 0.43 mg/mL in several studies (55). Due to complications (cf. "3.7 Complications") only one specimen in the 4.0 mg/mL group could be evaluated according to the protocol. In this sheep the cage was displaced at the interventional level (L3-4). Fused bone was found at the initial position of the cage as well as in the (displaced) cage itself. Overzealous bone growth was visible at the right-lateral side (side of cage insertion) as well as at the spinous processes of vertebral bodies L2, L3 and L4. No cage subsidence was observed.

At the control level (L1-2) of this sheep the cage was not displaced and no subsidence was observed after 3 months. Fused bone was found in the cage and medially from the cage (Figure 12). Like in the intervention group, overzealous bone growth was found around the spinous processes of the last thoracic vertebral body and vertebral body L1.

After reviewing literature (63), the highest rhBMP-2 concentration applied was reduced to 2.0 mg/mL (total dose of 2.0 mg per segment).

Drop-out in the follow-up data was due to animal sacrification for histological analysis according to the protocol (cf. Figure 5).

A   B   C
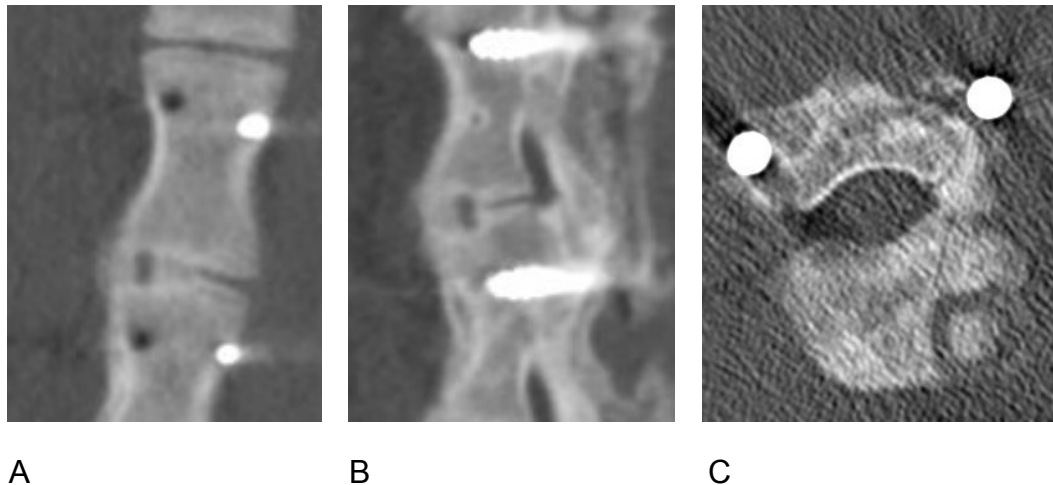
Figure 12. CT scan of internal control level L1-2 in a sheep where 4.0 mg/mL rhBMP-2 was applied in level L3-4 after 3 months showing "probable fusion" in all three planes.

3.2   New Bone Formation within the PEEK Cage (Fusion Status)

The fusion status in the intervention groups (rhBMP-2 applied) was more advanced in most cases than in the control group. It is important to note that the fusion status was determined in the cage pore and that it was not defined as any newly formed bone in the intervertebral disc space. Therefore, cage migration might have led to spinal fusion at the initial cage position but not in the cage pore. These results were considered unintended and therefore not included in the group of successful spinal fusion. Additionally, it is important to note that no osteoinductive material (e.g. autologous bone graft) was added at the control level. Thus, the fusion results in this group were expected to be unfavorable.

Fusion Status in the Control Group after 3 Months

In the control group 20 out of 29 specimens (69.0%) showed beginning new bone growth ("visible new bone") 3 months after surgery. In five specimens (17.2%), no radiographic signs of new bone growth in the cage pore were visible ("no new bone"). Probable fusion was observed in three specimens (10.3%; cf. Figure 13, Figure 14 and Figure 15). "Possible fusion" was found in only one specimen (3.4%).

A   B   C
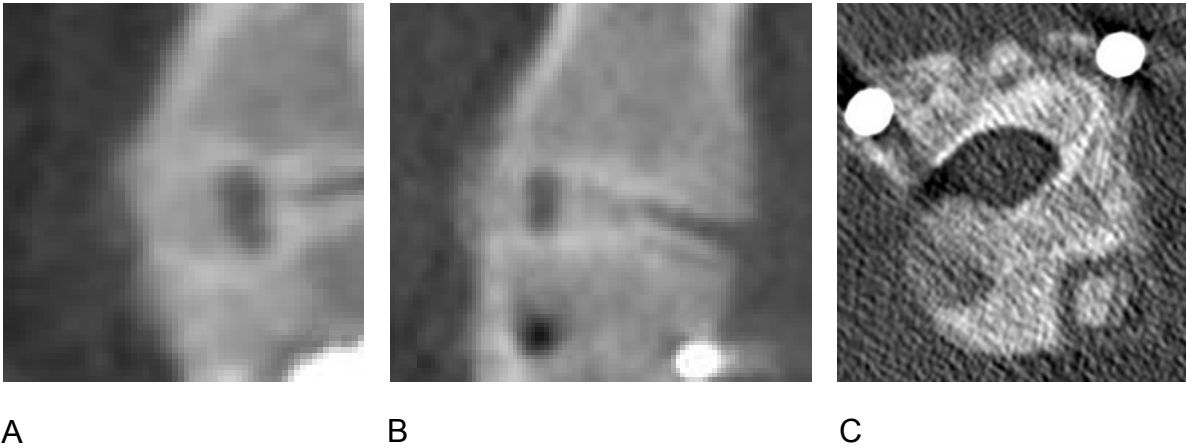
Figure 13. CT scan of control group after 3 months showing "probable fusion" in (A) the sagittal plane, (B) the coronal plane and (C) the transversal plane.

A   B   C

Figure 14. CT scan of control group after 3 months showing "probable fusion" in all three planes: (A) sagittal, (B) coronal, (C) axial.

A　　　　　　　　　B　　　　　　　　　C
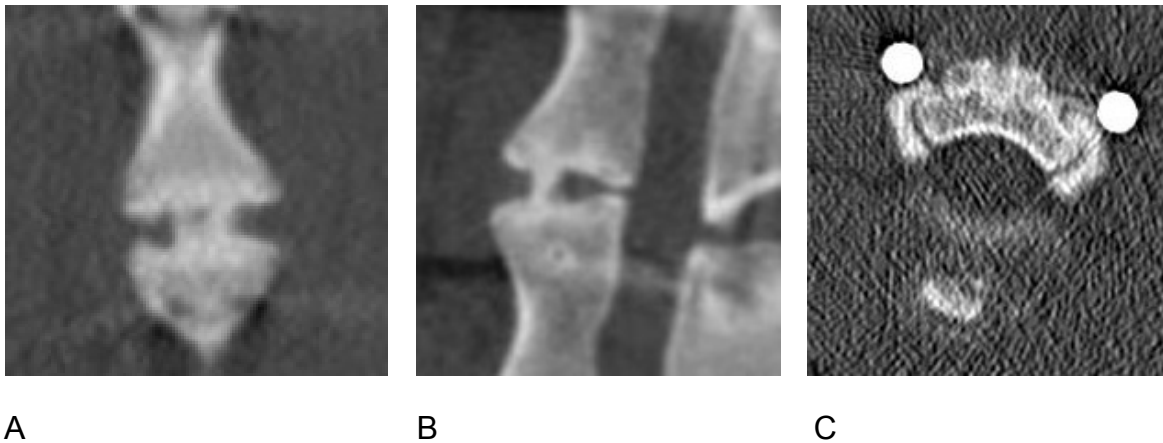
Figure 15. CT scan of the control group after 3 months showing "probable fusion" in all three planes. Unlike in the interventional level, the cage pore is not homogenously filled with new bone.

Fusion Status in the Intervention Groups after 3 Months

In the overall result of the intervention group, the fusion status was more advanced after 3 months compared to the control group. "No new bone" in the cage pore was only observed in 2 of 29 specimens (6.9%) (not subdividing the specimens according to the concentration and dose of rhBMP-2 applied). Since in the control group, only five specimens (17.2%) showed "no new bone", while most of the specimens showed "probable fusion" at all doses (group 1: 50%; group 2: 55.5%; and group 3: 70%), rhBMP-2 successfully initiated and promoted osteogenesis already after 3 months.

In group 1, five specimens (50%) showed "probable fusion" after 3 months (Figure 16). Two specimens (20%) in this group showed "possible fusion" and three specimens (30%) "visible new bone." Since new bone growth was found in all segments and advanced fusion states were found in 7 of 10 specimens, the lowest concentration and total dose of rhBMP-2 (0.5 mg/mL, total dose of 0.5 mg) was sufficient to induce and promote new bone growth in the cage pore. However, in two specimens, remarkable bone resorption at the endplates was observed (cf. "3.6 Bone Resorption and Overzealous Bone Growth").
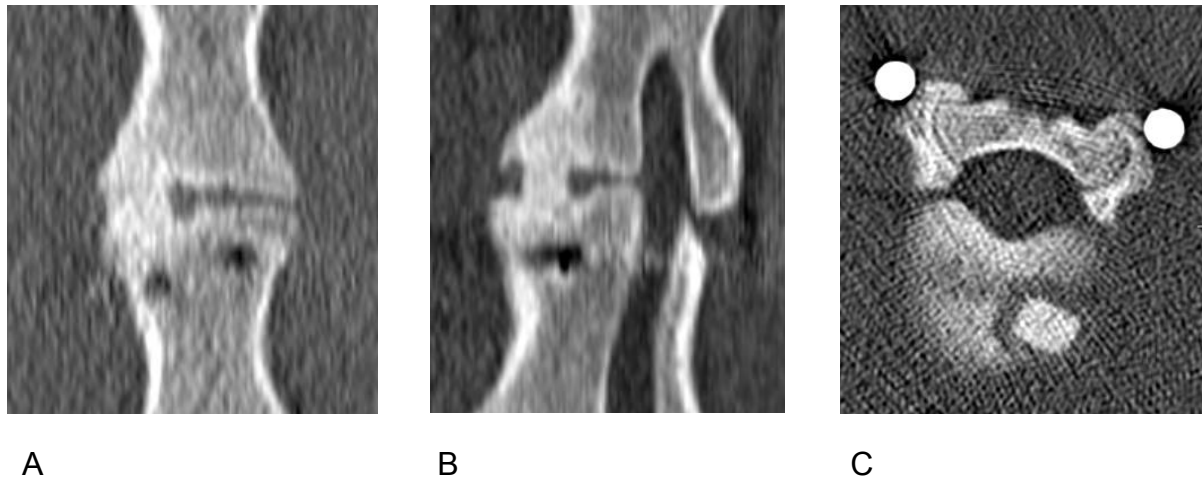
Figure 16. CT scan of group 1 specimen after 3 months showing "probable fusion" in (A) the coronal, (B) the sagittal and (C) the axial plane.

A dose of 1.0 mg/mL led to "probable fusion" in 5 of 10 specimens (22.2%), to "possible fusion" in two specimens (22.2%) and "visible new bone" in two specimens after 3 months. Fusion rates were thus only slightly improved compared to the application of the low dose (0.5 mg/mL) of rhBMP-2. In this group, no severe bone resorption at the endplates comparable to the findings in group 1 and group 2 was found.

Further increase of the rhBMP-2 dose per segment to 2.0 mg was associated with good overall fusion rates in 8 of 10 specimens (70% "probable fusion", 10% "possible fusion"; Figure 17). However, it is important to note that these excellent overall results in group 3 were compromised by two segments (20%) where no bone growth was present in the cage pore ("no new bone"). In these specimens – similar to the findings in group 1 – extensive bone resorption at the endplates and overzealous bone growth was observed.
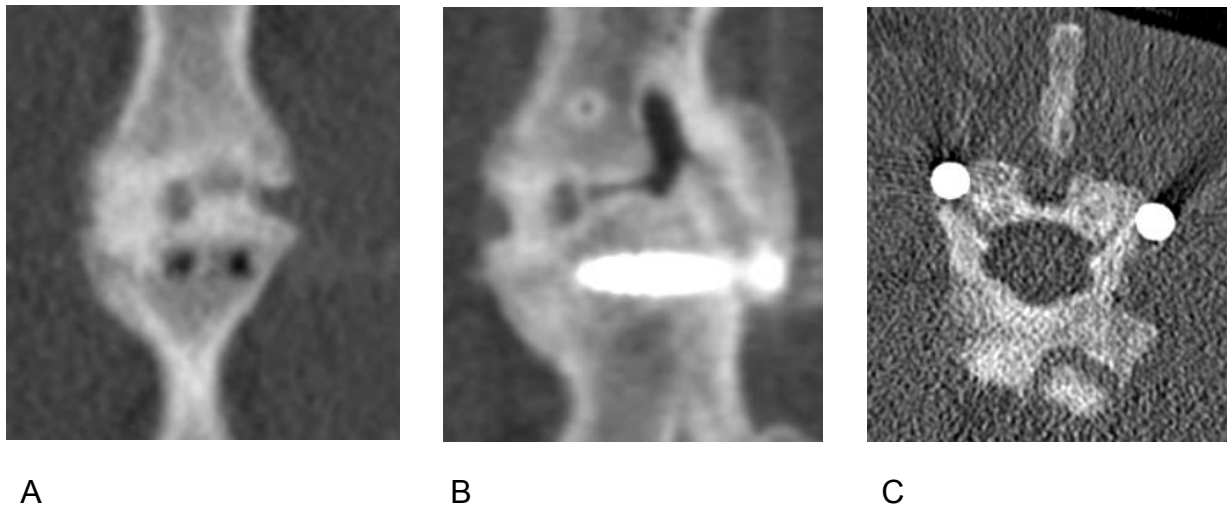
Figure 17. CT scan of group 3 specimen after 3 months showing "probable fusion" in (A) the coronal, (B) the sagittal and (C) the axial plane.
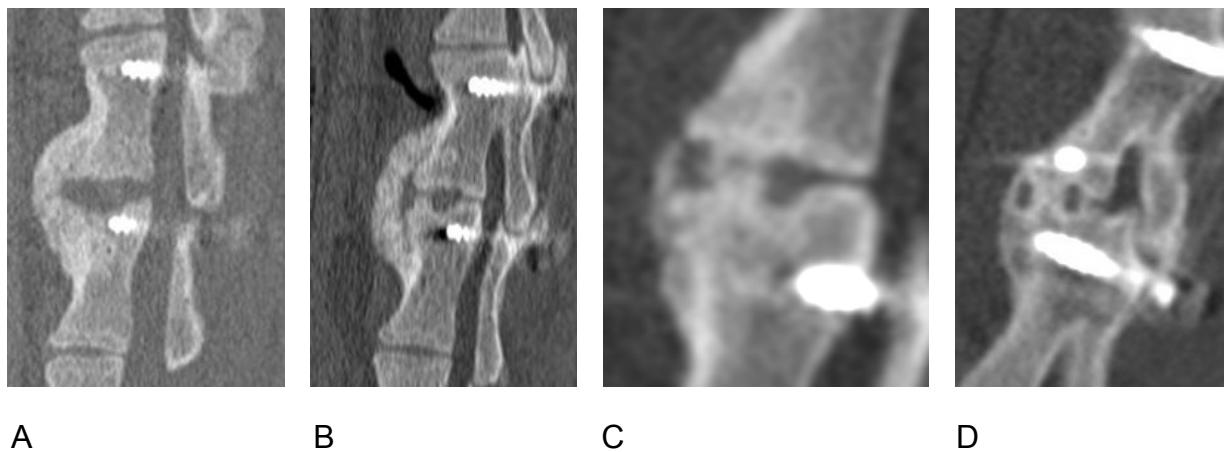
Figure 18. CT scan of group 3 specimen (L3-4) 3 months after intervention showing "no new bone" growth in the cage pore and massive bone resorption and overzealous bone growth (A).CT scan of group 1 level after 3 months showing bone resorption and expansive overzealous bone growth with "visible new bone" in the cage pore at level L3-4 in the sagittal plane (B). CT scan of group 2 specimen showing "possible fusion" after 3 months (C). CT scan of group 2 specimen after 3 months showing "probable fusion" in the sagittal plane (D).

Fusion Status in the Control Group after 6 Months

In the follow-up, the fusion status in the control group only reluctantly progressed between 3 months and 6 months. 20 of 29 segments (69%) were classified as "visible new bone" after 3 months. After 6 months, 14 of 20 segments (70%) were still found in this category. Nevertheless, progression of the fusion status was evident because after 6 months, 6 of 20 specimens (30%) showed advanced bone growth stages (10% "possible fusion" and 20% "probable fusion", cf. Figure 19) compared to only 4 of 29 specimens (13.7%) after 3 months (3.4% "possible fusion" and 10.3% "probable fusion").

A   B   C
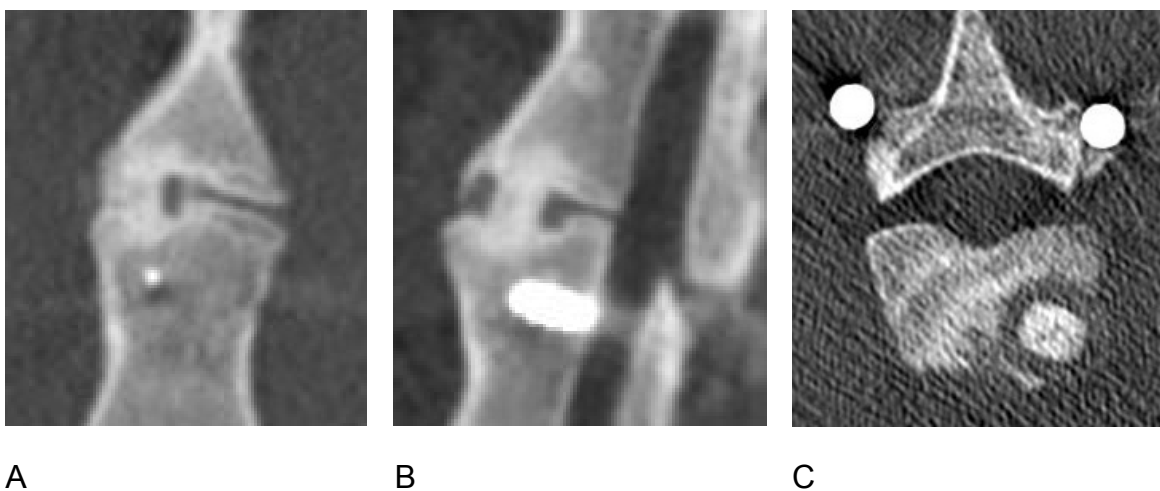
Figure 19. CT scan of control group after 6 months showing "probable fusion" and progressed bone growth in all three planes.

Fusion Status in the Intervention Groups after 6 Months

In the intervention groups, progression of fusion was more pronounced compared to the control group. After 6 months all specimens in group 1 as well as in group 2 were found to show advanced bone growth stages ("possible fusion" or "probable fusion").

In group 3, one specimen showed "no new bone" (due to massive bone resorption of the endplates and overzealous bone growth, cf. above). In group 1, 5 of 6 levels (83.3%) were classified as "probable fusion" after 6 months. One level (16.7%) was classified as "possible fusion". Early stages of new bone growth were not observed in this group at this time point. Comparable to the result after 3 months, a low dose of rhBMP-2 reliably promoted bone growth. In group 2, 7 of 8 specimens (87.5%) showed "probable fusion"

and one level (12.5%) was classified as "possible fusion" 6 months after surgery. In group 3, "probable fusion" was observed in 6 of 7 specimens (85.7%). This good result was compromised by one level (14.3%) still showing "no new bone" in the cage pore (cf. above; due to bone resorption).

Consequently, fusion rates in group 1 and group 2 were excellent and comparable after 6 months. Although most specimens in group 3 showed advanced fusion states, one specimen did not show any new bone growth in the cage but massive bone resorption.

Fusion Status in the Control Group after 12 Months

The fusion status in the control group showed a progression between 6 months and 12 months. Whereas most of the segments (14 of 21 specimens, 66.7%) were categorized as "visible new bone." after 6 months, most of the segments showed "probable fusion" (6 of 10 specimens, 60%) after 12 months. "Possible fusion" was found in one segment (10%) and an early fusion status marked by "visible new bone" in 3 segments (30%).

Fusion Status in the Intervention Groups after 12 Months

In the intervention group, excellent fusion results were observed after 12 months in group 1 as well as group 2. In these two groups, all specimens (n=4 in each group) showed "probable fusion". In group 1 endplate resorption was observed in 2 of 4 specimens which has to be taken into consideration for the overall result. The excellent overall fusion rate after 12 months in the intervention group was still compromised by two of three specimens in group 3. One segment (33.3%) showed no bone inside the cage pore (cf. above, bone resorption) and another segment (33.3%) showed "possible fusion." Only one segment in this group (33.3%) showed "probable fusion." The segment showing "possible fusion" in group 3 after 12 months deserves special consideration, since this segment was earlier categorized as "probable fusion" at the 3-month and 6-month time point. Therefore, it seems possible that the fusion status in this specimen regressed in the follow-up. In the control group and lower rhBMP-2 dose groups, no comparable cases were observed.

In our setting of lumbar interbody fusion, a dose of 1.0 mg/mL (group 2) showed the best overall fusion rates after 3 months, 6 months and 12 months.

In some specimens, a small void area in the center of the newly formed bone in the cage pore was observed (cf. "4.2 Spinal Interbody Fusion Status – Balancing Bone Resorption and Overzealous Bone Growth").

Although a higher dose of rhBMP-2 (group 3: 2.0 mg/mL) led to more advanced fusion states, at an earlier time point this dose was in 2 of 10 cases (20%) associated with extensive bone resorption at the endplates and was therefore not an advisable dose in the present study setting

Table 2. Fusion status after (A) 3 months, (B) 6 months and (C) 12 months.

Fusion Status	Control Group (n=29)	Group 1 0.5 mg/mL (n=10)	Group 2 1.0 mg/mL (n=9)	Group 3 2.0 mg/mL (n=10)
No new bone	5 (17.2%)	0 (0%)	0 (0%)	2 (20%)
Visible new bone	20 (69.0%)	3 (30%)	2 (22.2%)	0 (0%)
Possible Fusion	1 (3.4%)	2 (20%)	2 (22.2%)	1 (10%)
Probable Fusion	3 (10.3%)	5 (50%)	5 (55.5%)	7 (70%)

A

Fusion Status	Control Group (n=20)	Group 1 0.5 mg/mL (n=6)	Group 2 1.0 mg/mL (n=8)	Group 3 2.0 mg/mL (n=7)
No new bone	0 (0%)	0 (0%)	0 (0%)	1 (14.3%)
Visible new bone	14 (70%)	0 (0%)	0 (0%)	0 (0%)
Possible Fusion	2 (10%)	1 (16.7%)	1 (12.5%)	0 (0%)
Probable Fusion	4 (20%)	5 (83.3%)	7 (87.5%)	6 (85.7%)

B

Fusion Status	Control Group (n=10)	Group 1 0.5 mg/mL (n=4)	Group 2 1.0 mg/mL (n=4)	Group 3 2.0 mg/mL (n=3)
No new bone	0 (0%)	0 (0%)	0 (0%)	1 (33.3%)
Visible new bone	3 (30%)	0 (0%)	0 (0%)	0 (0%)
Possible Fusion	1 (10%)	0 (0%)	0 (0%)	1 (33.3%)
Probable Fusion	6 (60%)	4 (100%)	4 (100%)	1 (33.3%)

C

3.3 Change in Device Position (Cage Migration)

Migration of the cage after lumbar interbody fusion can lead to delayed bone union or even nonunion. Posterior cage migration may encroach the spinal canal requiring revision surgery.

In the control group, 19 of 28 cages (67.9%) did not migrate. 3 of 28 cages (10.7%) were displaced mildly (0.1-2.0 mm), 3 (10.7%) moderately (2.1-4.0 mm) and 3 (10.7%) severely (>4.0 mm). Migration ranged from 0.0 mm to 5.5 mm. The overall migration rate in the control group was relatively high (32.1%).

In group 1, 4 of 10 of the cages (40%) showed a stable position. No cage was displaced mildly, 4 cages (40%) were displaced moderately and 2 cages (20%) severely. Cage migration ranged from 0.0 mm to 5.4 mm. In group 2, only 1 of 9 cages was not displaced (11.1%). Cages migrated between 0.0 mm and 4.6 mm. 3 cages (33.3%) were displaced mildly, 3 cages (33.3%) moderately and 2 cages (22.2%) severely. In group 3, no spacer maintained its position (n=9). Migration ranged from 1.4 mm to 4.6 mm. 3 cages (33.3%) migrated mildly, 4 cages (44.4%) moderately and 2 cages (22.2%) severely. Interestingly, no progression of cage migration was observed in the follow-up in any specimen (after 3 months; cf. "4.3.3 Cage Migration"). In the intervention groups, cage migration occurred more frequently than in the control group. Cage migration was less common if the rhBMP-2 concentration and dose was lower. According to these data, the risk of cage migration increased with increased rhBMP-2 concentrations and doses. However, the extent of cage displacement was not dose-dependent (cf. Figure 20). The overall risk of cage migration might be reducible by decreasing the rhBMP-2 concentration and dose.

Given that even a low dose of rhBMP-2 led to a higher risk of cage migration compared to the control group, this adverse effect should be monitored continuously in postsurgical controls.

Figure 20. Extent of cage migration after 3 months in [mm] in (A) the control group, (B) the 0.5 mg/mL group, (C) the 1.0 mg/mL group, and (D) the 2.0 mg/mL group.

3.4 Maintenance of the Disc Space Height

In the control group, the disc space height was maintained in only 5 of 28 specimens (17.9%). The maximal loss of disc space height was -2.3 mm. No disc space was higher compared to the postoperative CT control in this group. The relatively high number of sintered disc spaces has to be interpreted in respect to the sophisticated technique applied to observe it.

In group 1, no case of disc space height maintenance was observed in all 10 specimens. The maximal loss of disc space height was -2.3 mm. In two specimens, a formally increased disc space height was measured when compared to the postoperative CT scan (by 1.2 mm and by 7.8 mm). Due to massive bone resorption at the endplates, the vertebral bodies were shorter and therefore, the distance measured between the cranial and caudal endplate was larger (comparable to the findings in the 0.5 mg/mL group). These two specimens (20%) were not included in Figure 21. In group 2, all 9 segments showed a reduced disc space height after 3 months in comparison to the postoperative control. Disc space height reduction varied between -0.8 mm and -1.7 mm (cf. Figure 21). In group 3, the disc space height was maintained in 1 of 10 segments (10%). Most of the specimens (7 of 10 specimens, 70%) were observed to have a reduced disc space height with a maximum disc space height reduction of -2.0 mm.

In the intervention groups, the extent of disc space height change did not show a dose-dependent pattern. The best overall result in the intervention group was observed in group 2, where the disc space height was neither severely reduced (due to cage subsidence) nor enlarged (due to resorption of the endplates).

Although the disc space height was best preserved in the control group, even in this group, the majority of specimens (23 of 28 specimens, 82.1%) showed a reduced disc space height. The disc space height did not further change after the 3-month time point in any group.
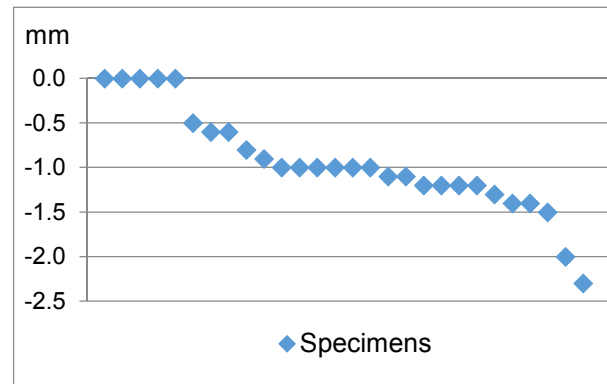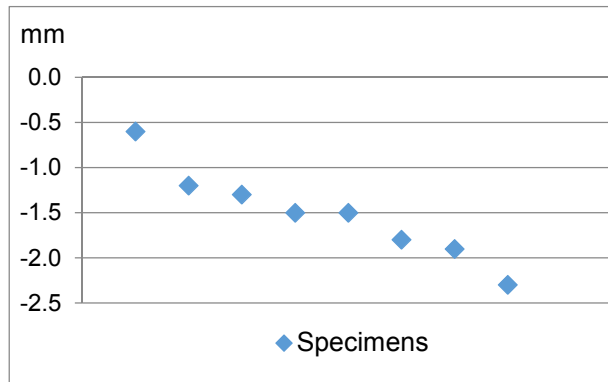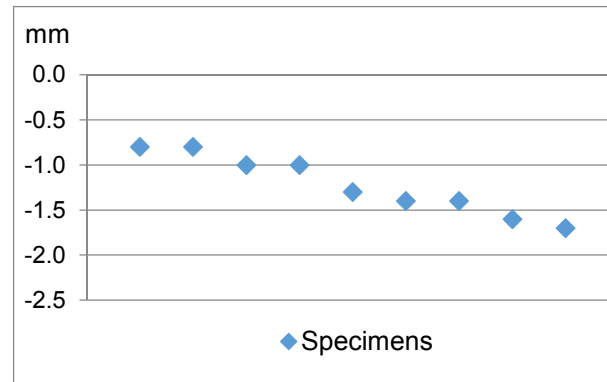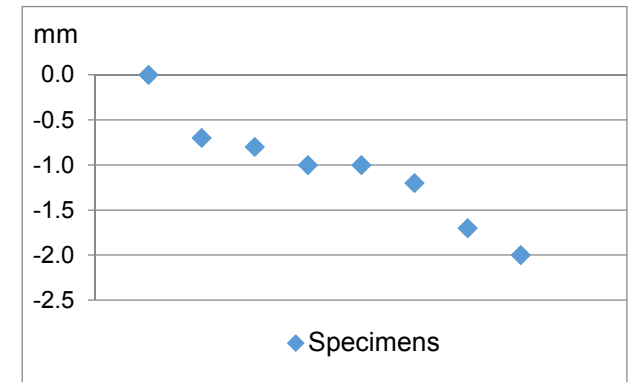
Figure 21. Disc space height difference after 3 months in [mm] in (A) the control group, (B) the 0.5 mg/mL group, (C) the 1.0 mg/mL group, and (D) the 2.0 mg/mL group.

3.5   Cage Subsidence

For the interpretation of the results, it is important to note that although cage subsidence was assessed for the cranial and caudal endplate separately, the two values were summarized for final results.

In the control group, most of the cages (16 of 28 specimens, 57.1%) did not subside in the endplates. In 2 specimens (7.1%), cage subsidence was severe (-3.4 mm and -4.2 mm). Three of 28 cages (10.7%) subsided mildly (-0.1 mm to -1.0 mm) and 7 cages (25.0%) moderately (-1.1 mm to -3.0 mm).

In the intervention groups, the cage subsidence rate was lowest in group 1. In this group, 7 of 10 cages (70.0%) did not subside in the endplates. One cage subsided mildly (-0.5 mm) and another cage moderately (-2.2 mm). In the specimen where bone resorption at the endplates was observed, the cage subsided severely (-4.7 mm). In group 2, 4 of 9 cages (44.4%) did not subside in the endplates. One cage (11.1%) subsided mildly (-1.0 mm) and four cages (44.4%) moderately. No cage subsided severely in this group. In group 3, 3 of 9 cages (33.3%) did not subside. Five cages (55.5%) subsided moderately and one cage severely (-8.2 mm). We would most likely attribute this enormous subsidence to massive bone resorption observed at the endplates. The other specimen in this group which showed massive bone resorption could not be analyzed for subsidence since the CT image taken immediately after surgery was not available for comparison.

In the intervention group, massive bone resorption at the endplates was associated with a high risk of cage subsidence (2 specimens in group 1: -2.2 mm and -4.7 mm; and one specimen in group 3: 8.2 mm). For a better overview, these specimens were not included in Figure 22. Like cage migration, subsidence did not continue in the follow-up after the 3-month time point.

According to these data the incidence of cage subsidence increased with increased rhBMP-2 concentrations and doses. However, the extent of cage subsidence was not dose-dependent (cf. Figure 22). The overall risk of cage subsidence might be reducible by decreasing the rhBMP-2 concentration and dose. Additionally, cage subsidence might also be influenced by other factors (e.g. cage design and material, primary position in the disc space).
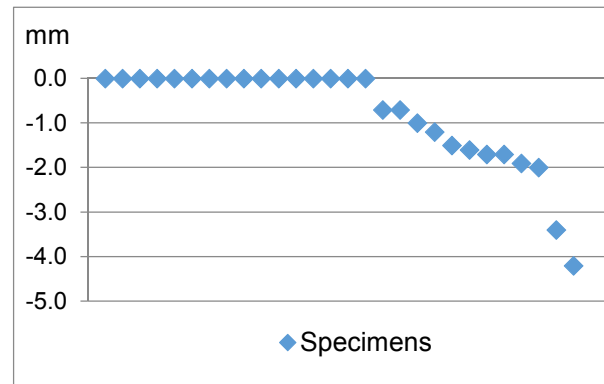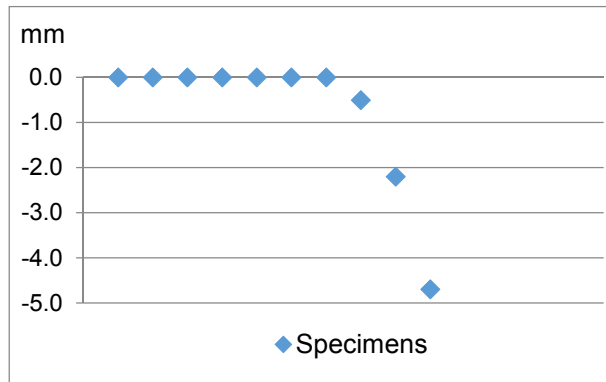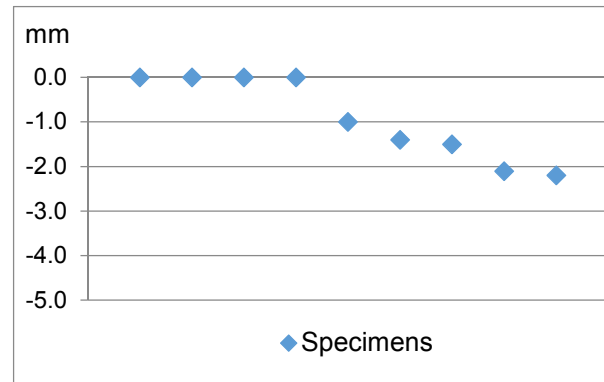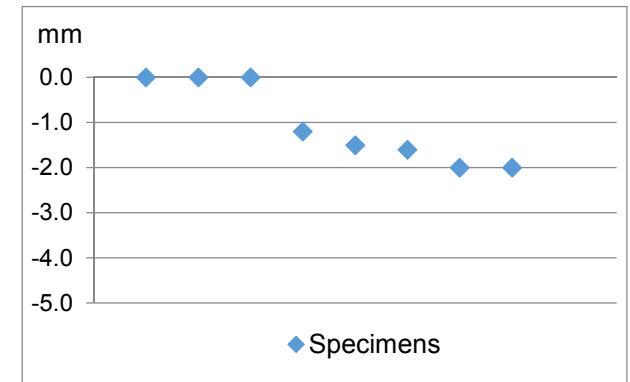
Figure 22. Extent of cage subsidence after 3 months in [mm] in (A) the control group, (B) the 0.5 mg/mL group, (C) the 1.0 mg/mL group, and (D) the 2.0 mg/mL group.

3.6   Bone Resorption and Overzealous Bone Formation

In our study we did not find radiological evidence of overzealous bone formation posteriorly around the spinal canal or neural foraminal encroachment in any specimen. There was no radiological evidence for ossification of muscles (myositis ossificans) or ligaments. A phenomena observed at all rhBMP-2 doses, though in varying extent, but only in 40% of the control group (12 of 30 specimens) and in minimal extent in this group, were bony on-growths anteriorly and right-laterally (probably due to the right-lateral approach) of the vertebral bodies. The greater extent of this bone growth can probably be directly attributed to the osteoinductive effect of rhBMP-2. The overzealous bone expanded cranially and caudally from the disc space where rhBMP-2 was used. This will be discussed elsewhere.

In the present study, persistent bone resorption at the endplates and overzealous bone formation was observed in two specimens of group 1 (cf. Figure 23) as well as two specimens of group 3 (cf. Figure 24). Additionally, two cases of minor resorptive defects (cf. Figure 25) were observed in group 3. No radiographic evidence of bone resorption was found in the control group.
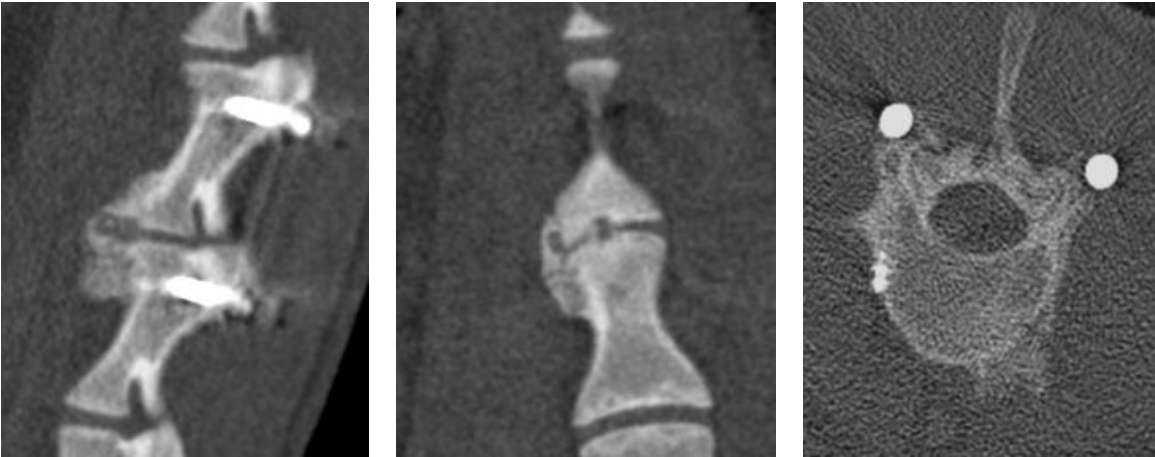
A
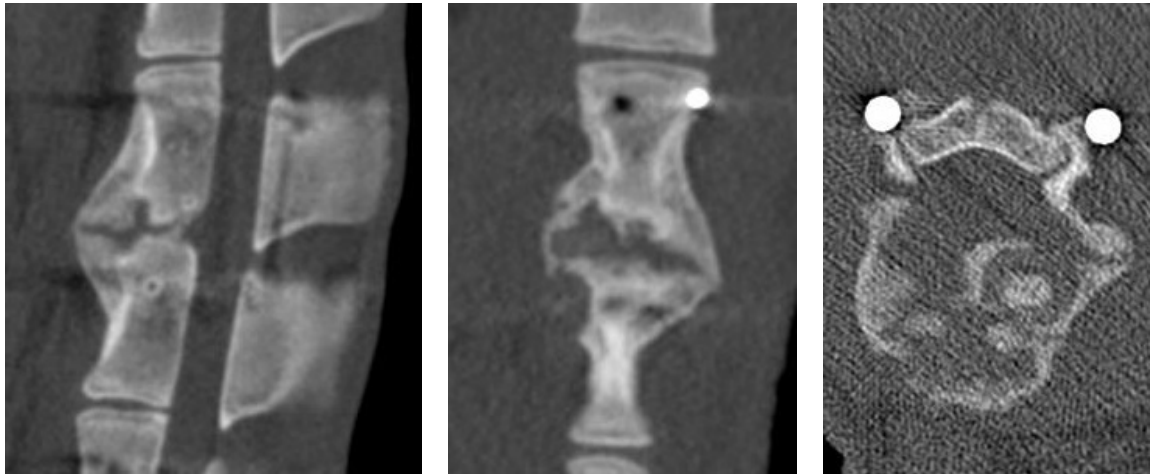
B

Figure 23. Group 1 specimens showing extensive bone resorption of the endplates and overzealous bone growth anteriorly and right-laterally in segment L3-4 (A) in all three planes and in segment L1-2 (B).
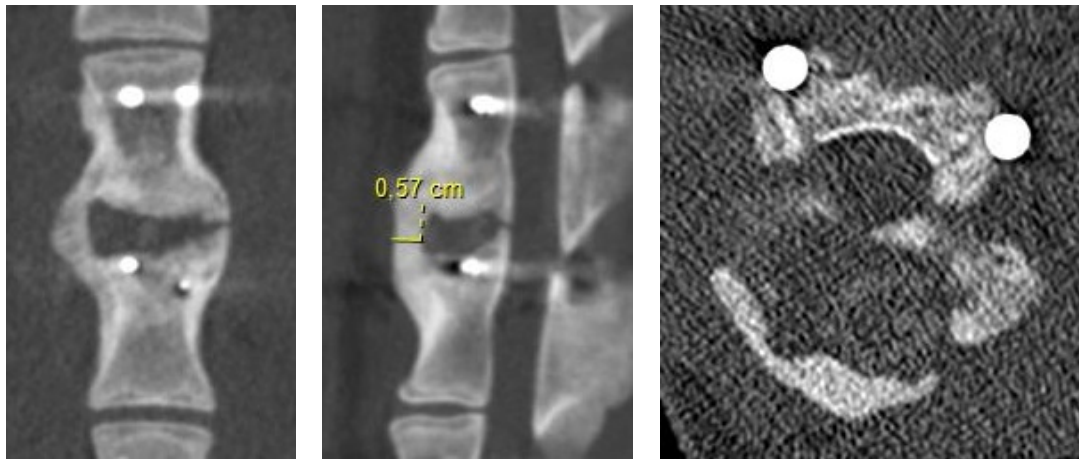
A
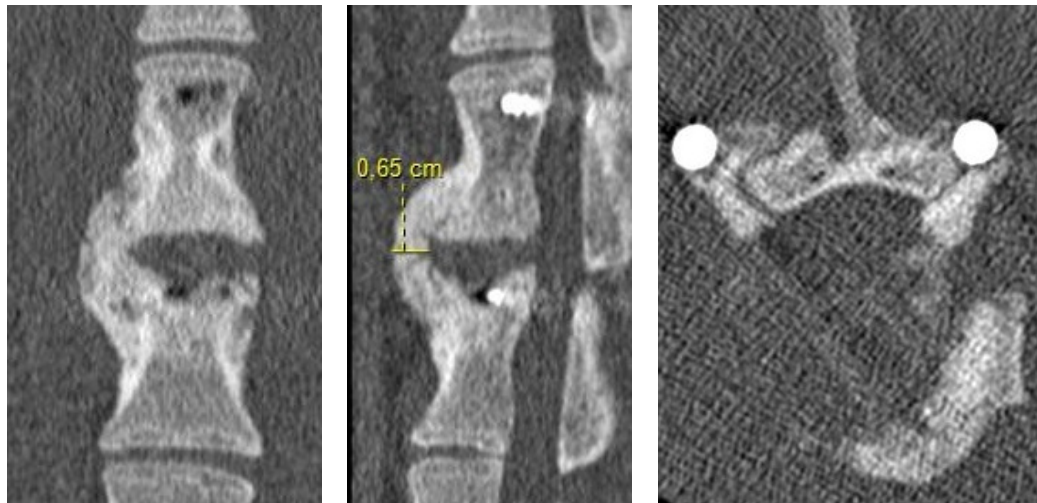
B

Figure 24. CT scan of group 3 after 3 months showing bone resorption and extensive overzealous bone growth in all three planes. Notice that no bone growth is present in the cage pore (A). CT scan of another specimen of group 3 after 3 months showing bone resorption and extensive overzealous bone growth in all three planes and no bone growth in the cage pore – possibly due to extreme cage movement and the resulting change in mechanical load (B).
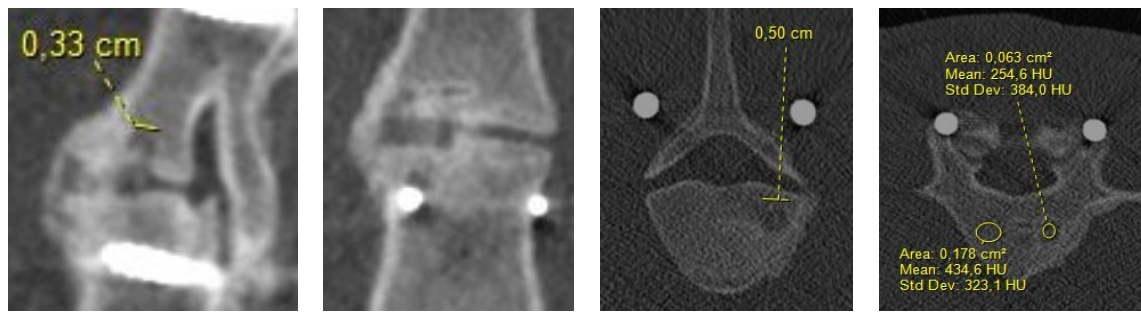
A
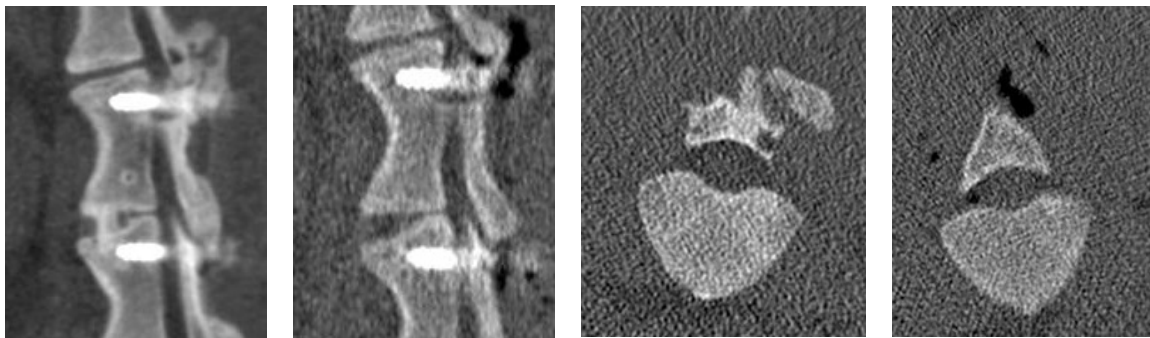
B

Figure 25. Group 3 specimen after 3 months showing resorptive defects in the caudal and cranial endplates in L3-4 in all three planes with reduced density (A) and group 3 specimen after 3 months showing bone resorption in the spinal process (right-laterally) of the last thoracic vertebral body which was not present on postoperative CT scan (B).

3.7   Complications

Perioperatively

One sheep of the pilot study group (4.0 mg/mL, n=3) was excluded from the study because dorsal fixation was attached at levels L1-2 and L3-4, while the cages were incorrectly implanted in levels L2-3 and L4-5. We observed in this specimen that the cages were severely displaced in both levels after three months. In the interventional level (4.0 mg/mL rh-BMP-2) fusion was observed at the initial postoperative cage position but not in the pore of the displaced cage (Figure 26, A). Overzealous bone growth was observed on the right-lateral side of the cage (side of cage insertion) and around the spinous processes of the last thoracic vertebral body and vertebral body L1.

At the control level, new bone growth (but no fused bone bridge) was observed in the displaced cage. On the right-lateral side of the displaced cage (side of cage insertion), a fused bone bridge was observed (Figure 26, B). No overzealous bone growth was present at the control level.

A   B
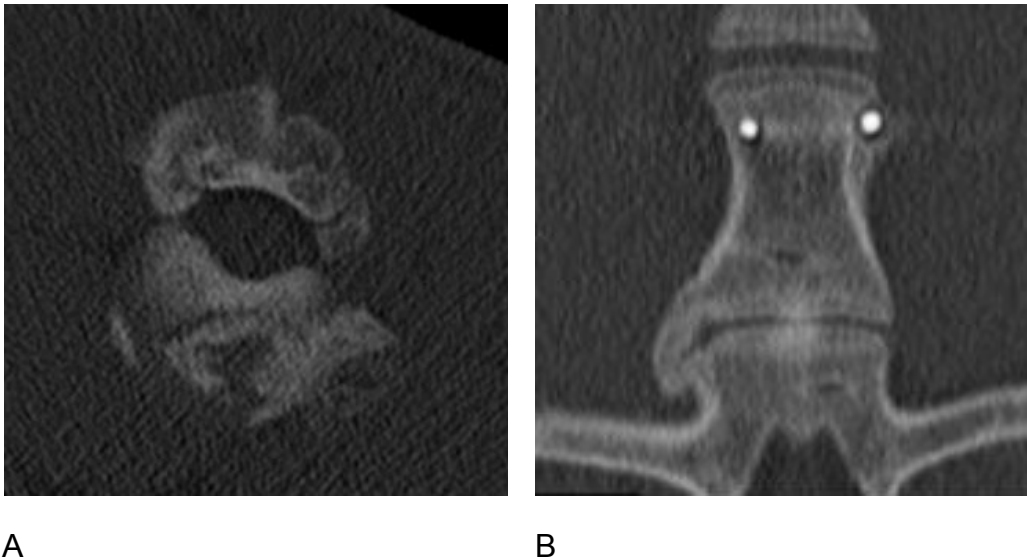
Figure 26. CT scan of interventional level L2-3 (4.0 mg/mL) with incorrectly localized dorsal fixation after 3 months showing severe cage displacement in the transversal axis. Overzealous bone growth on the spinous process of vertebral body L2 (A). Coronal CT image of control level L4-5 with incorrectly localized dorsal fixation and severely displaced cage after 3 months. Fused bone bridge on the right-lateral side of the displaced cage (B).

Another sheep of group 1 (0.5 mg/mL) was excluded from the study because of spinal instability due to intraoperative complications. The animal could not be oxygenated after repositioning for dorsal fixation of the spine. The inhalation tube was maintained in position. The cause leading to this complication could not be found. Therefore, the surgery had to be terminated before implantation of the dorsal fixation in both lumbar segments (interventional and control level).

One sheep of group 2 (1.0 mg/mL group) perioperatively suffered from peripheral hyperthermia, probably as anaphylactic reaction in response to Flunixine®. The surgery had to be terminated after preparation of the first lumbar level (interventional level) but before preparation of the control level. Therefore, only the interventional level of this sheep was included.

In one sheep of group 2 (1.0 mg/mL group), it was not possible to insert pedicle screws at the right-lateral side of the interventional level. The surgery had to be terminated with unilateral dorsal fixation of this segment. The resulting instability led to complete anterior cage dislodgement (Figure 27). Thus, only the control segment of this sheep was included in the study.

A   B   C

Figure 27. CT scan of interventional level L1-2 of group 2 (1.0 mg/mL) with unilateral fixation after 3 months showing complete cage dislodgement in the (A) transversal, (B) coronal and (C) sagittal axes.

One sheep in group 3 (2.0 mg/mL rhBMP-2) had to be excluded from the study because the OxPEKK cage at the interventional level broke perioperatively. Thus, only the control level was included in the study. In another specimen, the OxPEKK cage perioperatively broke at the control level. Therefore, only the interventional level in this sheep was included in the study. No PEEK cage broke in the present study.

Postoperatively

One sheep of the pilot study group (4.0 mg/mL) died seven days after surgery. Pathological examination diagnosed a diffuse typhlocolitis (inflammation of the cecum and the colon). It was not possible to find the cause of this inflammation or exclude an association to the use of rhBMP-2.

All remaining sheep recovered uneventfully from the surgical procedure so that normal activities in the pastures could be resumed 10 days after surgery.

CT Image Analysis

Some specimens were excluded from CT image analysis due to missing data sets. The assessment of cage migration, remaining disc space height and cage subsidence was not possible if the postoperative CT scan was missing (e.g. due to intraoperative complications) since comparison with the initial cage placement was essential. The fusion status was evaluated for all available CT scan images individually even if a CT scan in the follow-up was missing. Lumbar spine segments which were not operated according to the protocol (e.g. unilateral dorsal fixation) were excluded from the study.

4 Discussion

This thesis is part of a research project which was initiated in 2006 in cooperation with the Translational Centre for Regenerative Medicine (TRM) Leipzig, University of Leipzig (project number 1026AB). A previous clinical study on PLIF using rhBMP-2 (InductOs®) published in 2008 showed bone resorption at the vertebral endplates of the interventional levels in all 17 patients on CT images 3 months after surgery (45). In the follow-up, the osteolytic defects resolved and interbody fusion was observed. Additionally, overzealous bone growth was noticed which did not lead to clinically apparent symptoms (64). Subsequently, it was hypothesized that transient bone resorption caused by rhBMP-2 could potentially lead to severe adverse effects (e.g. cage subsidence, cage migration, temporary spinal instability). Therefore, this preclinical study was initiated to further elicit the dose-dependent effect of rhBMP-2 in lumbar interbody fusion in a large animal model.

4.1 Variations in Technique and Result Evaluation Hinder a Systematic Comparison of Study Results

Although many preclinical as well as clinical studies on the use of rhBMP-2 for spinal fusion exist, it is challenging – and in many cases impossible – to compare the findings and draw conclusions from the results because many variables and confounders influencing the outcome exist (cf. Table 3). The evaluation tools and criteria applied vary widely. Branch points out that "The Achilles Heel of contemporary health-care evidence is the inconsistent or highly variable methodologies of outcome assessment and reporting" (65). Branch further elaborates that "[…] meticulous observation of outcomes and imaging, is the major contributor to the knowledge base for any new technology" (65).

In the case of Infuse®, the data analysis is further impeded by the fact that several industry-sponsored trials were found to have misreported data, not disclosing information on adverse events (44). Fu et al. (26) conclude in their review of clinical studies on Infuse® that many published studies suffered from serious limitations (e.g. industry-sponsorship, not blinded, poor evaluation of associated harm).

Table 3. Possible variables and differences in study design in preclinical spinal fusion surgery

Species studied	Anatomical location	Surgical approach	Choice of fusion location	Choice of osteoinductive or –conductive agent and its dose/concentration	Choice of interbody spacer	Stand-alone vs. instrumented
human	cervical	anterior	interbody	DBM	PEEK cage	Dorsal pedicle screw fixation
primate	thoracic	posterior	intertransverse	Growth factor-based bone graft substitutes	Threaded titanium cage	Stand-alone
goat	lumbar	lateral	facet	BMP-7	BAK cage	
sheep		direct lateral		Ceramic-based bone graft substitute	LT cage	
dog		transforaminal		Cell-based bone graft substitutes	Titanium	
rabbit		extraforaminal		Beta TCP	Cortical femoral ring	
rat		extreme lateral		Autologous bone graft	Brantigan cage	
				Allogenic bone graft	Carbon fiber	
				Polymer-based bone graft substitutes		

4.2 Spinal Interbody Fusion Status – Balancing Bone Resorption and Overzelaous Bone Growth

Infuse® is FDA-approved as an osteoinductive material and a substitute for bone grafts in ALIF (17). Its indication is "[…] to help fuse vertebrae in the lower spine in order to treat degenerative disc disease" (17). Thus, most studies concentrate on the fusion status when analyzing the results. Unfortunately, no reliable data on fusion rates for spinal interbody fusion with or without rhBMP-2 exist. The fusion results are influenced by many variables (cf. Table 3) and the fusion status is assessed by different methods since no consensus on the definition of spinal fusion exists. At a symposium, McAfee (66) made a first attempt to find a consensus on how to define a successful ALIF.

In the present study, we found evidence in vivo that rhBMP-2 induces a coupled action of osteolysis and osteogenesis. RhBMP-2 reliably enhanced bone growth in the cage pore in the interventional level. The fusion status was better if a higher concentration resulting in a higher dose of rhBMP-2 was used but at the same time the incidence of adverse effects increased with increased dosing. For the overall result it is important to balance the fusion status against the adverse events associated with the procedure. Otherwise it is impossible to draw conclusions on the results of a new surgical intervention. The overall fusion result taking the adverse effects in account were not satisfactory in the interventional groups. In some specimens the bone column was bridging from one endplate to the other but its volume was low, possibly not leading to sufficient spinal stability in a clinical setting. The fused area was usually limited to the right-lateral and anterior sides from which the lumbar level was accessed. Hecht et al. (20) report an "[…] earlier rate of fusion and a greater fusion mass […]" when rhBMP-2 is used. Poynton et al. (54) state that "Findings have shown the fusion mass generated by rhBMP-2 and a collagen carrier to be more mature with more advanced remodeling and marrow formation than that generated by autograft." Many studies argue that rhBMP-2 should therefore be preferred as an osteoinductive agent because it avoids donor site morbidity from iliac crest bone harvest. However, in many cases, autologous bone grafts could be harvested locally at the area of surgical access without any additional donor site morbidity. Boden et al. (67) reported in 1998 that spinal fusion results were dose-dependent in a non-human primate model of lumbar interbody fusion. They compared the efficacy of rhBMP-2 in a dose of 0.75 mg/mL and 1.50 mg/mL. According to their study,

the higher concentration was able to produce an interbody fusion mass faster which was additionally thicker. Therefore, subsequent clinical trials used a concentration of 1.50 mg/mL with total doses varying according to the size of collagen sponge used.

One specimen in group 3 showing "possible fusion" after 12 months deserves special attention, since this segment was earlier categorized as "probable fusion" at the 3-month and 6-month time points. It seems therefore possible, that the fusion status in this case regressed in the follow-up. No comparable finding was observed in any other specimen.

In our study, bone fusion was assessed in the cage pore where rhBMP-2 was applied. Bone fusion in other areas, e.g. the initial cage placement if the cage had migrated, in the circumference of the cage or in the region of surgical access (right-lateral) was not included in the evaluation of the fusion status. Thus, fusion results might be underestimated. Since no osteoinductive material (empty or ACS only) was used in the control group, fusion results were expected to be unfavorable compared to the intervention group. Still, even in the control group, bone ingrowth into the cage pore was observed. The surgical intervention and/or postoperative mechanical loading might have induced bone growth in these specimens. Additionally, the disc space to be bridged is relatively small in the sheep which might partly explain the high fusion rates in the control group. However, the fusion process was delayed and less reliably initiated compared to the intervention groups. In most cases, the final results in the control group were unsatisfactory in terms of maturity degree and volume of the bridging bone.

In early industry-sponsored trials, rhBMP-2 was praised for leading to excellent fusion rates but recent reviews suggest that these fusion rates were overestimated, partly because the early industry-sponsored trials had serious methodological flaws (68). Fu et al. (26) show in their review that many studies actually could not prove significantly higher fusion rates than in the control group using ICBG in multiple locations. Some studies even report that the fusion rate and quality of bone fusion are inferior to ICBG when rhBMP-2 is used (in ALIF) (69). They found a significantly higher number of doubtful fusions when rhBMP-2 was used with centripetal voids and significantly smaller fusion masses observed on the 1-year CT images. Already in a trial which was cited in the FDA approval (17) on radius critical-sized defect repair in dogs with rhBMP-2 doses ranging from 0.0 mg/mL to 0.8 mg/mL a "Dose-dependent generation of excess bone and voids; voids observed especially in bone formed outside of implant area" were mentioned.

In the present study, void areas in the cage pore were observed in some specimens of the rhBMP-2 groups. In the present study no mechanism to explain its occurrence was found.

The "sentinel sign" defined as bone fusion anterior to the interbody cage is sometimes regarded as evidence of successful fusion but this is currently still being discussed (70). According to the findings in our study, anterior bone growth at least partly resulted from access to cancellous bone due to intraoperative Caspar pin placement and needs to be further analyzed in future studies. Burkus et al. (71) found in their study on radiographic assessment of interbody fusion devices for ALIF that "[…] the 'sentinel sign' of the progressive anterior bone formation frequently represented radial bone spur formation, not interbody fusion. The isolated sentinel sign may indicate progressive instability rather than progressive fusion." Williams et al. (60) suggest in their answer to a study by Lebwohl (70) that the anterior bone formation could be explained by "Wolff's law" as a physiological response to continued spinal instability. In our study, bone formation anterior to the cage even formed if a successful interbody fusion mass was observed which is in contradiction to Lebwohl et al.'s (70) standpoint.

In conclusion, we found excellent fusion rates in the intervention groups but also a higher incidence of adverse events, e.g. cage migration, bone resorption and overzealous bone growth, which possibly impaired the overall surgical outcome.

4.3 Adverse Effects Associated with RhBMP-2

In the present study – contrary to most studies – particular attention was paid to adverse effects associated with different doses and concentrations of rhBMP-2. Possible indirect markers for bone resorption, e.g. cage migration, cage subsidence and disc space height maintenance, were systematically assessed. The assessment of CT images was made according to a newly established protocol. This protocol was intended to objectively analyze the postoperative results at several follow-up time points with the first control 3 months after surgery. The follow-up images could be compared to images taken directly after surgery.

According to current literature, an osteoclastic phase marked by bone resorption always precedes the osteoblastic phase marked by bone apposition when rhBMP-2 is used (51).

Poynton and Lane (54) report relevant primary areas of concern regarding the safety of rhBMP-2:

- Overgrowth and uncontrolled bone formation (defined as overzealous bone growth in the present study)
- Osteoclastic activity (defined as bone resorption in the present, possibly leading to e.g. cage subsidence, migration and loss of fixation)
- Local safety (inflammation, edema, wound problems, and infection; not possible to analyze on CT images in the present study)
- Potential negative effects of BMPs on exposed dura and nerves (neurologic loss of function, retrograde ejaculation, early back pain, leg pain, radiculitis; not evaluated in the present study)
- Carcinogenicity (not evaluated in the present study)

Even after the publication of the YODA project results (25,26), it is difficult to appreciate the true safety profile of rhBMP-2 for lumbar interbody fusion. Among others an important reason is that in many clinical studies, the follow-up protocol did not include systematic imaging, despite the innovative and in some cases even off-label use of the growth factor. In some clinical studies, the final assessment of successful fusion result relied solely on subjective questionnaires which does not seem to be an appropriate approach because it is highly susceptible to bias (72).

A clear discrepancy was found between the data published in peer-reviewed journals and internal reports as well as those published in the FDA approval information (cf. Figure 28).
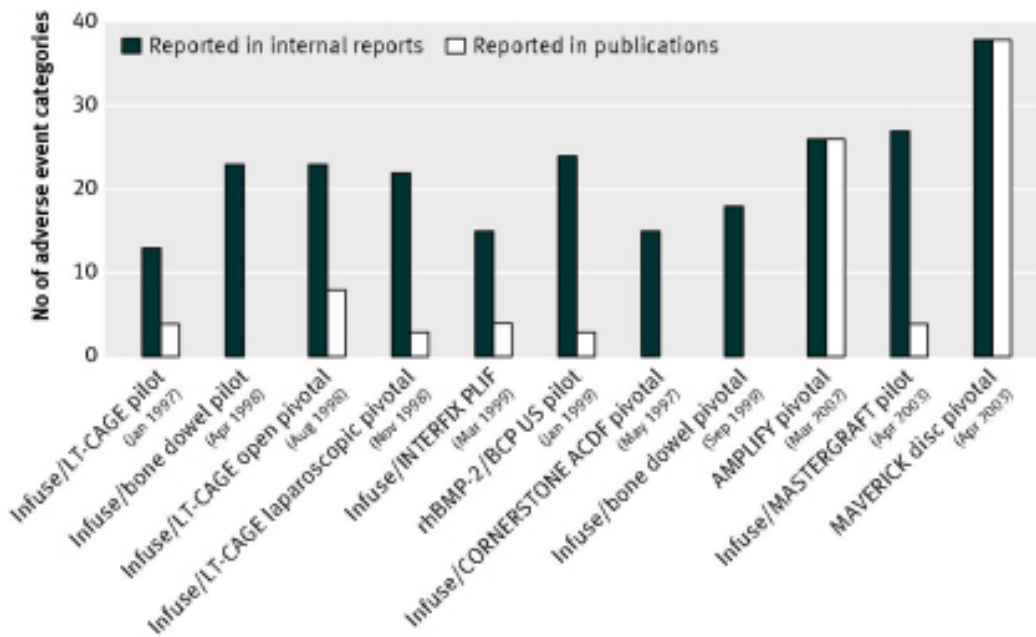
Figure 28. Number of adverse event categories reported in Medtronic internal reports and journal publications by date of first patient enrolment (73).

Smoljanovic et al. (74) analyzed published data on transient vertebral resorption and found that patients who developed vertebral resorption were "[…] more susceptible to spacer subsidence, loss of correction, graft migration, and failure of spinal interbody fusion." Carragee et al. (25) found that industry-sponsored trials on rhBMP-2 in ALIF underreported the incidence/prevalence of complications (osteolysis, device subsidence, reoperation rates, incidence of retrograde ejaculation and urinary retention, and infections) in the rhBMP groups. The findings from the systematic review of Fu et al. (26) mainly confirm Carragee et al.'s (25) theses. Little information on the local effects (inflammation, ectopic bone formation, osteolysis, subsidence) of Infuse® was found in the Medtronic datasets. Fu et al. (26) criticize that adverse events were not systematically analyzed. In their systematic review Simmonds et al. (49) point out that "Adverse event data in the literature raise concerns that rhBMP-2 may increase the risk for heterotopic bone formation, osteolysis, radiculitis, and retrograde ejaculation." Our results confirm that the application of rhBMP-2 might be associated with substance-specific adverse side effects like bone resorption and overzealous bone growth.

In conclusion, future efforts should concentrate on establishing standardized criteria for the evaluation of the overall success of spinal fusion surgeries in clinical and preclinical

studies. It would be crucial to generate a score to relate fusion results to adverse effects. Additionally, a postoperative CT scan taken immediately after surgery could prove very helpful for follow-up comparison even in clinical studies (already implemented in our department). Although regenerative medicine and tissue engineering are appealing fields, it is important to warrant patient safety – primum non nocere.

4.3.1 Bone Resorption

Initial trials on the use of rhBMP-2 for spinal fusion did not report resorption at the vertebral bodies. Already in 1999, Hecht et al. (20) suggested an association between rhBMP-2 application and bone resorption in spinal fusion surgery. In their study, they used threaded cortical allograft interbody dowels filled with a rhBMP-2-soaked ACS. After 6 months the allograft was completely resorbed and remodeled by the fusion mass. They therefore concluded that rhBMP-2 may not only enhance osteoblastic bone formation, but also osteoclastic bone resorption. This hypothesis is in line with the findings of massive bone resorption accompanied by abundant overzealous bone growth anteriorly and at the right-lateral side of the vertebral body observed in both specimens of group 1 and group 3 but not in group 2 in the present study. In the two specimens in group one new bone formation was present in the cage pore, whereas in the two specimens of group 3, no new bone growth was observed in the cage pore. Thus, the extent of bone resorption might increase with increasing concentration/doses of rhBMP-2. In summary, bone resorption probably also depends on factors other than concentration/dose of rhBMP-2. This is in line with Mannion et al. (63) who observed osteolysis in a clinical study even when a very low dose (1.4 mg) of rhBMP-2 was used.

In 2008 Smoljanovic et al. (75) revealed endplate resorption, osteolysis, and cage subsidence on original radiographs from an early industry-funded randomized controlled study by Burkus et al. (32). In the initial publication in 2002, these adverse effects were not commented on. Although Toth et al. (53) admit that vertebral endplate or vertebral body resorption was observed with expanded clinical use of rhBMP-2 for interbody fusion, they argue that "The assertion that cancellous bone resorption occurs in all cases in which rhBMP-2/ACS is used in the interbody space is not evidence-based. In fact, there were very few instances of resorption in the series of patients with the titanium trapezoidal fusion cages used to gain the original Food and Drug Administration approval." It still seems likely that temporary bone resorption present in the initial postoperative phase

(first 4-6 weeks) might have already resolved by the time of the first postoperative imaging. RhBMP-2 is reported to be most active during the first 4 weeks. Seeherman et al. (76) observed significant transient bone resorption at the contact areas 2 weeks after surgery in a femoral core defect in cynomolgus monkeys and proved that even a low total dose of only 360 μg rhBMP-2/ACS in a concentration of 1.5 mg/mL led to visible bone resorption associated with osteoclasts and receptor activation. Interestingly, this was not the case when a carrier with a slower release of rhBMP-2 was used. Thus, the rapid release of rhBMP-2 at the bone surface which is in contact with the collagen sponge might create favorable conditions for significant osteoclastic action. Seeherman et al. (77) point out that although inducing de novo and appositional bone formation, transient bone resorption induced by rhBMP-2 may lead to a loss of fixation or structural support. Knox et al. (51) report an osteolysis rate of 27.6% in TLIF with 5 mg rhBMP-2/level. In their study, routine CT scans were taken postoperatively, after 3 months and 6 months. In 2006, McClellan et al. (78) concluded that bone resorption observed at 22 of 32 lumbar levels after TLIF procedure was directly caused by rhBMP-2 and consequently led to cage subsidence and prevented radiographic union in a significant number of patients. Additionally, already in 2006, Hansen et al. (79) reported a case of endplate resorption, initially mistaken as an infection, associated with the use of   rhBMP-2 with a femoral ring. Some surgeons argue that bone resorption at the endplates might be due to an intraoperative vulneration of the endplates. As hypothesized by Toth et al. (53) the exposure of cancellous bone combined with the use of rhBMP-2 might cause a high risk of bone resorption. Toth et al. (53) reluctantly state that "[…] it is clear that access and proximity to cancellous bone is one important factor that can increase the risk of transient bone resorption." Interestingly, in early clinical trials on rhBMP-2 application in ALIF, a threaded titanium cage was countersunk into the vertebral bodies exposing cancellous bone but no bone resorption was reported (31,32). Michielsen et al. (80) found in their clinical study that rhBMP-2 (in a total dose of 8 mg) led to endplate osteolysis even without significant intraoperative endplate violation. They therefore conclude that "[…] osteolysis cannot be attributed to end-plate violation, as suggested in previous papers, and appears to be induced by rhBMP-2." Nor did they find any migration or subsidence of the titanium cages. Smoljanovic et al. (81) argue that the size of the contact surface between rhBMP-soaked absorbable collagen sponge and trabecular bone was responsible for the occurrence and clinical manifestations of

resorption. However, this hypothesis contradicts the finding that even when the FDA-approved cage, which does not allow direct contact of the ACS to the endplates, is used, bone resorption is observed. In some studies, rhBMP-2-loaded collagen sponges were not only placed into a cage but in direct contact to the endplate (as in many "off-label" studies), adjacent to the interbody cage, possibly increasing the risk for resorption. Lehman et al. (82) report that bone resorption was not fully resolved one year postoperatively which is in line with the findings in the present study that significant bone resorption did not resolve in the follow-up.

In 2007 Lewandrowski et al. (83) found vertebral osteolysis on CT images after PLIF with rhBMP-2 in five patients who complained about worsening back pain 4 weeks to 3 months postoperatively. Only small to medium kits of Infuse® were used. Thus, bone resorption might result in clinically apparent complications and will not always remain clinically silent as suggested by the NASS report (9). In Lewandrowski et al's (83) trial, all patients showed complete resolution of their symptoms within 12 weeks without additional intervention and the osteolytic defects seemed to be at least partially reconstituted. This is in line with the long-term findings in our clinical study on the use of rhBMP-2 in PLIF (unpublished data). Michielsen et al. (80) observed bone resorption and overzealous bone formation only if rhBMP-2 was applied and not in the control group with autologous bone in a randomized trial including 20 patients in each group undergoing single level PLIF.

In conclusion, given that rhBMP-2 seems to initially stimulate osteoclastic activity, rhBMP-2 should not be used as a stand-alone technique (no dorsal fixation system) in the lumbar spine as this can lead to severe adverse effects.

4.3.2 Overzealous Bone Growth

Overzealous bone formation, often referred to as "heterotopic/ectopic ossification" is associated with the use of rhBMP-2 in spinal fusion. Since "heterotopic ossification" refers to ossification of soft tissue, this term was considered inappropriate given that in the present study, no ossification of soft tissue was observed, although bone formation was found beyond the anterior confines of the vertebral bodies. Marshall Urist (11) demonstrated the osteoinductive properties of BMPs in ectopic areas, e.g. muscular tissue (heterotopic ossification). In a clinical study Shah et al. (84) used rhBMP-2 in a

patient suffering from multiple myeloma and observed the ossification of the retroperitoneum, psoas muscle, pelvis and abdominal wall, possibly associated with massive intraoperative bleeding. As early as 1999, overzealous bone growth was observed in a randomized trial of rhBMP-2 in PLIF sponsored by Medtronic (26). Subsequently, enrollment was suspended. However, in a Medtronic-sponsored supplement in the journal Spine, two articles discussed possible ectopic bone formation in the study mentioned above and concluded that the ectopic bone formation and complications were due to poor technique (26,54,85).

In the present study, overzealous bone growth was present mainly on the right-lateral side of the vertebral body which was the side of surgical access. Accordingly, Michielsen et al. (80) found that "Heterotopic bone [extended] posteriorly along the path of the cage implantation." Careful analysis and comparison of the formed overzealous bone masses in the present study resulted in the hypothesis that overzealous bone formation – at least partly – sprouted from the cancellous bone at the area of intraoperative Caspar pin placement. Klimo et al. (86) report in their study on the use of rhBMP-2 in the cervical spine that bone resorption at the endplates as well as overzealous bone growth posteriorly to the spine was observed. They used Caspar pins intraoperatively and applied an anterior plate fixation. According to the CT images included in the publication, it is possible that overzealous bone growth sprung from the raw decorticated bone left after removal of the Caspar pins. It remains unclear if a barrier and sealing cancellous bone might be helpful to prevent leaking of rhBMP-2 and overzealous bone growth. Patel et al. (87) suggest that fibrin glue application to areas where bone formation is unwanted could locally inhibit the effect of rhBMP-2. Rihn et al. (88) showed that a thin barrier helps to prevent rhBMP-2 from leaking through the annulotomy into the spinal canal and around the nerve root. They confirmed that rhBMP-2 itself can lead to postoperative radicular pain. Rihn et al. (88) found in their retrospective cohort study on complications in single-level TLIF with either autograft iliac crest or rhBMP-2 that a hydrogel sealant (Duraseal; Confluent Surgical Inc., Waltham, MA, USA) decreased the rate of postoperative radiculitis in the rhBMP-2 group from 20.4% to 5.4% (p=0.047). Poynton et al. (54) provide helpful advice on the handling of rhBMP-2: "Therefore, the key elements of safe rhBMP-2 usage are careful placement away from raw decompressed areas and retention of the rhBMP-2 within the fusion area by the carrier used." According to the NASS report (9) recent studies recommend to minimize "[…] osteolysis and ectopic bone

formation by providing a barrier between the rhBMP and the dura, lowering the dose used and carefully protecting the vertebral endplates." Future studies should analyze if the anterior and lateral overzealous bone growth found in our study can be reduced by sealing the Caspar pin holes.

In the present study overzealous bone growth was observed in all specimens in the intervention group independent of the dosing. Our study suggests that this effect is mainly associated with the application of rhBMP-2 since in the control group only some cases of minor overzealous bone growth were observed. This contradicts Goldschlager et al.'s (89) conclusion from a preclinical study who postulate that "[…] bone formation anterior to the cage […] occurred equally in both control and cell-treated animals […]." Overzealous bone growth might lead to complications by compressing anatomical structures. Accordingly, a posterior approach would risk bone growth close to the neuroforamen due to facetectomy. This hypothesis is supported by numerous clinical studies on PLIF reporting serious complications when rhBMP-2 is used. In our clinical study on rhBMP-2 application for PLIF overzealous bone formation was observed but fortunately remained clinically silent (45).

In the present study new bone growth was also observed at the spinous processes along the dorsal pedicle-screw system both in the intervention group and the control group without any clinical effect. This bone possibly formed in response to the mechanical stimulus generated by the dorsal fixation system.

Further research is clearly needed to analyze the exact mechanism of action of rhBMP-2 and to evaluate if overzealous/heterotopic bone formation can be avoided if rhBMP-2 is safely contained in the matrix and if exposed cancellous bone is occluded – and if this is technically feasible in all cases. It has to be evaluated which tissues are prone to the effect of rhBMP-2 and if e.g. fascia build a safe barrier.

4.3.3 Cage Migration

In the present study one hypothesis was that temporary bone resorption due to the action of rhBMP-2 leads to a loosening of the cage and hence cage migration. In the setting of lumbar interbody fusion, cage migration is a complication feared by surgeons because it might lead to or reflect spinal instability, and in rare cases the cage can even slip

posteriorly into the spinal canal compressing the neural structures. Usually, three possible reasons for cage-related complications in spinal interbody fusion exist: wrong placement, insufficient fixation, or failure of fusion (90). Cage malposition and undersizing or oversizing of the implant – might lead to cage migration and frequently also to cage subsidence.

In the present study, the incidence of cage migration was relatively high in both the control and the intervention groups. This suggests that the cage design was possibly inappropriate to maintain its position for several reasons. Firstly, the implant was relatively small compared to the surface of the endplates (cf. "4.6 Cage Design"). Due to the inflexible facet joints in the sheep disc space, distraction was very limited and therefore, only a small cage could pass. Since the cage covered only around 10% of the vertebral endplates it could not be ideally placed on the ring apophysis. Additionally, the cage design was very simple, e.g. there were no teeth to provide a better hold to the endplates.

It remains difficult to compare the migration rate found in the present study to other studies since the devices and techniques vary widely (cf. "4.1 Variations in Technique and Results Evaluation Hinder a Systematic Comparison of Study Results"). In the present study, the definition of cage migration was very strict and looked for on axial CT images in the follow-up using a sophisticated technique. Many studies rely on standard X-ray images to observe cage migration and in some cases post-operative images are not available to directly compare the cage position in the follow-up. An interesting finding was that even though the cage migration rate was high in the control group, it was even higher in the rhBMP-2 groups. Additionally, higher migration rates were observed in the intervention groups if a higher concentration and dose of rhBMP-2 was used. This suggests that temporary bone resorption due to rhBMP-2 may lead to a higher risk of cage migration and intermediate spinal instability. This phenomenon should be further examined in future studies.

Cage migration usually occurs in the early postoperative course and can remain asymptomatic. Sherman et al. (91) concluded from the findings in their study on lumbar interbody fusion in the sheep that cage migration took place early in the post-surgical course and was probably due to the lack of additional instrumentation. Our findings show that additional dorsal fixation is not sufficient to fully prevent cage migration – again the possibly unfavorable cage design has to be remembered. Unfortunately,

Sherman et al. (91) do not report the percentage of cage migration in their study on lumbar interbody fusion in the sheep model. Kim et al. (92) report an additional high risk of delayed union or nonunion associated with cage migration in the clinical setting which could not be proven in the present study.

Smoljanovic et al. (93) agree that the incidence of implant displacement/loosening and subsidence (possible clinical consequences of bone resorption) was greater in the rhBMP-2 group compared to the control group according to the FDA approval data (17). Vaidya et al. (52) as well as Knox et al. (51) also found an association between bone resorption due to rhBMP-2 application and cage migration. In their retrospective study on osteolysis and associated complications in transforaminal lumbar interbody fusion with rhBMP-2, Knox et al. (51) analyzed postoperative CT images of 57 patients. They found cage migration in 8.8% of the specimens. All patients with cage migration suffered from moderate or severe osteolysis. Their finding that the incidence and severity of cage migration did not change during the later months after surgery is in line with the findings in the present study since cage migration did not change in the follow-up after 3 months. The strength of Knox et al.'s (51) study is that consecutive patients received routine CT scans postoperatively as well as at their 3-month and 6-month postoperative follow-up. A total dose of 5 mg rhBMP-2 in a concentration of 1.5 mg/mL per level was applied and no additional sponges or rhBMP-2 were placed outside the PEEK Capstone/Perimeter cage. They finally concluded that "Cage migration is a potential complication from postoperative osteolysis and does not appear related to the severity of osteolysis" (51). In our study, cage migration occurred even if no osteolytic defects were apparent on the follow-up CT images. It is possible that the defects were already resolved by 3 months after the intervention (cf. "4.3.1 Bone Resorption"). Future studies should therefore plan the first follow-up imaging at an earlier time point, probably between 4-6 weeks after surgery.

From the findings in our study we suggest that rhBMP-2 causes transient postoperative spinal instability due to postoperative local bone resorption, which is best reflected by an elevated rate of cage migration. Given that even a low dose of rhBMP-2 led to a higher risk of cage migration compared to the control group, this adverse effect should be observed continuously in postsurgical controls. A standardized technique is needed to truly find all cases – even less pronounced ones – of cage migration.

4.3.4 Maintenance of the Disc Space Height

During spinal interbody fusion surgery, the disc space height is reconstructed by distraction to relieve the nerve roots and a cage is implanted as a spacer. Postoperative disc space height reduction might lead to compression of nerve roots exiting through the neural foramen and thus lead to neurological deficits. The disc space height is consequently an important factor for the overall surgical result. In our study the disc space height was rarely fully preserved. However, it is important to bear in mind that CT imaging allows a more detailed analysis of the disc space height than X-ray images and that stringent criteria were applied to evaluate the reduction of the disc space height. Thus, even minor changes of the disc space height, which would possibly remain clinically silent, were reported in the present study. Additionally, a single cage placed somewhat laterally on the endplate, like in the present study, might not be sufficient to reliably retain the disc space height.

Interestingly, the incidence of disc space height reduction in the present study was higher in the intervention groups and showed a dose-dependent pattern with a higher incidence of disc space height reduction if a higher dose of rhBMP-2 dose was used. However, the extent of disc space height reduction was not dose-dependent. The disc space height did not further change in the follow-up after 3 months. Disc space height reduction was probably partly due to cage migration which naturally results in instability of the segment and compromised ligamentotaxis. Since cage migration was observed more often in the intervention group than in the control group and was more frequent if a higher concentration/dose of rhBMP-2 was applied, it is possible that an initial osteoclastic activity triggered by rhBMP-2 results in an instability and loosening of the cage in the intervertebral disc space. It is difficult to compare the disc space height maintenance in the present study to former studies since no consensus on the exact definition or the assessment criteria exists (cf. "4.1 Variations in Technique and Result Evaluation Hinder a Systematic Comparison of Study Results").

Technically, it is not advisable to measure the disc space height as described by Easley et al. (94) if rhBMP-2 is used, since the vertebral bone structure can be altered significantly by the (resorptive) effect of rhBMP-2: "The disc height measurements used were collected by first identifying the four 'corners' of the disc located on both the anterior and the posterior edges of the two adjoining end plates […]."

In conclusion, disc space height reduction along with cage migration and cage subsidence might be appropriate indirect markers of intermediate bone resorption in interbody fusion when rhBMP-2 is used.

4.3.5 Cage Subsidence

Cage subsidence, meaning that the cage sinks into one or both adjacent vertebral bodies, can lead to a loss of structural stability (60) if ligamentotaxis is compromised. Clinically, this adverse effect can increase the incidence of nonunions. At the start of the present study, it was hypothesized that bone resorption at the vertebral endplates due to rhBMP-2 could give way to a sinking of the cage. It was found that the incidence of cage subsidence was relatively high both in the control group as well as in the intervention groups, suggesting that it was partly influenced by factors other than rhBMP-2, e.g. physiological postoperative bone remodeling at the endplates, the cage design with thin walls and a small load bearing area and the endplate preparation.

Still, cage migration was more frequent in the interventional level than in the control group. The subsidence rate in the intervention group was lowest in group 2 in which it was comparable to the control group. The highest dose used in the present study (2.0 mg/mL) resulted in a higher incidence of subsidence, whereas the extent of cage subsidence was not dose-/concentration-dependent. This is in line with Fu et al.'s (26) report on two small single-center cohort studies in which higher rates of implant subsidence were found in patients who received rhBMP-2 for ALIF (95,96). Vaidya et al.(95) report subsidence in ALIF in 70% (14 of 20) of the cases with BMP-2 and 6% without BMP-2. They are convinced that the increased incidence of subsidence associated with rhBMP-2 occurred due to bone resorption at the endplates triggered by the growth factor. Sethi et al. (50) agree from the findings in their clinical study on rhBMP-2-assisted ALIF that "[…] subsidence may be attributed to the resorption of endplates, allowing penetration of the cage into the vertebral body." In their study, they report a subsidence rate of 50% at 3 months after surgery. Unfortunately, they only indirectly evaluated subsidence: "Radiographically, subsidence presents as narrowing of the n space […]." An advantage of the present study is that cage subsidence was evaluated by directly comparing the cage position immediately after surgery with its position in the follow-up on coronal CT images and in detail on thin-cut CT scans (cf. "2.6.3 CT Interpretation") and that is was

not defined as any reduction of the disc space height. On X-ray images, only severe cage subsidence if any is visible. Therefore, cage subsidence might be underestimated in clinical studies, but this preclinical study cannot predict if even minor cage subsidence leads to clinical symptoms. If the cage migrates, it is difficult to define and measure cage subsidence. In the present study, cage subsidence was measured at the final position of the cage. Another factor which can hinder an adequate evaluation of cage subsidence is newly formed bone around the cage. If no postoperative images are available, this bone formation can easily mimic cage subsidence.

In the present study, the cage design has possibly contributed to the relatively high incidence of cage subsidence for several reasons (cf. "4.3.3 Cage Migration"). According to Polikeit et al. (97), cages should be designed to rely on the strong peripheral portion of endplates (ring apophysis) and offer a large volume for the graft and growing bone column. Additionally, the cage material and design probably influence the cage subsidence rate. Le et al. (98) suggest that a large anterior-posterior diameter of the cage and a smaller intraoperative distraction of the vertebral segment might be helpful to prevent cage subsidence. In the present study, the cage was too small to be mainly based on the ring apophysis (cf. "4.6 Cage Design"). The relatively large cage pore allowed the ingrowth of a large bony column. Lee et al. (99) noted that "[…] the success of the fusion and the fused area inside cages are more important than the cage footprint per se in terms of final subsidence amount […]". Additionally, Willliams et al. (60) associated subsidence with an increased incidence of failed fusions due to cage migration caused by the loss of mechanical structural support. In the present study, the fusion status correlated neither with cage migration nor cage subsidence.

Sethi et al. (50) report that cage subsidence occurs between six weeks and three months after surgery. According to the findings in our study we agree that bone resorption occurs in the early postoperative phase. Unfortunately, the first follow-up CT image was not taken until three months after the intervention. Cage subsidence did not continue after three months.

According to a protocol proposed by Ha et al. (100) subsidence, was initially measured on coronal reconstructions. However, after measuring several anatomical structures on follow-up images, the reliability of the measurements highly depended on the exact positioning of the animal as well as choosing the same sectional plane in the follow-up.

In conclusion, there is a need for a consensus on the definition of subsidence, even though it might not be an ideal quality marker for the overall result of spinal interbody fusion. Additionally, the concentration- and dose-dependent effect of rhBMP-2 on cage subsidence needs to be further explored.

4.4 Thin-Cut CT Imaging as an Evaluation Tool

For lumbar interbody fusion assessment, a wide variety of individual criteria exist. In many cases, X-ray images are used for evaluation. However, until now, no expert consensus on the appropriate non-invasive evaluation tools and criteria for the assessment of spinal interbody fusion exists (66). Surgical exploration is clearly the most reliable method to diagnose spinal nonunion (66,101). However, in most cases, it is impractical because of its invasiveness. Commonly applied radiological investigations include static and dynamic radiographs, computed tomography, and (rarely) magnetic resonance imaging (102).

Although postoperative imaging is an integral part of many follow-up protocols, no consensus regarding the definition of radiographic successful interbody fusion and the best diagnostic imaging modality for its assessment exists (66,103,104). Unfortunately, valid data regarding imaging diagnosis of spinal nonunions, e.g. trials to compare imaging results from surgical exploration, are sparse. Currently, the favored technique is CT imaging because it is more reliable than plain or flexion-extension radiographs in evaluating the fusion status in spinal fusion (60,103,105–107). Bio-mechanical evaluation methods, e.g. dynamic X-ray, are often used to assess spinal fusion in pre-clinical and clinical studies. However, Lee et al. (108) conclude in their clinical study on PLIF in which they compared dynamic radiographs and thin-section three-dimensional computed tomography to evaluate the fusion status that "[…] assessment using plain radiographs is inappropriate." Furthermore, they found that "For an objective and accurate assessment of fusion after PLIF surgery, it would be more appropriate to look for interbody bridging bone formation at 12 months by 3D thin-section CT rather than dynamic flexion-extension." The editors correctly point out that the advantages of CT imaging mentioned have to be balanced against a high radiation exposure of the patient and increased cost. Therefore, it is not advisable to use this technique for frequent follow-up imaging. CT imaging is often not applied in routine follow-up but only if clinical evidence for complications exist. Clinical outcomes are reported to correlate poorly with

the observed CT-morphological fusion status (108). However, since the exact effect of rhBMP-2 in spinal fusion is still under debate, imaging contributes significantly to the available data and should be made in the follow-up to exclude (clinically unapparent) adverse events and to intervene if indicated (65). Interestingly, in many published articles, images are not provided along with the publication. Thus, it is difficult to form an independent opinion of the morphologic fusion results. In many clinical studies, X-ray imaging and subjective questionnaires are the only evaluation tools used to assess fusion results. In the context of an experimental technology, more sophisticated technologies should be used to reveal possible (unknown) complications.

In the present study, thin-cut CT imaging was chosen to evaluate bone structures in the follow-up. Thin-cut CT imaging is a standard clinical method and regarded as the "best available" (109) technology to assess spinal fusion, since it provides high resolution images without an overlay effect present on plain radiographs. Metallic cages or instrumentation may create artifacts impeding an accurate interpretation of CT scans (110,111). However, if a radiolucent PEEK cage is used – as in the present study - artifacts on the CT scans are avoided and the fusion status in the cage pore can be evaluated more easily. CT scans are widely used to diagnose spinal nonunion. Compared to plain radiographs, the high resolution images provide more bony details and the location and status of the fusion mass can be better assessed (102) as well as possible adverse events (e.g. cage subsidence, overzealous bone growth) without the above mentioned overlay effect. Although plain axial CT images are probably not sufficient to detect transverse clefts in a fusion mass (112), the advent of spiral CT scan thin-slice images and multiplanar reformatted images further improved its accuracy (113). CT scans, including reformatted images, have been proven to provide more information regarding spine fusion status than plain radiographs (107,114–120) and are essential for a more detailed analysis of the surgical outcome. Additionally, a more thorough assessment of peri-implant lucencies and bone formation within the interbody device (71), especially if it is made of a radiolucent material like PEEK, is possible. Haid et al. (27) agree that CT scans are the preferred mean for cage placement assessment. MRI imaging is not an appropriate imaging method to assess bony structures. In 2011, Sethi et al. (50) contributed decisively to establishing evaluation criteria for the analysis of radiographic and CT imaging results when rhBMP-2 is used. They saw the need for establishing consistent radiological evaluation criteria. In conclusion, CT scans are

commonly used to assess spine fusions and may be considered the preferred radiographic method (71,121).

In their study on the reliability and accuracy of thin-cut computed tomography scans to determine the fusion status of ALIF, Carreon et al. (122) compared the radiographic classification by five spine surgeons with the findings on surgical exploration. They concluded that reviewers usually overstated fusion according to the images. However, in the present study, metallic cages were used so that artifacts on the CT images impeding the assessment of the fusion status could not be avoided. In their study, Lee et al. (108) compared fusion results on plain radiographs with thin-section three-dimensional computed tomography after PLIF. They concluded that thin-cut CT is an adequate tool for the assessment of fusion. The advantage of CT imaging is that it is non-invasive and allows direct assessment of the bony bridge. According to Santos et al. (107), radiographs tend to over-estimate the presence of successful fusion compared to thin-slice CT scans with sagittal and coronal reconstructions in ALIF using carbon fiber cages and autologous bone. However, in their study, fusion assessment was not compared to surgical exploration results.

In a histological, radiographic, and CT imaging study with 31 pig-tail macaques undergoing interbody fusion with autologous iliac crest bone and titanium interbody fusion device, Cook et al. (123) found CT imaging techniques to be superior to radiographs in determining the presence of histologically-controlled fusion in ALIF. They observed that CT imaging results were in line with histological analysis in 24 of 29 cases (83%), whereas radiographic assessment was adequate compared to histological findings in only 12 of 29 cases. In their cohort study on PLIF and posterolateral fusion to compare fusion assessment using CT and radiographs compared to surgical exploration, Fogel et al. (104) examined 90 consecutive patients of whom 54 underwent CT-scanning. Successful interbody fusion was defined as bridging bone between vertebral endplates filling half of the fusion area. Fogel et al. (104) demonstrated excellent sensitivity (100%) and similar values of specificity for plain radiographs and CT scans in diagnosing nonunion of PLIF in the presence of radiolucent cages (104).

Unfortunately, CT images cannot give detailed information on the composition of the tissue. Therefore, it will be important to compare the findings of the present study to the histological results (discussed elsewhere). Although micro-CT images would have

provided a more detailed analysis of the newly grown tissue, it was not applicable in the present study for multiple reasons. The specimens needed to be decalcified immediately after sacrification for histological analysis which renders a micro-CT analysis impossible. Furthermore, it would not have been possible to take micro-CT images at several follow-up time points since only small specimens can be analyzed and it could not have been performed in vivo.

Bone density measurements could provide additional information on details of the bone bridge after spinal fusion. However, measuring Hounsfield Units, the quantitative scale for describing radiodensity in CT images, as suggested by several authors (124,125), did not prove to be precise in the present study. In the present study no phantoms were used to calibrate density measurements. Generally, it is essential for bone density measurements on CT images to include phantoms with standardized density and calibrate measured HU values accordingly (126). Interestingly, the calibration process is not mentioned in detail (61,125) or not at all (61,112) in some publications, leaving uncertainty if it was conducted.

In the present study, it was not possible to find adequate regions of interest (ROI) to measure and compare bone density in the follow-up:

- Bone density in the cage pore could not be measured because the area of interest was too small to compensate for possible inaccuracy of data
- Calculated mean values of several successive CT images to compensate for possible variations could not be generated because of several artefacts (e.g. dorsal fixation system)

An interesting addition for the measurement of distances (e.g. cage migration, cage subsidence) would be to assess 3D CT image reconstructions. They could provide a good impression of the fusion mass in the cage pore. Additionally, linear distance (e.g. cage migration/subsidence) could be measured in the three-dimensional space and would not be reduced to a planar image, potentially leading to inaccuracy. Whyms et al. (127) investigated the effect of 3D-CT volume rendering techniques on the accuracy of linear, angular, and volumetric measurements. They concluded that linear measurements were accurate compared to the true anatomic measurements, irrespective of scanner parameters or rendering technique. In this context, it is important to point out that a stable

position of the spine on all follow-up images is essential to warrant comparable measurements.

Since the effect of rhBMP-2 was well advanced at the 3-month control and since several studies declare that the growth factor is most effective between two and four weeks after surgery (53,128), future studies should take the first follow-up CT image earlier than in the present study. Already in 2009 Smoljanovic et al. (129) pointed out that early postoperative CT-imaging (one to three months after surgery) will prove helpful in visualizing resorptive defects which are generally not visible on plain radiographs. Additionally, findings on CT images should be correlated with the clinical outcome. In the clinical setting, the first follow-up exam including imaging is usually not performed before 6 months postoperatively because it generally aims at assessing the fusion status rather than adverse effects unless the patient presents clinical symptoms suggesting surgical complications.

In conclusion, thin-cut CT imaging with multiplanar reconstruction was the best applicable method to observe the development of spinal fusion and adverse events in vivo in the postoperative course in the present study. Multiplanar reconstructions provide a coherent impression of the fusion status and adequate images to assess associated complications.

4.5 Animal Model

Animal models are helpful to systematically analyze new surgical approaches and technologies. It is known that rhBMP-2 shows species-specific differences in its action. Nonhuman primates have the best homology with humans (size, upright posture, genetic makeup) and are considered the most valid and critical preclinical model (130). However, due to massive ethical concerns ruminants are preferred in many cases since dimensions of the vertebral bodies are closer related to human beings than in rodents. Additionally, experience regarding the application of rhBMP-2 in sheep exist. The surgical technique applied in human beings has to be adjusted to be practicable in the sheep. Mageed et al. (131) analyzed the anatomy of the ovine spine and found good similarity to the human spine in terms of vertebral endplates and spinal canal dimensions. Differences exist regarding dimensions of the vertebral bodies. Additionally, the sheep lumbar spine is reported to have biomechanical similarities to the human spine (132–135). Wilke et al. (136) and Smit (133) proved that the loading of the quadrupedal spine shows

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more similarity to humans than one would expect. A dorsal fixation system was used to reduce the effect of loading differences in the sheep spine. According to Foster et al. (137), the sheep model is the model of choice if pedicle screws are required because the pedicles in other animals are too thin. Turner (138) agrees that "Sheep have become a convenient, economical and practical large animal model for orthopaedic research. For spine surgery, they are useful for investigating fusion devices, growth factors and their carriers, instrumentation methods, vertebroplasty-kyphoplasty models, disc replacement and vertebral body corpectomy." An interesting remark by Turner (138) is that skeletally mature animals should be preferred. He usually uses sheep which are older than 3.5 years. In the present study 2-year old sheep were used. Hence, they might not have been fully skeletally mature and were evidently not suffering from degeneration of the spine.

In conclusion, animal models are helpful in medical research to provide a basic and systematic understanding of experimental procedures although its limitations have to be considered when the results are interpreted. Following "The Three Rs" (replacement, reduction, refinement) suggested by Russell and Burch in 1959 (139,140), animal testing should be avoided for ethical reasons whenever possible. Joint research center like the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) were established to actively support the development, validation, and acceptance of methods to replace, reduce, or refine animal testing (141).

4.6   Cage Design

In the present study, a PEEK cage with a simple design was chosen (cf. "2.3 Cage Design and Material"). PEEK has an excellent modulus of elasticity, closely resembling that of cortical bone, and is radiolucent, avoiding artifacts on CT images and allowing an unobstructed look into the cage pore (cf. "4.4 Thin-Cut CT Imaging as an Evaluation Tool"). As Sethi et al. (50) agree PEEK cages allow "[…] a clearer, unobstructed radiographic view of new bone formation during follow-up examinations […]" and are "[…] ideal spacers to study the imaging cascade of changes [after spinal fusion surgery]." Since it was important in the present study to observe the changes inside the cage pore in the follow-up, the radiolucent material turned out to be well-suited. Schimmel et al. (142) report in their study on PEEK cages for ALIF that 23 of 26 patients had to be re-

operated because of symptomatic spinal nonunion. They suggest that PEEK cages might lead to insufficient initial stability compromising bony bridging. However, in the cited study, no dorsal fixation system was used to improve immediate postoperative spinal stability. Toth et al. (143) concluded from their study on lumbar interbody spinal fusion using a PEEK threaded interbody fusion device packed with either autograft or rhBMP-2 on an ACS that "[…] PEEK may be a useful biomaterial for interbody fusion cages due to the polymer's increased radiolucency and decreased stiffness." The observed mild chronic inflammation around the PEEK implant was unfortunately not further discussed.

The cage design in the present study was chosen to provide sufficient mechanical support, while leaving a relatively large cage pore to place the rhBMP-2-soaked ACS (intervention group) or ACS only (control group) and allow bone ingrowth. The cage covered only around 10% of the vertebral endplates (based on CT-morphological measurements) and was therefore undersized according to current recommendations to completely place it on the strong ring apophysis of the vertebral body (144). Thus, the resulting bone column inside the cage pore was relatively small compared to the intervertebral disc space volume. Kandziora et al. (145) analyzed the effect of cage design in spinal interbody fusion. They showed in their in-vitro and in-vivo study that the endplate-implant contact area did not determine the subsidence rate of the cages (Harms cage vs. SynCage-C). However, the maximal contiguous cage pore correlated significantly with bone formation inside the cage and spinal stability in vivo. Similarly, Kanayama et al. (146) reported that a larger cage pore leads to a reduction of "stress shielding" resulting in a stimulation of bone formation. In future studies, the cage should probably be larger to lie mainly on the ring apophysis so that the mechanical load is evenly distributed in the vertebral segment. However, a larger cage will be difficult to insert into the ovine disc space because the inflexible facet joints hinder an adequate distraction of the disc space. Additionally, the cage position plays an important role for the resulting bone column and final stability. In the clinical setting it is uncommon to position the cage mainly on one lateral side of the vertebral endplates (in the present study at the right side). Still, according to Denis' (147) three-column theory, the segments in our study can be assumed to be stable because of the addition of a bilateral dorsal fixation system.

Subsequent studies should include visible markers (e.g. titanium markers in the inner and outer circumference as well as at the inferior and superior rim) in the (radiolucent) PEEK

cage to better assess its position on CT images. In the present study, it was challenging to identify the cage and its position, especially when it was not surrounded by new bone to be silhouetted against the intervertebral disc space.

Two out of seven OxPEKK cages broke (cf. page 59). This material thus might be inappropriate to resist the applied intraoperative/postoperative mechanical load if designed like in the present study.

In conclusion, the cage design was not ideal for the present study and could partly be responsible for the relatively high incidence of cage migration found even in the control group (cf "3.3 Change in Device Position (Cage Migration)").

4.7   Control Group

The present study was performed using intra-individual controls. Specifically, the same surgical procedure was performed both in the interventional and in the control spinal level. However, at the control level no osteoinductive material was added. Consequently, confounders inherent to other osteoinductive materials were eliminated. Additionally, it was possible to analyze the incidence of adverse events, e.g. cage migration, cage subsidence and disc space height reduction independent of other substances – including bone autografts – interfering with the local bone metabolism. It is important to note that the approach at the control level does not correspond to the "gold standard" (autologous bone graft) and that the fusion rate at the control level was therefore expected to be inferior to the interventional level. Generally, it is important to note that the disc space height to be bridged is relatively small in sheep which might partly explain why the fusion results in the control group were better than initially expected if no osteoinductive material is used.

The study design included a randomization regarding the vertebral level at which either procedure was performed. This aimed at excluding any possible impact of mechanical differences at the spine segments as confounder. Intra-individual control groups are an interesting approach to reduce the number of animal testing. RhBMP-2 is proven to have a short systemic half-life and therefore probably does not have any systemic effect if applied on a collagen sponge (148). Thus, the growth factor is expected to be effective only locally at the lumbar segment where it was applied, not influencing the control level.

To reduce a possible effect per continuitatem and to balance mechanical loading, a vertebral level was left untouched between the interventional and control level (manipulation of levels L2/3 and L4/5 but not L3/4).

4.8   Device vs. Drug - Concentration and Total Dose Matter

The FDA states on its homepage: "This definition [meaning the definition of a medical device] provides a clear distinction between a medical device and other FDA regulated products such as drugs. If the primary intended use of the product is achieved through chemical action or by being metabolized by the body, the product is usually a drug" (149). Although the effect of Infuse® is mainly achieved by the biochemical cascade triggered by rhBMP-2, it was accepted as a medical device for the approval in 2002 (17). Consequently, Infuse® was handed like an "implant" for off-label indications by many surgeons (9). From a biomedical point of view, it is not surprising that the concentration as well as the total dose play a decisive role for the clinical effect of the growth factor rhBMP-2. However, in multiple studies, information on these two factors was not provided. Interestingly, e.g. Pradhan et al. (96) did not report the dosing of rhBMP-2 in their study and still their report was accepted in a peer-reviewed journal. Even in Medtronic-sponsored studies the concentration and total dose of rhBMP-2 varied (concentration of 1.5 mg/mL with total doses ranging from 0.6 to 16.8 mg, higher concentrations and total doses in some PLIF studies). Fu et al. (26) conclude in their systematic review that "Information to adequately evaluate the effects of dose on risk for effectiveness and harms was also insufficient." Ultimately, Fu et al. (26) could not determine the effects of rhBMP-2 dosage because of differences in surgical approach, rhBMP-2 carrier, and fusion hardware (cf. "4.1 Variations in Technique and Result Evaluation Hinder a Systematic Comparison of Study Results").

In the present study, the effect of three different concentrations – resulting in three different total doses – of rhBMP-2 in lumbar interbody fusion was assessed. In the sheep, rhBMP-2 was used in a concentration of 0.43 mg/mL on an ACS in multiple trials (150). However, even after the analysis of publicly available data and the FDA approval data (17), it remains difficult to understand how this concentration was determined. Additionally, there is no recommendation on the total dose to be applied in sheep.

In our clinical study bone resorption was observed if a total dose of 12 mg rhBMP-2 per disc space was used (45). Since the volume of the ovine disc space is only around one third that of humans, a total dose of 4 mg rhBMP-2 per disc space was used in the pilot study. Based on this high dose, three lower doses were used to find the safest dose. The lowest concentration used in the present study was approximately the concentration recommended for sheep (0.5 mg/mL vs. 0.43 mg/mL).

However, a volume of 1.0 mL might have led to a local total dose which was too high or the ACS might have been oversaturated: According to the instructions provided by Medtronic, it is recommended to distribute 1.4 mL of the dissolved rhBMP-2 on a collagen sponge of 2.5 cm x 5 cm (=12.5 cm²). This equals 1 mL/8.92 cm². In our study a collagen sponge of the same thickness measuring 1 cm x 5 cm (=5 cm²) was used. The recommended volume for an ACS of 5 cm² would be 0.56 mL. Therefore, the volume in our study was higher by a factor of 1.79 than the recommendation (=1.0 mL/0.56 mL). It is not clear if an over-saturated ACS leads to a changed release rate of rhBMP-2. Interestingly, the FDA approval data state that "[…] the release of rhBMP-2 in vivo is independent of the binding of rhBMP-2 to the sponge in vitro, and may be diffusion-controlled" (17). Thus, it remains unclear if an oversaturated sponge influences the dosing in vivo. According to the FDA approval data, the relative retention of rhBMP-2 is unaffected by the concentration of rhBMP-2 administered (17). The amount of rhBMP-2 incorporated into ACS had a minimal effect on rhBMP-2 retention in vivo, and no effect on the rate of release of rhBMP-2 into serum in vitro.

The total doses applied in the present study are high compared to current clinical recommendations that even low doses of rhBMP-2 might sufficiently enhance spinal interbody fusion. However, in preclinical studies, low concentrations and total doses of rhBMP-2 were associated with unfavorable fusion results (17). In the present study, even the lowest concentration of rhBMP-2 (group 1: 0.5 mg/mL) led to massive bone resorption and overzealous new bone growth in two specimens. Two comparable cases were observed in group 3 but not in group 2. The present study could therefore not prove that the incidence of adverse events with rhBMP-2 application is purely concentration- or dose-dependent.

In summary, we could not establish a safe concentration or dose for use in ovine lumbar interbody fusion. This finding is in line with clinical studies in which bone resorption at the

vertebral endplates was observed even when rhBMP-2 was used at the clinically recommended concentration of 1.5 mg/mL (45,151). It was consequently suggested that the total dose applied in these studies was too high and responsible for the complication. The results from the present study suggest that high concentrations and high doses of rhBMP-2 can be associated with a high risk of (transient) bone resorption and other – probably related – adverse events in lumbar spinal interbody fusion surgery. The systematic analysis of possible adverse events – bone resorption, overzealous bone growth, cage migration, cage subsidence and disc space height reduction – suggests that the effect of rhBMP-2 risks transient instability of the spine in the postoperative course. In the clinical setting this calls for additional intraoperative stabilization, e.g. dorsal fixation system, to improve postoperative spinal stability. Unfortunately, it cannot ensure complete spinal stability since 20% of the load will still be exerted on the vertebral bodies and cage migration can probably not be fully avoided.

Studies have shown that the effect of rhBMP-2 highly depends on the species studied (67). Thus, as for many pharmaceuticals, it is not possible to directly extrapolate information on the dosing from the sheep model to humans. However, the results of the present study prove that intra-individual differences in the reaction to and effect of rhBMP-2 – just like any other drug – could possibly exist and should be further analyzed. Fu et al. (26) conclude from their systematic review with access to individual patient data that the existing data on the dosing of rhBMP-2 for interbody fusion are incomplete and that no definite recommendations on the effective and safe dose can be made. Bolesta (152) agrees that "The optimal dose is influenced by the surgical site, the surgical approach, and patient-related factors. Clinical judgment comes into play." Klimo et al. (86) state that the application of universal doses is oversimplified and that – just like for any pharmaceutical – inherent patient biology plays a significant role for its effect regarding its safety as well as the adverse events. They further elaborate that patient size and gender might be relevant.

Many clinical off-label studies reflect the uncertainty about the adequate rhBMP-2 dose to be used for spinal fusion. Several publications on associated – partly severe – adverse events exist. After clinical approval, rhBMP-2 was frequently used in doses much higher than the recommended dose according to the saying "the more the better." The manufacturer evidently also believed that increasing the concentration and total dose of

rhBMP-2 would further decrease the fusion time and improve the fusion results because FDA-approval was sought for a new product containing a higher concentration and a very high total dose of rhBMP-2 (AMPLIFY; 2 mg/mL, 40 mg per level, carrier comprised of collagen and resorbable ceramic). However, approval for this preparation was rejected by the FDA because of increased cancer rates in the intervention group compared to the control group, which were possibly related to rhBMP-2 (25).

After serious adverse effects associated with rhBMP-2 were reported, most surgeons either stopped its application or used a decreased dose (153). However, it is not yet proven that low doses of rhBMP-2 can fully prevent bone resorption, since the underlying mechanism of action is suggested to always comprise a coupled action of osteoclastic and osteoblastic activity, reportedly initiated by osteoclastic action. It is important to achieve a balance between the action of osteoclasts and osteoblasts. Another factor possibly influencing but neglected by many surgeons is that if Infuse® is used with autogenous bone grafts containing bone cells and mediators themselves it probably changes the local total dose of the growth factor (154).

In conclusion, according to the current literature and the findings in the present study, Infuse® should be regarded and handled as a drug rather than a medical device. It is important to further evaluate its concentration-/dose-dependent effect on spinal interbody fusion.

4.9   Reports on Adverse Effects

The reported benefits and extremely positive initial publications on rhBMP-2 led to its frequent application in spinal fusion. Although rhBMP-2 was (FDA-/EMA-) approved only for a specific spinal fusion technique, widespread off-label use of rhBMP-2 was reported over the past decade (155). This is clearly mirrored by Mesfin et al.'s (48) statement that "[…] rhBMP-2 is routinely used off-label for posterolateral fusions, posterior lumbar interbody fusions, transforaminal lumbar interbody fusions, and cervical fusions." According to the American Nationwide Inpatient Service (NIS) database, 328 468 spine fusion procedures were performed between 2002 and 2006, and in 45 871 cases rhBMP-2 was used. Since its FDA-approval in 2002, the use of rhBMP-2 has substantially increased from 429 cases (0.69% of all fusions) to 17 623 cases in 2006 (24.3% of all primary spinal fusions and 36.6% of revision fusions) (156). Vaidya (157) clearly states

into which trap many spine surgeons have fallen: "We have used it [rhBMP-2] in ways that were not originally approved by the FDA because we felt if it works so well for one indication; why not try it for others. Many of us read early articles on off label use which showed the results were excellent in the c-spine [cervical spine] and in PLIF or TLIF surgery."

Some of the reported clinical complications could have possibly been avoided if more attention had been paid to early studies on the safety profile of rhBMP-2, including in-vitro studies. As early as 2002, Poynton et al. (54) published a study on the safety profile of rhBMP-2. They concluded that rhBMP-2 is safe in spine surgery if applied accurately and if the carrier material adequately retains the protein. Interestingly, they already mentioned bone resorption as a possible adverse side effect but concluded that "Osteoclastic overstimulation does not appear to be a significant problem with rhBMP-2" (54). Furthermore, some study results were initially withheld from public. Later, it became public that already in 1999 a trial by Haid et al. (27) was halted by the FDA because heterotopic bone formation in the spinal canal was observed in 75% of the patients (24 of 32) who received rhBMP-2/ACS (absorbable collagen sponge). Interestingly, their study remained unreported until 2004. McKay et al. (85) reported in 2002 that heterotopic bone formation adjacent to the cages and even along their insertions tracts was present in the human posterior lumbar interbody fusion when rhBMP-2 was used. They stated that "It is not clear whether the formation of this bone is technique related" (85). The study was reported to have been halted temporarily.

The evident discrepancy between the results of case reports with severe complications and the extraordinarily positive results of first publications on Infuse® in which no adverse side effects were reported led to doubts on the initial trials. Carragee et al. (25) critically reviewed the available data on the clinical use of rhBMP-2 in spinal surgery and conclude the following: "Notably, with each new industry-sponsored trial publication the safety findings were identical: no reported observed adverse events associated with rhBMP-2. Given that 780 patients received rhBMP-2 in these industry-sponsored publications and that not a single adverse event had been reported, the estimated risk of rhBMP-2 use could be calculated to be less than 0.5% with 99% certainty. That is, the reported risk of an adverse event with rhBMP 2, based upon the industry-sponsored data, was less than 1/40th the risk of a course of commonly used anti-inflammatory or antibiotic medications."

E.g. in 2001 Kleeman et al. (158) report in their first clinical study on rhBMP-2 application for lumbar interbody fusion that "[…] 95% (21 of 22 [patients]) were available for follow-up; 100% were satisfied with treatment at 12 months. Concerning their symptoms, 100% reported relief of back pain, 100% had improvement of leg pain, and 100% described significant functional improvement." These extraordinarily positive results seem unrealistic. The reported complications show that rhBMP-2 is a highly potent pharmaceutical drug rather than a medical device – as which it was approved by the FDA – and that it can potentially cause severe adverse side effects and even lead to death in extreme cases (e.g. due to throat and neck swelling in cervical spinal fusion). In 2010 Smoljanovic et al. (93) concluded from the FDA premarket approval data of rhBMP-2 that the incidence of implant displacement/loosening and subsidence possibly as a consequence of bone resorption were higher in the rhBMP-2 group compared to the control group. Additional safety concerns associated with the use of rhBMP-2 for spondylodesis exist. Postoperative imaging e.g. showed an increased risk of local inflammation, edema, wound problems, and infections (37,44,52,159). Furthermore, BMPs might have a negative effect on exposed dura and nerves. The incidence of retrograde ejaculation was reported to be higher if rhBMP-2 is used (160,161).

According to Carragee et al. (25) "[…] it was clear at the time [of clinical approval] that the nature and diversity of adverse events could not be well predicted given that rhBMP-2 appeared to be involved in a multiplicity of physiological and pathological events, including, but not limited to, inflammatory response, bone induction and resorption pathways, abnormal growth signaling pathways, certain malignancy pathways and induction of an altered immune response." In summary, further studies on the safety profile of rhBMP-2 for spinal interbody fusion surgery are needed.

4.10   Limitations of the Present Study

In the present study only a small number of specimens per dosing group were analyzed. In the intervention groups, only 11 sheep per dosing were analyzed. It was therefore not possible to generate statistically significant results.

CT images provide a helpful insight into the effect of rhBMP-2 in spinal interbody fusion. In this preclinical study, it was not possible to correlate the clinical outcome with the presence of bone resorption, overzealous bone growth or other analyzed adverse effects.

As mentioned earlier (cf. "4.8 Device vs. Drug – Concentration and Total Dose Matter"), the doses and concentrations of rhBMP-2 were probably too high. The measurements on CT images are susceptible to errors. Technically, distances on planar CT images are translated from three-dimensional distances in vivo. The measurements can be impaired according to geometrical rules if an angle change is not avoided in the follow-up imaging. It could be helpful to measure distances on three-dimensional reconstructions if well-defined landmarks are found which remain at the same location during the follow-up. Additionally, the PEEK cage was completely radiolucent and its position was therefore occasionally difficult to assess. In future studies incorporated markers visible on the CT images into the cage design could be helpful. Although CT imaging is an excellent tool to get an overall impression of the interventional result, it does not provide detailed information on the bone microstructure or on the overall fusion outcome. Bone micro-CT could provide excellent bone details, but it is associated with a high load of radiation and cannot be performed in vivo. The histological analysis (discussed elsewhere) of the specimens will provide additional insight into the tissue composition.

5 Conclusion and Suggestions for Future Studies

This thesis was intended to systematically analyze the dose-/concentration-dependent effect of rhBMP-2 on the fusion status and the adverse effects in a large animal model. During the present study, it became evident that a safe dose/concentration of rhBMP-2 avoiding bone resorption and overzealous bone growth as well as associated adverse effects might not exist. According to the study results and available literature information on rhBMP-2, there is a lack of information regarding its clinical use. Riew et al. (162) clearly state that "BMP (Bone Morphogenetic Protein) is a naturally occurring substance that your body normally makes to heal bone. As such, it is similar to insulin. Used incorrectly, or inappropriately at the wrong doses, by untrained people, it can kill or harm." Riew et al. (162) further elaborate that "Therefore, it is obvious that minute doses do not appear to cause problems, whereas large doses result in a high incidence of complications. In theory, at least, there may exist an ideal dose at which bone healing is reproducibly enhanced, but the incidence and severity of adverse reactions are minimized." Unfortunately, we cannot report a safe dose/concentration from our study results in the sheep.

In conclusion, it will be important to find an expert consensus on quality markers for "successful spinal fusion" (in correlation with clinical parameters). It is decisive to agree on parameters which define a "good outcome" in interbody fusion surgery. The experience with Infuse® shows how important it is to establish and adhere to quality standards for studies on innovative surgical approaches. Given that rhBMP-2 is probably most active during the first four weeks, the first follow-up control including imaging, e.g. CT scans, should be scheduled as early as two to four weeks after surgery. This will also play a role for establishing a clinical algorithm for follow-up examinations. Bone resorption seems to be a transient effect in most cases which will (partly) be reconstituted by bone remodeling in the follow-up. An off-label study in the cervical spine proved bone resorption already two weeks after surgery (50). Although bone resorption was rarely observed in the present study, indirect marker of its occurrence, e.g. cage migration and cage subsidence, were frequently noticed. Therefore, an earlier follow-up image might have allowed to directly detect resorptive defects in more specimens. In summary, a stable spine fixation is needed to warrant stability in the post-operative course. Knox et al. (51) emphasize "[…] the need for secure fixation during the osteolytic phase." The importance

of containment of rhBMP-2 as well as sealing cancellous bone surfaces needs to be further evaluated.

Furthermore, the practicability, validity and reliability of CT scan measurements compared to in vivo measurements needs to be analyzed. From a technical point it would be helpful to include a radiographically visible tracer in radiolucent cages.

Future studies should be carried out to obtain detailed information on the application of rhBMP-2 with all necessary details (e.g. total dose, concentration, carrier used, location of application, binding time of rhBMP-2). It would be welcomed if the adverse events could be clearly stated already in the abstract, even if their cause remains unclear. Phrasings like "no device-/drug-related complications" seem misleading since it is often not possible to find the cause of an adverse effect.

It is important to know that spine surgery has rapidly evolved in recent decades, which has resulted in the development of many new technologies. It is surprising that despite technological advances, the total number of revision spinal surgeries has increased according to Martin et al. (163). The YODA working groups concluded that ''[…] rhBMP-2 provided little or no benefit compared to bone graft and may be associated with more harms, possibly including cancer'' (164). What a disappointment! Similarly, Fu et al. (26) conclude that "In spinal fusion, rhBMP-2 has no proven clinical advantage over bone graft and may be associated with important harms, making it difficult to identify clear indications for rhBMP-2." They further elaborate that "On the basis of the currently available evidence, it is difficult to identify clear indications for rhBMP-2 in spinal fusion."

An important question to answer is: Would I as a surgeon recommend the procedure to a loved family member or a close friend? In the case of rhBMP-2 for lumbar interbody fusion, skepticism remains given the lack of information and recently reported serious adverse effects.

6   Bibliography

1.   AAOS. Low Back Pain-OrthoInfo [Internet]. 2013. Available from:
http://orthoinfo.aaos.org/topic.cfm?topic=a00311

2.   Müller-Gerbl M, Weisser S, Linsenmeier U. The distribution of mineral density in the cervical
vertebral endplates. Eur Spine J. 2008 Mar;17(3):432–8.

3.   Young B, Woodford P, O'Dowd G. Wheater's Functional Histology: A Text and Colour Atlas.
Elsevier Health Sciences; 2013. 195 p.

4.   AAOS. Spinal Fusion Glossary-OrthoInfo [Internet]. 2010 [cited 2015 Sep 13]. Available from:
http://orthoinfo.aaos.org/topic.cfm?topic=A00599

5.   Orndorff DG, Youssef JA, Scott MA. Minimally Invasive Approaches for Spine Surgery. J Spinal
Res Found. 2013;8(1).

6.   Sobotta J. Atlas and Text-book of Human Anatomy [Internet]. Рипол Классик; [cited 2014 Aug 1].
Available from: http://books.google.com/books?id=3toQAwAAQBAJ&pgis=1

7.   Ma B. Spinal fusion - series: MedlinePlus Medical Encyclopedia [Internet]. 2014 [cited 2015 Sep
13]. Available from: https://www.nlm.nih.gov/medlineplus/ency/presentations/100121_6.htm

8.   Ullrich PF. Bone Graft for Spine Fusion [Internet]. 1999 [cited 2015 Sep 13]. Available from:
http://www.spine-health.com/treatment/spinal-fusion/bone-graft-spine-fusion

9.   North American Spine Society. Recombinant Human Bone Morphogenetic Protein (rhBMP-2)
[Internet]. 2014. p. 30. Available from:
https://www.spine.org/Documents/PolicyPractice/CoverageRecommendations/rhBMP.pdf

10.   McKay WF, Peckham SM, Badura JM. A comprehensive clinical review of recombinant human
bone morphogenetic protein-2 (INFUSE Bone Graft). Int Orthop. 2007/07/20 ed. 2007;31(6):729–
34.

11.   Urist MR. Bone: formation by autoinduction. Science (80-). 1965/11/12 ed. 1965;150(3698):893–
9.

12.   Calhoun JH, Mader JT. Musculoskeletal infections. New York: M. Dekker; 2003. xvii, 625 p. p.

13.   Chen D, Zhao M, Mundy GR. Bone morphogenetic proteins. Growth factors. 2004/12/29 ed.
2004;22(4):233–41.

14.   Reddi AH, Reddi A. Bone morphogenetic proteins (BMPs): from morphogens to metabologens.
Cytokine Growth Factor Rev. 2009/11/11 ed. 2009;20(5-6):341–2.

15.   Sipe JB, Zhang J, Waits C, Skikne B, Garimella R, Anderson HC. Localization of bone
morphogenetic proteins (BMPs)-2, -4, and -6 within megakaryocytes and platelets. Bone.
2004/12/14 ed. 2004;35(6):1316–22.

16.   Tsiridis E, Upadhyay N, Giannoudis P. Molecular aspects of fracture healing: which are the
important molecules? Injury. 2007/03/27 ed. 2007;38 Suppl 1:S11–25.

17. FDA. InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device -P000058. U.S. Food and Drug Administration, Department of Health and Human Services, Center for Devices and Radiological Health. Center for Devices and Radiological Health; 2002.

18. EMA. InductOs, INN-dibotermin alfa [Internet]. European Medicines Agency (EMA). 2008 [cited 2014 Jul 31]. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000408/WC500032317.pdf

19. Burkus JK, Gornet MF, Schuler TC, Kleeman TJ, Zdeblick TA. Six-year outcomes of anterior lumbar interbody arthrodesis with use of interbody fusion cages and recombinant human bone morphogenetic protein-2. J Bone Joint Surg Am. 2009/05/05 ed. 2009;91(5):1181–9.

20. Hecht BP, Fischgrund JS, Herkowitz HN, Penman L, Toth JM, Shirkhoda A. The use of recombinant human bone morphogenetic protein 2 (rhBMP-2) to promote spinal fusion in a nonhuman primate anterior interbody fusion model. Spine (Phila Pa 1976). 1999/04/21 ed. 1999;24(7):629–36.

21. Sandhu HS. Anterior lumbar interbody fusion with osteoinductive growth factors. Clin Orthop Relat Res. 2000/02/29 ed. 2000;(371):56–60.

22. Alt V, Chhabra A, Franke J, Cuche M, Schnettler R, Le Huec JC. An economic analysis of using rhBMP-2 for lumbar fusion in Germany, France and UK from a societal perspective. Eur Spine J. 2009/03/21 ed. 2009;18(6):800–6.

23. FDA. INFUSE® Bone Graft - P00054 [Internet]. 2004 [cited 2014 Aug 7]. Available from: http://www.accessdata.fda.gov/cdrh_docs/pdf/p000054a.pdf

24. FDA. INFUSE® Bone Graft - P050053 [Internet]. 2007 [cited 2014 Jun 25]. Available from: http://www.accessdata.fda.gov/cdrh_docs/pdf5/p050053a.pdf

25. Carragee EJ, Hurwitz EL, Weiner BK. A critical review of recombinant human bone morphogenetic protein-2 trials in spinal surgery: emerging safety concerns and lessons learned. Spine J. 2011/07/07 ed. 2011;11(6):471–91.

26. Fu R, Selph S, McDonagh M, Peterson K, Tiwari A, Chou R, et al. Effectiveness and harms of recombinant human bone morphogenetic protein-2 in spine fusion: a systematic review and meta-analysis. Ann Intern Med. 2013/06/20 ed. 2013;158(12):890–902.

27. Haid Jr. RW, Branch Jr. CL, Alexander JT, Burkus JK. Posterior lumbar interbody fusion using recombinant human bone morphogenetic protein type 2 with cylindrical interbody cages. Spine J. 2004/09/15 ed. 2004;4(5):527–9.

28. Boden SD, Zdeblick TA, Sandhu HS, Heim SE. The use of rhBMP-2 in interbody fusion cages. Definitive evidence of osteoinduction in humans: a preliminary report. Spine (Phila Pa 1976). 2000/03/07 ed. 2000;25(3):376–81.

29. Dimar 2nd JR, Glassman SD, Burkus JK, Pryor PW, Hardacker JW, Carreon LY. Clinical and radiographic analysis of an optimized rhBMP-2 formulation as an autograft replacement in posterolateral lumbar spine arthrodesis. J Bone Joint Surg Am. 2009/06/03 ed. 2009;91(6):1377–86.

30. Boden SD, Kang J, Sandhu H, Heller JG. Use of recombinant human bone morphogenetic protein-2 to achieve posterolateral lumbar spine fusion in humans: a prospective, randomized clinical pilot trial: 2002 Volvo Award in clinical studies. Spine (Phila Pa 1976). 2002/12/04 ed. 2002;27(23):2662–73.

31. Burkus JK, Gornet MF, Dickman CA, Zdeblick TA. Anterior lumbar interbody fusion using rhBMP-2 with tapered interbody cages. J Spinal Disord Tech. 2002/10/24 ed. 2002;15(5):337–49.

32. Burkus JK, Transfeldt EE, Kitchel SH, Watkins RG, Balderston RA. Clinical and radiographic outcomes of anterior lumbar interbody fusion using recombinant human bone morphogenetic protein-2. Spine (Phila Pa 1976). 2002/11/20 ed. 2002;27(21):2396–408.

33. Baskin DS, Ryan P, Sonntag V, Westmark R, Widmayer MA. A prospective, randomized, controlled cervical fusion study using recombinant human bone morphogenetic protein-2 with the CORNERSTONE-SR allograft ring and the ATLANTIS anterior cervical plate. Spine (Phila Pa 1976). 2003/06/18 ed. 2003;28(12):1219–24; discussion 1225.

34. Dimar JR, Glassman SD, Burkus KJ, Carreon LY. Clinical outcomes and fusion success at 2 years of single-level instrumented posterolateral fusions with recombinant human bone morphogenetic protein-2/compression resistant matrix versus iliac crest bone graft. Spine (Phila Pa 1976). 2006/10/19 ed. 2006;31(22):2534–9; discussion 2540.

35. Dawson E, Bae HW, Burkus JK, Stambough JL, Glassman SD. Recombinant human bone morphogenetic protein-2 on an absorbable collagen sponge with an osteoconductive bulking agent in posterolateral arthrodesis with instrumentation. A prospective randomized trial. J Bone Joint Surg Am. 2009/07/03 ed. 2009;91(7):1604–13.

36. Ong KL, Villarraga ML, Lau E, Carreon LY, Kurtz SM, Glassman SD. Off-label use of bone morphogenetic proteins in the United States using administrative data. Spine (Phila Pa 1976). 2010/08/12 ed. 2010;35(19):1794–800.

37. Epstein NE. Pros, cons, and costs of INFUSE in spinal surgery. Surg Neurol Int. 2011/02/08 ed. 2011 Jan;2:10.

38. Shields LBE, Raque GH, Glassman SD, Campbell M, Vitaz T, Harpring J, et al. Adverse effects associated with high-dose recombinant human bone morphogenetic protein-2 use in anterior cervical spine fusion. Spine (Phila Pa 1976). 2006 Mar 1;31(5):542–7.

39. Smucker JD, Rhee JM, Singh K, Yoon ST, Heller JG. Increased swelling complications associated with off-label usage of rhBMP-2 in the anterior cervical spine. Spine (Phila Pa 1976). 2006 Nov 15;31(24):2813–9.

40. Smoljanovic T, Pecina M. RE: complications attributable to the use of rhBMP-2 inside the femoral ring allograft during anterior lumbar interbody fusion. Spine J. 2008/01/08 ed. 2008;8(2):413–4; author reply 414.

41. Health C for D and R. Public Health Notifications (Medical Devices) - FDA Public Health Notification: Life-threatening Complications Associated with Recombinant Human Bone Morphogenetic Protein in Cervical Spine Fusion. Center for Devices and Radiological Health;

42. Kuklo TR, Groth AT, Anderson RC, Frisch HM, Islinger RB. Recombinant human bone morphogenetic protein-2 for grade III open segmental tibial fractures from combat injuries in Iraq. J Bone Joint Surg Br. 2008/08/02 ed. 2008;90(8):1068–72.

43. Scott J. Withdrawal of a paper. J Bone Joint Surg Br. 2009/03/05 ed. 2009;91(3):285–6.

44. Carragee EJ, Baker RM, Benzel EC, Bigos SJ, Cheng I, Corbin TP, et al. A biologic without guidelines: the YODA project and the future of bone morphogenetic protein-2 research. Spine J. 2012/12/04 ed. 2012;12(10):877–80.

45. Meisel HJ, Schnoring M, Hohaus C, Minkus Y, Beier A, Ganey T, et al. Posterior lumbar interbody fusion using rhBMP-2. Eur Spine J. 2008/10/08 ed. 2008;17(12):1735–44.

46. Howell TH, Fiorellini J, Jones A, Alder M, Nummikoski P, Lazaro M, et al. A feasibility study evaluating rhBMP-2/absorbable collagen sponge device for local alveolar ridge preservation or augmentation. Int J Periodontics Restorative Dent. 1997/04/01 ed. 1997;17(2):124–39.

47. Laursen M, Hoy K, Hansen ES, Gelineck J, Christensen FB, Bunger CE. Recombinant bone morphogenetic protein-7 as an intracorporal bone growth stimulator in unstable thoracolumbar burst fractures in humans: preliminary results. Eur Spine J. 2000/02/09 ed. 1999;8(6):485–90.

48. Mesfin A, Buchowski JM, Zebala LP, Bakhsh WR, Aronson AB, Fogelson JL, et al. High-dose rhBMP-2 for adults: major and minor complications: a study of 502 spine cases. J Bone Joint Surg Am. 2013/09/06 ed. 2013;95(17):1546–53.

49. Simmonds MC, Brown JVE, Heirs MK, Higgins JPT, Mannion RJ, Rodgers MA, et al. Safety and effectiveness of recombinant human bone morphogenetic protein-2 for spinal fusion: a meta-analysis of individual-participant data. Ann Intern Med. 2013 Jun 18;158(12):877–89.

50. Sethi A, Craig J, Bartol S, Chen W, Jacobson M, Coe C, et al. Radiographic and CT evaluation of recombinant human bone morphogenetic protein-2-assisted spinal interbody fusion. AJR Am J Roentgenol. 2011/06/28 ed. 2011;197(1):W128–33.

51. Knox JB, Dai 3rd JM, Orchowski J. Osteolysis in transforaminal lumbar interbody fusion with bone morphogenetic protein-2. Spine (Phila Pa 1976). 2011/01/11 ed. 2011;36(8):672–6.

52. Vaidya R, Sethi A, Bartol S, Jacobson M, Coe C, Craig JG. Complications in the use of rhBMP-2 in PEEK cages for interbody spinal fusions. J Spinal Disord Tech. 2008/12/06 ed. 2008;21(8):557–62.

53. Toth JM, Boden SD, Burkus JK, Badura JM, Peckham SM, McKay WF. Short-term osteoclastic activity induced by locally high concentrations of recombinant human bone morphogenetic protein-2 in a cancellous bone environment. Spine (Phila Pa 1976). 2009/02/26 ed. 2009;34(6):539–50.

54. Poynton AR, Lane JM. Safety profile for the clinical use of bone morphogenetic proteins in the spine. Spine (Phila Pa 1976). 2002/09/03 ed. 2002;27(16 Suppl 1):S40–8.

55. Smoljanovic T, Bojanic I, Bicanic G, Delimar D. Re: Toth JM, Boden SD, Burkus JK, et al. Short-term osteoclastic activity induced by locally high concentrations of recombinant human bone morphogenetic protein-2 in a cancellous bone environment. Spine 2009;34:539-50. Spine (Phila Pa 1976). 2010/03/02 ed. 2010;35(5):597; author reply 597–8.

56. Tang S, Rebholz BJ. Does anterior lumbar interbody fusion promote adjacent degeneration in degenerative disc disease? A finite element study. J Orthop Sci. 2011/02/12 ed. 2011;16(2):221–8.

57. Hilibrand AS, Robbins M. Adjacent segment degeneration and adjacent segment disease: the consequences of spinal fusion? Spine J. 2004/11/16 ed. 2004;4(6 Suppl):190S – 194S.

58. Levin DA, Hale JJ, Bendo JA. Adjacent segment degeneration following spinal fusion for degenerative disc disease. Bull NYU Hosp Jt Dis. 2007/06/02 ed. 2007;65(1):29–36.

59. Hsu HP, Zanella JM, Peckham SM, Spector M. Comparing ectopic bone growth induced by rhBMP-2 on an absorbable collagen sponge in rat and rabbit models. J Orthop Res. 2006/06/17 ed. 2006;24(8):1660–9.

60. Williams AL, Gornet MF, Burkus JK. CT evaluation of lumbar interbody fusion: current concepts. AJNR Am J Neuroradiol. 2005/09/13 ed. 2005;26(8):2057–66.

61. Burkus JK, Dorchak JD, Sanders DL. Radiographic assessment of interbody fusion using recombinant human bone morphogenetic protein type 2. Spine (Phila Pa 1976). 2003/02/19 ed. 2003;28(4):372–7.

62. Singh K, Smucker JD, Gill S, Boden SD. Use of recombinant human bone morphogenetic protein-2 as an adjunct in posterolateral lumbar spine fusion: a prospective CT-scan analysis at one and two years. J Spinal Disord Tech. 2006/08/08 ed. 2006;19(6):416–23.

63. Mannion RJ, Nowitzke AM, Wood MJ. Promoting fusion in minimally invasive lumbar interbody stabilization with low-dose bone morphogenic protein-2--but what is the cost? Spine J. 2010/08/27 ed. 2011;11(6):527–33.

64. Hohaus C MHGT. gms | 131. Kongress der Deutschen Gesellschaft für Chirurgie | Long time follow up after posterior lumbar interbody fusion with bone morphogenetic protein-2.

65. Branch Jr. CL. Physican-directed (off-label) use of recombinant bone morphogenic protein-2: let us do it well! Spine J. 2011/07/07 ed. 2011;11(6):469–70.

66. McAfee PC, Boden SD, Brantigan JW, Fraser RD, Kuslich SD, Oxland TR, et al. Symposium: a critical discrepancy-a criteria of successful arthrodesis following interbody spinal fusions. Spine (Phila Pa 1976). 2001/02/27 ed. 2001;26(3):320–34.

67. Boden SD, Martin Jr. GJ, Horton WC, Truss TL, Sandhu HS. Laparoscopic anterior spinal arthrodesis with rhBMP-2 in a titanium interbody threaded cage. J Spinal Disord. 1998/05/20 ed. 1998;11(2):95–101.

68. Carragee EJ, Bono CM, Scuderi GJ. Pseudomorbidity in iliac crest bone graft harvesting: the rise of rhBMP-2 in short-segment posterior lumbar fusion. Spine J. 2009/10/24 ed. 2009;9(11):873–9.

69. Flouzat-Lachaniette C-H, Ghazanfari A, Bouthors C, Poignard A, Hernigou P, Allain J. Bone union rate with recombinant human bone morphogenic protein-2 versus autologous iliac bone in PEEK cages for anterior lumbar interbody fusion. Int Orthop. 2014 Mar 14;

70. Lebwohl NH, Williams AL, Gornet MF, Burkus JK. Radiographic Evaluation of the Postoperative Interbody Fusion Patient: Is CT the Study of Choice? AJNR Am J Neuroradiol. 2005 Sep 1;26(8):1885–6; discussion 1886–7.

71. Burkus JK, Foley K, Haid RW, LeHuec JC. Surgical Interbody Research Group--radiographic assessment of interbody fusion devices: fusion criteria for anterior lumbar interbody surgery. Neurosurg Focus. 2006/05/31 ed. 2001;10(4):E11.

72. Choi BCK, Pak AWP. A catalog of biases in questionnaires. Prev Chronic Dis. 2005;2:A13.

73. Rodgers MA, Brown JVE, Heirs MK, Higgins JPT, Mannion RJ, Simmonds MC, et al. Reporting of industry funded study outcome data: comparison of confidential and published data on the safety and effectiveness of rhBMP-2 for spinal fusion. BMJ. 2013 Jan;346:f3981.

74. Smoljanović T, Bojanić I, Pećina M. The Use of Bone Morphogenetic Protein in Lumbar Spine Surgery. J Bone Jt Surg. The Journal of Bone and Joint Surgery, Inc.; 2009 Aug 1;91(8):2045–6.

75. Smoljanovic T, Pecina M. Re: Burkus J K, Transfeldt E E, Kitchel S H, et al. Clinical and radiographic outcomes of anterior lumbar interbody fusion using recombinant human bone morphogenetic protein-2. Spine 2002;27:2396-408. Spine (Phila Pa 1976). 2008/01/17 ed. 2008;33(2):224.

76. Seeherman HJ, Li XJ, Bouxsein ML, Wozney JM. rhBMP-2 induces transient bone resorption followed by bone formation in a nonhuman primate core-defect model. J Bone Joint Surg Am. 2010/02/04 ed. 2010;92(2):411–26.

77. Seeherman H, Li R, Bouxsein M, Kim H, Li XJ, Smith-Adaline EA, et al. rhBMP-2/calcium phosphate matrix accelerates osteotomy-site healing in a nonhuman primate model at multiple treatment times and concentrations. J Bone Joint Surg Am. 2006/01/05 ed. 2006;88(1):144–60.

78. McClellan JW, Mulconrey DS, Forbes RJ, Fullmer N. Vertebral bone resorption after transforaminal lumbar interbody fusion with bone morphogenetic protein (rhBMP-2). J Spinal Disord Tech. 2006/10/06 ed. 2006;19(7):483–6.

79. Hansen SM, Sasso RC. Resorptive response of rhBMP2 simulating infection in an anterior lumbar interbody fusion with a femoral ring. J Spinal Disord Tech. 2006/06/09 ed. 2006;19(2):130–4.

80. Michielsen J, Sys J, Rigaux A, Bertrand C. The effect of recombinant human bone morphogenetic protein-2 in single-level posterior lumbar interbody arthrodesis. J Bone Joint Surg Am. 2013/05/17 ed. 2013;95(10):873–80.

81. Smoljanovic T, Bojanic I, Pecina M, Rihn JA, Gates C, Glassman SD, et al. The use of bone morphogenetic protein in lumbar spine surgery. J Bone Joint Surg Am. 2009/04/24 ed. The Journal of Bone and Joint Surgery, Inc.; 2009 Aug 1;58(8):677–88.

82. Lehman Jr. RA. Vertebral body osteolysis after minimal-access transforaminal interbody fusion. Spine J. 2011/07/07 ed. 2011;11(6):581–2.

83. Lewandrowski KU, Nanson C, Calderon R. Vertebral osteolysis after posterior interbody lumbar fusion with recombinant human bone morphogenetic protein 2: a report of five cases. Spine J. 2007/05/29 ed. 2007;7(5):609–14.

84. Shah RK, Moncayo VM, Smitson RD, Pierre-Jerome C, Terk MR. Recombinant human bone morphogenetic protein 2-induced heterotopic ossification of the retroperitoneum, psoas muscle, pelvis and abdominal wall following lumbar spinal fusion. Skeletal Radiol. 2010/02/18 ed. 2010;39(5):501–4.

85. McKay B, Sandhu HS. Use of recombinant human bone morphogenetic protein-2 in spinal fusion applications. Spine (Phila Pa 1976). 2002/09/03 ed. 2002;27(16 Suppl 1):S66–85.

86. Klimo Jr. P, Peelle MW. Use of polyetheretherketone spacer and recombinant human bone morphogenetic protein-2 in the cervical spine: a radiographic analysis. Spine J. 2009/07/04 ed. 2009;9(12):959–66.

87. Patel V V, Zhao L, Wong P, Pradhan BB, Bae HW, Kanim L, et al. An in vitro and in vivo analysis of fibrin glue use to control bone morphogenetic protein diffusion and bone morphogenetic protein-stimulated bone growth. Spine J. 2006/07/11 ed. 2006;6(4):397–403; discussion 404.

88. Rihn JA, Patel R, Makda J, Hong J, Anderson DG, Vaccaro AR, et al. Complications associated with single-level transforaminal lumbar interbody fusion. Spine J. 2009/06/02 ed. 2009;9(8):623–9.

89.   Goldschlager T, Rosenfeld J V, Ghosh P, Itescu S, Blecher C, McLean C, et al. Cervical interbody fusion is enhanced by allogeneic mesenchymal precursor cells in an ovine model. Spine (Phila Pa 1976). 2010/12/31 ed. 2011;36(8):615–23.

90.   Pradhan B, Wang J. Complications of Interbody Fusion Cages. Complications of Pediatric and Adult Spinal Surgery. CRC Press; 2004. p. 439–56.

91.   Sherman BP, Lindley EM, Turner AS, Seim 3rd HB, Benedict J, Burger EL, et al. Evaluation of ABM/P-15 versus autogenous bone in an ovine lumbar interbody fusion model. Eur Spine J. 2010/08/10 ed. 2010;19(12):2156–63.

92.   Kim PE, Zee CS. Imaging of the Postoperative Spine: Cages, Prostheses, and InstrumentationSpinal Imaging. In: Goethem JWM, Hauwe L, Parizel PM, editors. Springer Berlin Heidelberg; 2007. p. 397–413.

93.   Smoljanovic T, Bicanic G, Bojanic I. Re: Kleeman TJ, Ahn UM, Talbot-Kleeman A. Laparoscopic anterior lumbar interbody fusion with rhBMP-2: a prospective study of clinical and radiographic outcomes. Spine 2001;26:2751-6. Spine (Phila Pa 1976). 2010/09/17 ed. 2010;35(20):E1013.

94.   Easley NE, Wang M, McGrady LM, Toth JM. Biomechanical and radiographic evaluation of an ovine model for the human lumbar spine. Proc Inst Mech Eng H. 2008/10/22 ed. 2008;222(6):915–22.

95.   Vaidya R, Weir R, Sethi A, Meisterling S, Hakeos W, Wybo CD. Interbody fusion with allograft and rhBMP-2 leads to consistent fusion but early subsidence. J Bone Joint Surg Br. 2007/03/16 ed. 2007;89(3):342–5.

96.   Pradhan BB, Bae HW, Dawson EG, Patel V V, Delamarter RB. Graft resorption with the use of bone morphogenetic protein: lessons from anterior lumbar interbody fusion using femoral ring allografts and recombinant human bone morphogenetic protein-2. Spine (Phila Pa 1976). 2006/05/02 ed. 2006;31(10):E277–84.

97.   Polikeit A, Ferguson SJ, Nolte LP, Orr TE. The importance of the endplate for interbody cages in the lumbar spine. Eur Spine J. 2003 Dec;12(6):556–61.

98.   Le T V, Baaj AA, Dakwar E, Burkett CJ, Murray G, Smith DA, et al. Subsidence of polyetheretherketone intervertebral cages in minimally invasive lateral retroperitoneal transpsoas lumbar interbody fusion. Spine (Phila Pa 1976). 2012/06/15 ed. 2012;37(14):1268–73.

99.   Lee JH, Jeon DW, Lee SJ, Chang BS, Lee CK. Fusion rates and subsidence of morselized local bone grafted in titanium cages in posterior lumbar interbody fusion using quantitative three-dimensional computed tomography scans. Spine (Phila Pa 1976). 2010/05/01 ed. 2010;35(15):1460–5.

100.   Ha SK, Park JY, Kim SH, Lim DJ, Kim SD, Lee SK. Radiologic Assessment of Subsidence in Stand-Alone Cervical Polyetheretherketone (PEEK) Cage. J Korean Neurosurg Soc. 2009/01/13 ed. 2008;44(6):370–4.

101.   Raizman NM, O'Brien JR, Poehling-Monaghan KL, Yu WD. Pseudarthrosis of the spine. J Am Acad Orthop Surg. 2009/08/05 ed. 2009;17(8):494–503.

102.   Goldstein C, Drew B. When is a spine fused? Injury. 2010/12/15 ed. 2011;42(3):306–13.

103.   Tuli SK, Chen P, Eichler ME, Woodard EJ. Reliability of radiologic assessment of fusion: cervical fibular allograft model. Spine (Phila Pa 1976). 2004/04/15 ed. 2004;29(8):856–60.

104. Fogel GR, Toohey JS, Neidre A, Brantigan JW. Fusion assessment of posterior lumbar interbody fusion using radiolucent cages: X-ray films and helical computed tomography scans compared with surgical exploration of fusion. Spine J. 2007/06/05 ed. 2008;8(4):570–7.

105. Cizek GR, Boyd LM. Imaging pitfalls of interbody spinal implants. Spine (Phila Pa 1976). 2000/10/18 ed. 2000;25(20):2633–6.

106. Christensen FB, Laursen M, Gelineck J, Eiskjaer SP, Thomsen K, Bunger CE. Interobserver and intraobserver agreement of radiograph interpretation with and without pedicle screw implants: the need for a detailed classification system in posterolateral spinal fusion. Spine (Phila Pa 1976). 2001/03/10 ed. 2001;26(5):534–8.

107. Santos ER, Goss DG, Morcom RK, Fraser RD. Radiologic assessment of interbody fusion using carbon fiber cages. Spine (Phila Pa 1976). 2003/05/28 ed. 2003;28(10):997–1001.

108. Lee HS, Lee JH. A comparison of dynamic views using plain radiographs and thin-section three-dimensional computed tomography in the evaluation of fusion after posterior lumbar interbody fusion surgery. Spine J. 2013/10/01 ed. 2013;13(10):1200–7.

109. Ho JM, Ben-Galim PJ, Weiner BK, Karbach LE, Reitman CA, Heggeness MH, et al. Toward the establishment of optimal computed tomographic parameters for the assessment of lumbar spinal fusion. Spine J. 2011/06/21 ed. 2011;11(7):636–40.

110. Larsen JM, Rimoldi RL, Capen DA, Nelson RW, Nagelberg S, Thomas Jr. JC. Assessment of pseudarthrosis in pedicle screw fusion: a prospective study comparing plain radiographs, flexion/extension radiographs, CT scanning, and bone scintigraphy with operative findings. J Spinal Disord. 1996/04/01 ed. 1996;9(2):117–20.

111. Lee C, Dorcil J, Radomisli TE. Nonunion of the spine: a review. Clin Orthop Relat Res. 2004/03/17 ed. 2004;(419):71–5.

112. Brodsky AE, Kovalsky ES, Khalil MA. Correlation of radiologic assessment of lumbar spine fusions with surgical exploration. Spine (Phila Pa 1976). 1991/06/01 ed. 1991;16(6 Suppl):S261–5.

113. Hilibrand AS, Dina TS. The use of diagnostic imaging to assess spinal arthrodesis. Orthop Clin North Am. 1998/10/03 ed. 1998;29(4):591–601.

114. Epstein NE, Silvergleide RS. Documenting fusion following anterior cervical surgery: a comparison of roentgenogram versus two-dimensional computed tomographic findings. J Spinal Disord Tech. 2003/06/07 ed. 2003;16(3):243–7.

115. Lang P, Genant HK, Chafetz N, Steiger P, Morris JM. Three-dimensional computed tomography and multiplanar reformations in the assessment of pseudarthrosis in posterior lumbar fusion patients. Spine (Phila Pa 1976). 1988/01/01 ed. 1988;13(1):69–75.

116. Ploumis A, Mehbod A, Garvey T, Gilbert T, Transfeldt E, Wood K. Prospective assessment of cervical fusion status: plain radiographs versus CT-scan. Acta Orthop Belg. 2006/08/08 ed. 2006;72(3):342–6.

117. Rothman SL, Glenn Jr. W V. CT evaluation of interbody fusion. Clin Orthop Relat Res. 1985/03/01 ed. 1985;(193):47–56.

118. Shah RR, Mohammed S, Saifuddin A, Taylor BA. Comparison of plain radiographs with CT scan to evaluate interbody fusion following the use of titanium interbody cages and transpedicular instrumentation. Eur Spine J. 2003/05/08 ed. 2003;12(4):378–85.

119. Siambanes D, Mather S. Comparison of plain radiographs and CT scans in instrumented posterior lumbar interbody fusion. Orthopedics. 1998/03/21 ed. 1998;21(2):165–7.

120. Zinreich SJ, Long DM, Davis R, Quinn CB, McAfee PC, Wang H. Three-dimensional CT imaging in postsurgical "failed back" syndrome. J Comput Assist Tomogr. 1990/07/01 ed. 1990;14(4):574–80.

121. Kaiser MG, Mummaneni P V, Matz PG, Anderson PA, Groff MW, Heary RF, et al. Radiographic assessment of cervical subaxial fusion. J Neurosurg Spine. 2009/09/23 ed. 2009;11(2):221–7.

122. Carreon LY, Glassman SD, Schwender JD, Subach BR, Gornet MF, Ohno S. Reliability and accuracy of fine-cut computed tomography scans to determine the status of anterior interbody fusions with metallic cages. Spine J. 2008 Jan;8(6):998–1002.

123. Cook SD, Patron LP, Christakis PM, Bailey KJ, Banta C, Glazer PA. Comparison of methods for determining the presence and extent of anterior lumbar interbody fusion. Spine (Phila Pa 1976). 2004/05/08 ed. 2004;29(10):1118–23.

124. Schreiber JJ, Hughes AP, Taher F, Girardi FP. An association can be found between hounsfield units and success of lumbar spine fusion. HSS J. 2014/02/01 ed. 2014;10(1):25–9.

125. Spruit M, Meijers H, Obradov M, Anderson PG. CT density measurement of bone graft within an intervertebral lumbar cage: increase of hounsfield units as an indicator for increasing bone mineral content. J Spinal Disord Tech. 2004/05/29 ed. 2004;17(3):232–5.

126. Kandziora F, Pflugmacher R, Scholz M, Schafer J, Schollmeier G, Schmidmaier G, et al. Dose-dependent effects of combined IGF-I and TGF-beta1 application in a sheep cervical spine fusion model. Eur Spine J. 2003/11/18 ed. 2003;12(5):464–73.

127. Whyms BJ, Vorperian HK, Gentry LR, Schimek EM, Bersu ET, Chung MK. The effect of computed tomographic scanner parameters and 3-dimensional volume rendering techniques on the accuracy of linear, angular, and volumetric measurements of the mandible. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013 May;115(5):682–91.

128. Boerckel JD, Kolambkar YM, Dupont KM, Uhrig BA, Phelps EA, Stevens HY, et al. Effects of protein dose and delivery system on BMP-mediated bone regeneration. Biomaterials. 2011 Aug;32(22):5241–51.

129. Smoljanovic T, Bojanic I, Dapic T. Significance of early CT evaluation after lumbar interbody fusions using recombinant human bone morphogenetic protein-2. AJNR Am J Neuroradiol. 2009/02/28 ed. 2009;30(5):e71.

130. Drespe IH, Polzhofer GK, Turner AS, Grauer JN. Animal models for spinal fusion. Spine J. 2005/11/18 ed. 2005;5(6 Suppl):209S – 216S.

131. Mageed M, Berner D, Julke H, Hohaus C, Brehm W, Gerlach K. Is sheep lumbar spine a suitable alternative model for human spinal researches? Morphometrical comparison study. Lab Anim Res. 2014/01/08 ed. 2013;29(4):183–9.

132. Wilke HJ, Kettler A, Wenger KH, Claes LE. Anatomy of the sheep spine and its comparison to the human spine. Anat Rec. 1997/04/01 ed. 1997;247(4):542–55.

133. Smit TH. The use of a quadruped as an in vivo model for the study of the spine - biomechanical considerations. Eur Spine J. 2002/04/17 ed. 2002;11(2):137–44.

134. White 3rd AA. Clinical biomechanics of cervical spine implants. Spine (Phila Pa 1976). 1989/10/01 ed. 1989;14(10):1040–5.

135. Sandhu HS, Khan SN, Suh DY, Boden SD. Demineralized bone matrix, bone morphogenetic proteins, and animal models of spine fusion: an overview. Eur Spine J. 2001/11/22 ed. 2001;10 Suppl 2:S122–31.

136. Wilke HJ, Kettler A, Claes LE. Are sheep spines a valid biomechanical model for human spines? Spine (Phila Pa 1976). 1997/11/14 ed. 1997;22(20):2365–74.

137. Foster MR, Allen MJ, Schoonmaker JE, Yuan HA, Kanazawa A, Park S-A, et al. Characterization of a developing lumbar arthrodesis in a sheep model with quantitative instability. Spine J. Jan;2(4):244–50.

138. Turner AS. Spine Surgery in a Large Animal Model: Experiences and Limitations [Internet]. 2005 [cited 2014 Jul 23]. Available from: http://www.ecmjournal.org/journal/supplements/vol010supp03/pdf/vol010supp03a34.pdf

139. Russell WMS. The development of the three Rs concept. Altern Lab Anim. Jan;23(3):298–304.

140. Johns Hopkins University. The Principles of Humane Experimental Technique by W.M.S. Russell and R.L. Burch [Internet]. 2015 [cited 2014 Jul 31]. Available from: http://altweb.jhsph.edu/pubs/books/humane_exp/het-toc

141. European Commission. European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) — EURL ECVAM [Internet]. 2015 [cited 2015 Sep 16]. Available from: https://eurl-ecvam.jrc.ec.europa.eu/

142. Schimmel JJ, Poeschmann MS, Horsting PP, Schonfeld DH, van Limbeek J, Pavlov PW. PEEK Cages in Lumbar Fusion: Mid-term Clinical Outcome and Radiological Fusion. J Spinal Disord Tech. 2012/08/22 ed. 2012;

143. Toth JM, Wang M, Estes BT, Scifert JL, Seim 3rd HB, Turner AS. Polyetheretherketone as a biomaterial for spinal applications. Biomaterials. 2005/08/24 ed. 2006;27(3):324–34.

144. Zavatsky JM, Serhan H, Agarwal AK, et al.Zavatsky JM, Serhan H, Agarwal AK et al. Would resting a lateral interbody cage across the ring apophysis in the lumbar spine mitigate endplate violation? Poster presented at: 13th Annual Conference of the International Society for the Advancement of Spine Surgery Vancouver, BC. 2013.

145. Kandziora F, Schollmeier G, Scholz M, Schaefer J, Scholz A, Schmidmaier G, et al. Influence of cage design on interbody fusion in a sheep cervical spine model. J Neurosurg. 2002/05/07 ed. 2002;96(3 Suppl):321–32.

146. Kanayama M, Cunningham BW, Haggerty CJ, Abumi K, Kaneda K, McAfee PC. In vitro biomechanical investigation of the stability and stress-shielding effect of lumbar interbody fusion devices. J Neurosurg. 2000/09/30 ed. 2000;93(2 Suppl):259–65.

147. Denis F. Spinal instability as defined by the three-column spine concept in acute spinal trauma. Clin Orthop Relat Res. 1984/10/01 ed. 1984;(189):65–76.

148. Friess W, Uludag H, Foskett S, Biron R, Sargeant C. Characterization of absorbable collagen sponges as rhBMP-2 carriers. Int J Pharm. 1999;187:91–9.

149. Center for Devices and Radiological HealthCenter for Devices and Radiological Health. Classify Your Medical Device - Is The Product A Medical Device? Center for Devices and Radiological Health; 2014.

150. Sandhu HS, Toth JM, Diwan AD, Seim 3rd HB, Kanim LE, Kabo JM, et al. Histologic evaluation of the efficacy of rhBMP-2 compared with autograft bone in sheep spinal anterior interbody fusion. Spine (Phila Pa 1976). 2002/03/09 ed. 2002;27(6):567–75.

151. Crandall DG, Revella J. Transforaminal lumbar interbody fusion versus anterior lumbar interbody fusion as an adjunct to posterior instrumented correction of degenerative lumbar scoliosis: three year clinical and radiographic outcomes. Spine (Phila Pa 1976). 2009/09/16 ed. 2009;34(20):2126–33.

152. Bolesta MJ. Recombinant human bone morphogenetic protein-2: what's a spine surgeon to do?: commentary on an article by J. Michielsen, MD, et al.: "The effect of recombinant human bone morphogenetic protein-2 in single-level posterior lumbar interbody arthrodesis." J Bone Joint Surg Am. 2013/05/17 ed. 2013;95(10):e72.

153. Wang JC. Debates on the Use of BMP in Spine Surgery [Internet]. 2013 [cited 2014 Jul 22]. p. 6. Available from: http://www2.aaos.org/anmeet/anmt2013/debates.pdf

154. Joseph V, Rampersaud YR. Heterotopic bone formation with the use of rhBMP2 in posterior minimal access interbody fusion: a CT analysis. Spine (Phila Pa 1976). 2008/02/05 ed. 2007;32(25):2885–90.

155. Epstein NE, Schwall GS. Costs and frequency of "off-label" use of INFUSE for spinal fusions at one institution in 2010. Surg Neurol Int. 2011/09/03 ed. 2011;2:115.

156. Cahill KS, Chi JH, Day A, Claus EB. Prevalence, complications, and hospital charges associated with use of bone-morphogenetic proteins in spinal fusion procedures. JAMA. 2009/07/02 ed. 2009;302(1):58–66.

157. Vaidya R. Transforaminal interbody fusion and the "off label" use of recombinant human bone morphogenetic protein-2. Spine J. 2009/07/23 ed. 2009;9(8):667–9.

158. Kleeman TJ, Ahn UM, Talbot-Kleeman A. Laparoscopic anterior lumbar interbody fusion with rhBMP-2: a prospective study of clinical and radiographic outcomes. Spine (Phila Pa 1976). 2001/12/12 ed. 2001;26(24):2751–6.

159. Vaidya R, Carp J, Sethi A, Bartol S, Craig J, Les CM. Complications of anterior cervical discectomy and fusion using recombinant human bone morphogenetic protein-2. Eur Spine J. 2007/03/28 ed. 2007;16(8):1257–65.

160. Smoljanovic T, Cimic M, Bojanic I. Aggressive end plate decortication as a cause of osteolysis after rhBMP-2 use in cervical spine interbody fusion. Spine J. 2010/02/10 ed. 2010;10(2):187–8; author reply 188.

161. Carragee EJ, Mitsunaga KA, Hurwitz EL, Scuderi GJ. Retrograde ejaculation after anterior lumbar interbody fusion using rhBMP-2: a cohort controlled study. Spine J. 2011/05/27 ed. 2011;11(6):511–6.

162. Riew KD, Carragee EJ. Commentary: Despite reports of catastrophic complications, why recombinant human bone morphogenetic protein-2 should be available for use in anterior cervical spine surgery. Spine J. 2012/12/04 ed. 2012;12(10):900–1.

163. Martin BI, Mirza SK, Comstock BA, Gray DT, Kreuter W, Deyo RA. Are lumbar spine reoperation rates falling with greater use of fusion surgery and new surgical technology? Spine (Phila Pa 1976). 2007/09/01 ed. 2007;32(19):2119–26.

164. Weiner BK, Hurwitz EL, Schoene ML, Carragee EJ. Moving forward after YODA. Spine J. 2013/09/14 ed. 2013;13(9):995–7.

Eidesstattliche Versicherung

„Ich, Gerlinde S. Heil, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „The Dose-Dependent Effect of Bone Morphogenetic Protein 2 on Spinal Fusion Status and Adverse Effects in Right-Lateral Lumbar Interbody Fusion in the Sheep Model" selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

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Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem Betreuer, angegeben sind. Sämtliche Publikationen, die aus dieser Dissertation hervorgegangen sind und bei denen ich Autor bin, entsprechen den URM (s.o) und werden von mir verantwortet.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst."

24. November 2015   Gerlinde S. Heil

Anteilserklärung an etwaigen erfolgten Publikationen

Gerlinde S. Heil hatte folgenden Anteil an den folgenden Publikationen:

Publikation 1 (Abstract for Oral Presentation): Heil G.S., Hohaus C., Gerlach K., Vergroesen P.P., Meisel H.J., Balancing bone resorption and overzealous bone growth in Anterior Lumbar Interbody Fusion (ALIF) with RhBMP-2 in the sheep model: A question of dose and/or concentration?, European Spine Journal, Volume 24, Number 3, March 2015.
Beitrag im Einzelnen: Datenerfassung, Datenauswertung, Verfassen des Abstracts und Kongress-Präsentation.

Publikation 2 (Abstract for Poster Presentation): Heil G.S., Meisel H.J., Gerlach K., Hohaus C., Balancing bone resorption and overzealous bone growth in anterior lumbar interbody fusion (ALIF) with rhBMP-2 in the sheep model - a question of dose and/or concentration?, Regenerative Medicine, Volume 10, Number 7s, October 2015.
Beitrag im Einzelnen: Datenerfassung, Datenauswertung, Verfassen des Abstracts.

Gerlinde S. Heil

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

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