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TRAUMA SURGERY

Osseointegration of hollow cylinder based spinal implants in normal and osteoporotic vertebrae: a sheep study

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Abstract

Introduction Osteoporosis is not only responsible for an increased number of metaphyseal and spinal fractures but it also complicates their treatment. To prevent the initial loosening, we developed a new implant with an enlarged implant/bone interface based on the concept of perforated, hollow cylinders. We evaluated whether osseointegration of a hollow cylinder based implant takes place in normal or osteoporotic bone of sheep under functional loading conditions during anterior stabilization of the lumbar spine.

Materials and methods Osseointegration of the cylinders and status of the fused segments (ventral corpectomy, replacement with iliac strut, and fixation with testing implant) were investigated in six osteoporotic (age 6.9 ± 0.8 years, mean body weight 61.1 ± 5.2 kg) and seven control sheep (age 6.1 ± 0.2 years, mean body weight 64.9 ± 5.7 kg). Osteoporosis was introduced using a combination protocol of ovariectomy, high-dose prednisone, calcium and phosphor reduced diet and movement restriction. Osseointegration was quantified using fluorescence and conventional histology; fusion status was determined using biomechanical testing of the stabilized segment in a six-degree-of-freedom loading device as well as with radiological and histological staging.

Results Intact bone trabeculae were found in 70% of all perforations without differences between the two groups ($P = 0.26$). Inside the cylinders, bone volume/total volume was significantly higher than in the control vertebra (50 ± 16 vs. 28 ± 13%) of the same animal ($P<0.01$), but significantly less ($P<0.01$) than in the near surrounding (60 ± 21%). After biomechanical testing as described in Sect. "Materials and methods", seven spines (three healthy and four osteoporotic) were classified as completely fused and six (four healthy and two osteoporotic) as not fused after a 4-month observation time. All endplates were bridged with intact trabeculae in the histological slices.

Conclusions The high number of perforations, filled with intact trabeculae, indicates an adequate fixation; bridging trabeculae between adjacent endplates and tricortical iliac struts in all vertebrae indicates that the anchorage is adequate to promote fusion in this animal model, even in the osteoporotic sheep.

Keywords Animal model · Osteoporosis · Fracture treatment · Spine · Spinal implant · Sheep

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Introduction

Osteoporosis is not only responsible for an increased number of metaphyseal and spinal fractures [29] but it also complicates their treatment [5]. The rarefaction of the trabecular network that leads to spontaneous fracture persists also during fracture healing. This complicates the anchorage of stabilizing implants significantly. It results in an increased number of implant failures during the surgical treatment of osteoporotic fractures [6, 21].

The process of implant loosening begins with local failure of the surrounding bone structure at the point of peak stress during loading of the bone/implant construct. If bone trabeculae are significantly reduced in number and thickness, as in osteoporosis, the load-bearing interface between bone and implant is reduced and therefore subjected to higher stress levels even under a given load. Thus, the risk of local failure increases. Upon breakage of the initial load-bearing trabeculae, the remaining trabeculae in contact with the interface are forced to withstand an even greater load. This ultimately leads to successive, non-linear failure of the interface that becomes clinically evident as "cutting-through" or "cutting-out" phenomenon [11].

Several attempts were made to prevent this initial loosening of the implant. Several authors have argued that enlargement of the implant could lead to better anchorage [8, 15]. However, enlargement is limited by the anatomical boundary conditions and biological compatibility.

To prevent the initial loosening, we developed a new implant with an enlarged implant/bone interface based on the concept of perforated, hollow cylinder [32]. Mechanical testing revealed in the meantime that cutting through the adjacent bone is significantly less than with conventional screws [13]. However, osseointegration of the implant in osteoporotic bone is a prerequisite for maintaining the fixation during fracture healing and yet is not well investigated.

Therefore, the present study was designed to evaluate whether osseointegration of a hollow cylinder based implant takes place in normal bone of sheep as well as in osteoporotic structure under functional loading conditions during anterior stabilization of the lumbar spine. A further aim was to examine whether this type of implant provides enough stability to promote fusion of the anterior column.

Materials and methods

Osteoporosis animal model

Osteoporosis was introduced in ten female white alpine sheep (age 6.9 ± 0.8 years, mean body weight 61.1 ± 5.2 kg) using a combination protocol of ovariectomy, high-dose prednisone, calcium and phosphor reduced diet and movement restriction [19, 20]. A drug-free period of 2 months before spinal surgery ensured normalization of the hormonal status to avoid influences on the osseointegration and fusion processes [31]. At the time of operation, the cancellous bone mineral density at the distal radius was reduced in average by 30% compared to the initial values and did not recover until the end of the experiment [12].

Eight healthy sheep (age 6.1 ± 0.2 years, mean body weight 64.9 ± 5.7 kg) served as a control. For this animal study, approval was obtained according to the applicable state and federal guidelines. The side effects of the osteoporosis introduction via high-dose steroids resulted in the loss of two animals that did not survive anaesthesia during spinal surgery. Implant dislocation associated with neurological symptoms were the reason for the loss of two more osteoporotic and one control sheep. The analysis is based on the values of six osteoporotic and seven control sheep.

Implants and implant procedure

The newly developed implant based on hollow, perforated cylinders was downscaled to meet the sheep anatomy [25, 37]. An anti-rotation screw was placed in continuation of the bridging element to prevent rotation around the longitudinal axis of the cylinder (Fig. 1). The cylinders were coated with Bonit® (hydroxyapatite/brushite 1.67/1.1, DOT, Rostock, Germany) in order to enhance osseointegration.

After sterile preparation, the anterior lumbar spine was exposed via a lateral, retroperitoneal approach as described by Baramki et al. [3]. The anterior part of L4 was dissected with an oscillating saw up to the anterior edges of both processus lateralis (ventral corpectomy).

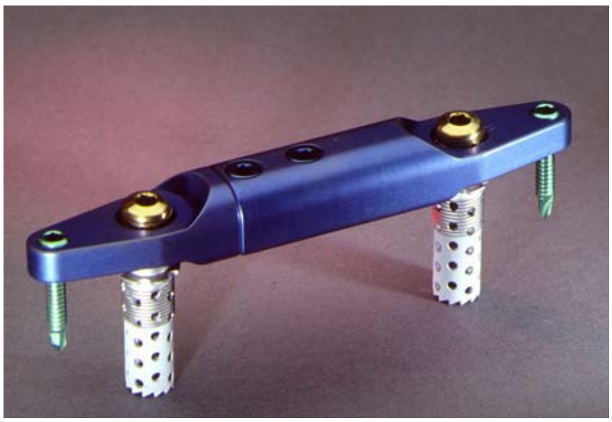

Fig. 1 Sheep implant based on hollow, perforated cylinders with dimensions adapted to sheep geometry (locked anti-rotation screw in line with the cylinders)

Both adjacent discs were removed and the endplates of L3 and L5 cleaned and scraped. The distance between endplates was measured and a tricortical strut from the ipsilateral iliac crest of the corresponding size was used for replacement [18]. One cylinder was inserted after pre-drilling with a biopsy drill (7.35/6.45 outer/inner diameter) into the distal part of L3 and one into the proximal part of L5. The cylinders were oriented within a mean angle of 10° to the adjacent endplate. The angle of the cylinders was fixed with the locking mechanism at the end of the cylinder-plate junction and an additional anti-rotation screw was added into each vertebra (Fig. 1). The iliac crest defect was covered with polyurethane sponges to reduce the risk of major bleedings. The sheep were supported with slings for 1 week and received adequate pain treatment. Neurological status as well as health status were controlled weekly, X-ray controls (a.p.) were taken immediately after operation, 1 and 2 weeks later, and then on a monthly base. All animals were labelled with Calceingreen (weeks 5, 7, and 9) and Xylenolorange (weeks 11, 13, and 15) according to Rahn [28]. The animals were sacrificed 16 weeks after operation using an overdose of Vetanarcol.

Outcome variables

Macroradiography

The lumbar spines were harvested and soft tissue was removed carefully. The fused segments from L3 to L5 were dissected leaving ligaments intact. Macroradiographs were taken in a.p. and lateral direction using Faxitron 804 (Faxitron Company, IL, USA) and repeated after the biomechanical testing and removal of the bridging bar. Two independent observers evaluated all macroradiographs with respect to fusion, signs for lyses around the implant, and possible implant failure.

Biomechanical testing

The upper part of L3 and the lower part of L5 were embedded in the potting jig of the testing machine. Cancellous bone screws were introduced into both outer endplates and embedded into PMMA. The specimens were loaded in a six-degree-of-freedom loading device [22]. The tests were carried out by application of moments in the three principal directions separately (flexion/extension—left/right lateral bending—left/right axial rotation). The loads were applied continuously with a sinusoidal shape, a magnitude of approximately ± 6.0 N m for *flexion/extension* and lateral bending and 7.5 N m for torsion. All tests were performed twice: first, with the complete implant and second, without the bridging bar. The following values were calculated out of the hysteresis curves: range of motion with ± 6 N m, high stiffness (HS), low stiffness (LS), and ratio between both. Stiffness was derived from the ratio between load and displacement. In fused segments, stiffness over the whole range of motion is expected; in non-fused segments, the typical double-S shape is expected. In the latter case, the LS zone corresponds to the "neutral zone" and the HS zone to both ends of the curve [23]. Additionally, the loss of stiffness as well as the increase of ROM due to the removal of the connecting bar was calculated [17].

Histology

After the non-destructive biomechanical testing, the specimens were immediately processed for histological evaluation. Twenty-eight slices per vertebra including the fusion zone with a thickness of 200 μm were cut perpendicular to the main axis of the cylinders. Four representative localizations over the whole length of the cylinder were chosen for further evaluation under light and fluorescence microscope. Macroradiographs were taken from every slice (Faxitron 804, Faxitron Company) and digitized afterwards (Fig. 2). One half of the slices was stained with basic fuchsine and light green; the other one was prepared for fluorescence microscopy.

The following outcome variables were derived from the measurements described before: number of perforations with bony content and condition (none, partial, or complete ingrowth), bone volume (BV) per total volume (TV) as a surrogate for bone mineral density (BV/TV) inside the cylinder and outside (defined as circle ring around the cylinder with an outer diameter

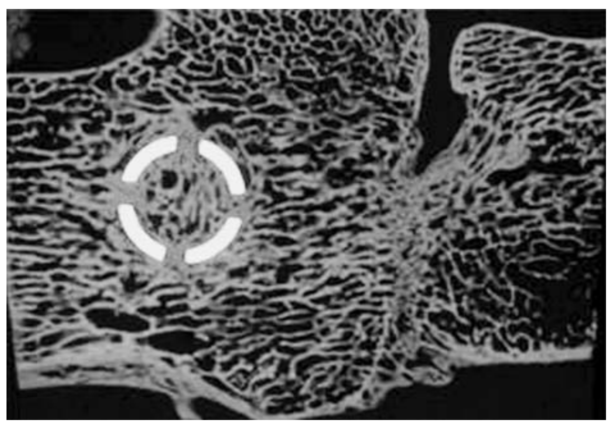

Fig. 2 Example of a cylinder with bony ingrowth into the perforations, and completed fusion (*right*)

of 1.5 times the outer cylinder diameter), presence and thickness of any capsule around the cylinder (straight contact, fibrous layer between 0.025 and 0.25 mm, and complete capsule with a thickness of more than 0.25 mm), percentage of new formed bone in the first half of fracture healing (green) and second half (red), fusion status of every endplate (none, single trabeculae, more than 50% bridged with trabeculae), fusion of the complete motion segment, described biomechanically and coincidence of fusion process (ratio of green to red labelled bone inside the cylinder and the adjacent, fused endplate), and bony ingrowth into the perforations. The vertebra L6 of each sheep served as a control for the BV per TV.

Statistics

The necessary sample size of each group was determined during the planning of the experiment using statistical power estimation. A difference of more than 25% can be detected with a statistical power of $\beta = 0.8$, assuming a standard deviation of 8.6% by using more than six sheep per group. Our zero hypothesis was that the cancellous bone inside the cylinder will reduce compared with the control of a healthy vertebra due to the missing mechanical stimulation (outcome variable BV/TV), and the osteoporotic sheep will show a lower fusion rate (outcome variable fusion status of the endplate) and less ingrowth into the holes (outcome variable ingrowth). Parameter differences were tested with the program SPSS 11.5 using the non-parametric Wilcoxon ranking test and Fisher's exact test with $\alpha = 0.05$.

Results

Osseointegration

Overall, continuous bone trabeculae were found in 70% of all perforations without differences between the two groups ($P = 0.26$). There were also no differences between the ingrowth into the cylinders of the cranial vertebra L3 and the caudal vertebra L5 ($P = 0.88$). Interrupted trabeculae were observed in 16 perforations and additional 13 were filled with fibrous tissue. The fluorescence pictures revealed that 12% of all perforations were filled within the first 9 weeks after operation (green) and the majority (88%) in the second half of fracture healing (see Fig. 4). Inside the cylinders, BV/TV was significantly higher than in the control vertebra L6 (50 ± 16% vs. 28 ± 13%) of the same animal ($P<0.01$), but significantly less ($P<0.01$) than in the near surrounding (60 ± 21%). Osteoporotic sheep showed lower mean values of BV/TV compared to the non-osteoporotic sheep in the control vertebra (−6%), inside the cylinder (−13%) and outside (−12%) (Fig. 3). No differences were found between L3 and L5. In the second half of fracture healing, significantly more (+30%) bone was formed in- and outside the cylinders ($P<0.001$) without any differences between both groups.

The evaluation of the macroradiographs by two independent observers revealed the same results in 80% of all cases. No radiological signs for lyses were found in 7 (observer 1) resp. 8 (observer 2) of 12 cylinders of the osteoporosis group. The control group revealed no differences in the percentage of macroradiographs without signs for lyses (9 of 14).

The evaluation of the histological slices of all cylinders (26 cylinders in the pooled group) revealed a complete capsule around three cylinders (mean thickness 0.8 mm). All of these animals showed signs of a secondary dislocation of the implant during the healing process. Around six cylinders (23%), no fibrous tissue could be detected and direct bony contact to the implant surface was observed. A thin of fibrous layer

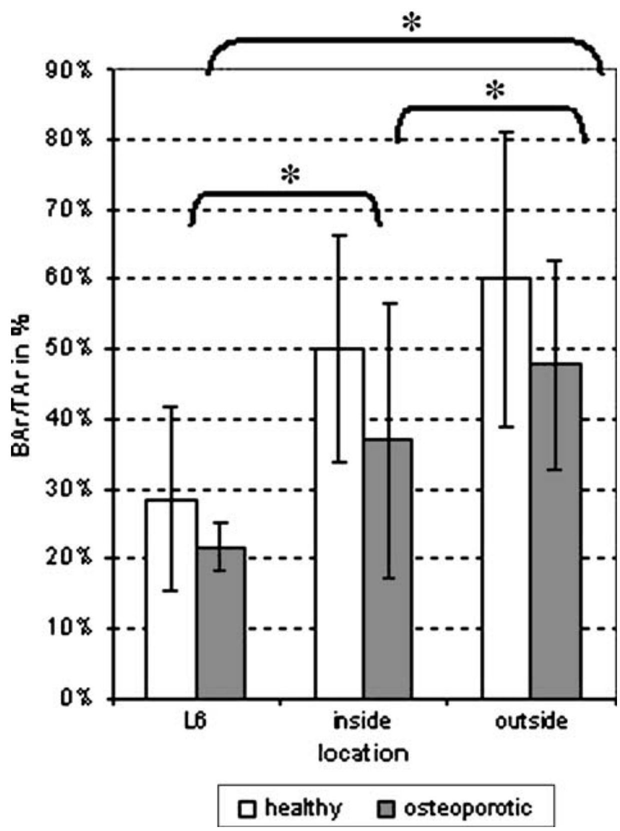

Fig. 3 Comparison of bone content (Bar/Tar) inside the cylinder, outside, and in a control vertebra (L6) of the same animal; note significant more bone inside and around the cylinders compared with the control vertebra, marked with *asterisk*

covered all other cylinders (65%). No difference was found between L3 and L5 ($P = 0.59$) and between both groups ($P = 0.32$).

Fusion

Nine of 13 spines were regarded as fused after examination of the X-rays. The group of osteoporotic sheep showed a higher, non-significant percentage (67% in osteoporotic sheep in contrast to 43% in normal sheep) of fused segments ($P = 0.59$ with Fisher's exact test).

Whereas specimens that were not fused completely after 4 months showed the characteristic bilinear hysteresis curve in the mechanical tests, fused spines showed an almost linear hysteresis curve. Additionally, the increase in range of motion in extension/flexion was significantly higher after implant removal in animals with non-fused spines (Table 1). At the same time, stiffness in lateral bending decreased in not fused segments significantly more than in fused segments ($P = 0.006$). Using these variables to describe the hysteresis curve for every specimen, seven spines (three healthy and four osteoporotic) were classified as completely fused and six (four healthy and two osteoporotic) as not fused after a 4-month observation time.

All endplates were bridged with intact trabeculae in the histological slices (Fig. 2). Six of the seven fused segments showed signs for resorption in the middle part where the autologous bonegraft was inserted.

Coincidence of osseointegration and fusion

Fusion took place prior to the osseointegration in 50% of all fused endplates ($n = 14$). Only in 21%, the osseointegration of the cylinder was faster than the fusion of the adjacent endplate. No significant differences were found between both testing groups with respect to the relationship between osseointegration and fusion.

Discussion

Although hollow cylinders were introduced for the anchorage of implants in dental surgery nearly 25 years ago [32], the results cannot be extrapolated to other locations and indications due to differences in the loading of the implant and the differences between the two-step implantation of dental prosthesis and the one-step treatment of a fracture. Whereas in dental implants osseointegration has to take place prior to load carrying, in fracture treatment both have to progress at the same time and will interact with each other. This assumption is supported by our findings about the coincidence of fusion and osseointegration. Hollow cylinders without a large thread have to be osseointegrated to fulfil their function as a load-carrying anchorage device. A complete capsule formation would reduce the pullout forces significantly and result in unstable fixation. However, the high number of perforations, filled with intact trabeculae, indicates an adequate fixation (Fig. 4). It would not be possible under micromotion with an amplitude over 0.5 mm according to the findings from Aspenberg et al. [2].

The increased BV inside and around the cylinders also indicates an adequate bony incorporation. The biological stimulation subsequent to the preparation of the implant bed seems to overcompensate the mechanical environment due to the stiff cylinder wall [24, 36, 38]. A longer follow-up period could help to clarify whether the overcompensation inside and around the

Table 1 Differences in range of motion and stiffness of the stabilized segments with respect to fusion status after 4 months ($n = 13$), values are provided as means ± standard deviation, for definition of low and high stiffness please see "Materials and methods"

	Fused	Not fused	*P*
Osteoporosis status	3 Healthy and 4 osteoporotic	4 Healthy and 2 osteoporotic	0.391[a]
ROM *Ex/Flex* (deg)	2.33 ± 0.44	4.58 ± 1.72	0.023
ROM *lateral bending* (deg)	1.60 ± 0.61	4.17 ± 1.91	0.020
ROM *torsion* (deg)	1.12 ± 0.12	1.76 ± 0.38	0.007
Increase ROM due to implant removal (%)	12.41 ± 8.02	34.9 ± 15.08	0.013
Low stiffness *Ex/Flex* (N m/deg)	4.11 ± 1.29	1.84 ± 0.82	0.003
Low stiffness lateral bending (N m/deg)	6.92 ± 2.68	2.23 ± 1.41	0.003
Low stiffness torsion (N m/deg)	9.93 ± 1.77	5.51 ± 1.55	0.002
Decrease of low stiffness *Ex/Flex* after implant removal (%)	−6.75 ± 8.27	−31.04 ± 14.09	0.006
Decrease of low stiffness lateral bending after implant removal (%)	−4.98 ± 7.43	−25.45 ± 23.85	0.091
Stiffness ratio (low stiffness/high stiffness)	0.12 ± 0.11	0.36 ± 0.13	0.006

[a] Percentage of fusion in the osteoporosis group vs. control group

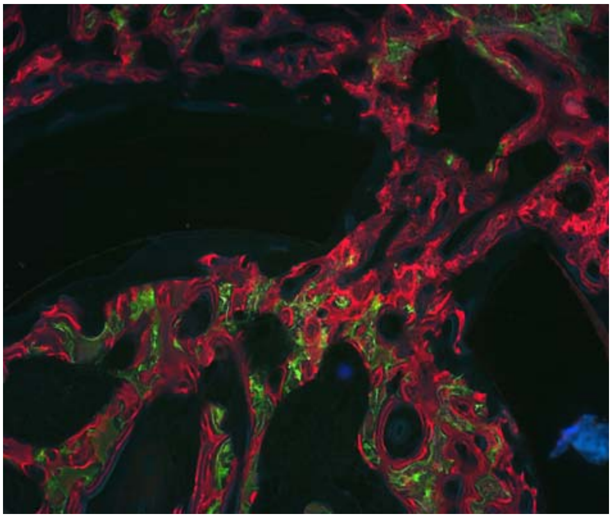

Fig. 4 Detail of a cylinder showing ingrowth through a perforation into the cylinder (fluorescence microscopy). Ingrowth took place in the second half of the fracture healing period, marked with *red fluorescence labelling*

cylinder will remain or if the bony content will be resorpted later on. The increased bone formation enhances the bony incorporation of the implant and decreases the risk of implant failure by cutting through the adjacent bone. However, only a small amount of the cylinders (23%) can be regarded as fully osseointegrated according to the strict definition of osseointegration given by Branemark [4]. The observed thin layers around the majority of the cylinders prevent the direct structural and functional connection between ordered, living bone and the surface of a load-carrying implant. Beside micromotion, the relatively fast acting coating could also be the reason for the fibrous layers [27]. Initially, the coating with hydroxyapatite/brushite was chosen to enhance osseointegration as it was shown by other groups [1, 30]. Since the resorption process of this coating is unknown in spinal applications, this is now the subject of further investigations.

The histological observation of bridging trabeculae between adjacent endplates and tricortical iliac struts in all vertebrae indicates that the anchorage is adequate to promote fusion, even in the osteoporotic sheep. These results would better meet the biomechanical definition of osseointegration; the term osseointegration should be reserved to the case of full force-transmitting attachment [35]. The fusion rate in osteoporotic spines can be the same, if the implant can be anchored sufficiently. Whereas during histological investigation the fusion of every endplate can be evaluated separately, the biomechanical evaluation describes the whole testing segment. Typical bilinear curves, similar to a normal motion segment, were found in specimens with histological signs for resorption in the middle of the iliac strut. The graft resorption is known from clinical studies and may result in unloading either due to an inadequate surgical technique or stress shielding in combination with a stiff implant [7, 10]. In these cases, the remaining stabilizing effect of the implant could be demonstrated in the non-fused group, where stiffness decreased after removal of the implant more than 25% in contrast to fused spines with less than 7% decrease on average. The parameters chosen to describe the hysteresis curves, e.g. the stiffness ratio showed highly significant differences between completely fused and non-fused spines and are in line with other groups [16]. They are suitable in distinguishing between fused and non-fused spines and help to evaluate the shape of spinal hysteresis curve. The comparison of the timing of fusion and the ingrowth into the cylinders revealed that both processes go parallel. This is only possible if the primary stability is adequate [26].

The results of the study have to be discussed with respect to the limitations of an animal model. Although sheep were used for several studies on spinal implants [14, 16, 34], biological and biomechanical boundary conditions are different. The ratio between anchorage depth of the cylinders in the sheep vertebra and the bridging length is much smaller than in humans. The lever arm on each cylinder becomes bigger. Additionally, the diameter of the cylinders is less than half of the human implant (Fig. 5). This results in

Fig. 5 Antero-posterior and lateral view on a fused segment after disassembling of the bridging part. Note the complete fusion but the *thin radiolucent line* around the proximal cylinder, visible in both planes

a decreased implant/bone interface and less active cancellous bone for osseointegration. In conclusion, our model represents a situation that is close to a biomechanical worst-case scenario in humans [33]. The osteoporosis induction including high-dose prednisone treatment seems to reduce the general health of the animals despite the termination of induction 2 months before operation. It is in our opinion responsible for the high dropout rate. Although several groups work with this osteoporosis model, the question remains whether it is appropriate to study fracture healing in osteoporotic bone [9]. In summary, full force-transmitting osseointegration of the testing implant is possible. However, in further studies, the choice of some influencing factors such as appropriate animal model or coating of the implant has to be reconsidered.

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