To my parents

Bone regeneration for spinal fusion – translational studies and the pathway to the patient

Diyar Delawi

Colofon

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Bone regeneration for spinal fusion – translational studies and the pathway to the patient

Bot regeneratie voor wervelfusies – translationele studies en de route naar de patiënt

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This thesis is based upon the following publications

1. The incidence of donor site pain after bone graft harvesting from the posterior iliac crest may be overestimated: a study on spine fracture patients. D Delawi, WJA Dhert, RM Castelein, AJ Verbout, FC Oner Spine, 2007 Aug 1;32(17):p1865-8

2. Relevance of bone graft viability in a goat transverse process model. MC Kruyt, D Delawi, P Habibovic, FC Oner, CA van Blitterswijk, WJA Dhert Journal of Orthopaedic Research, 2009 Aug;27(8):p1055-9.

3. Platelet leukocyte gel facilitates bone substitute growth and autologous bone growth in a goat model. PA Everts, D Delawi, CB Mahoney, A van Erp, EP Overdevest, A van Zundert, JT Knape, WJA Dhert Journal of Biomedical Materials Research, Part A, 2010 Feb;92(2):p746-53.

4. Comparing Autograft, Allograft, and Tricalcium Phosphate Ceramic in a Goat Instrumented Posterolateral Fusion Model. D Delawi, MC Kruyt, Y Huipin, KL Vincken, JD de Bruijn, FC Oner, WJA Dhert Accepted in Tissue engineering, Part C

5. Case study regenerative medicine: spinal fusion. D Delawi, FC Oner, WJA Dhert Book chapter in Converging Technologies 2006, Study Centre for Technology Trends, Edited by M Doorn, The Netherlands, ISBN-10: 90-809613-3-7, p74-91

6. A prospective, randomized, controlled, multicenter study of osteogenic protein-1 in instrumented posterolateral fusions: report on safety and feasibility. D Delawi, WJA Dhert, L Rillardon, E Gay, D Prestamburgo, C Garcia-Fernandez, E Guerado, N Specchia, JL van Susante, N Verschoor, HM van Ufford, FC Oner Spine, 2010 May 20;35(12):p1185-91

7. OP-1 compared to iliac crest autograft in instrumented posterolateral fusion: a randomized multi-center non-inferiority trial. D Delawi, W Jacob, JLC van Susante, L Rillardon, D Prestamburgo, N Specchia, E Gay, N Verschoor, C Garcia-Fernandez, E Guerado, J van Ufford, MC Kruyt, WJA Dhert, FC Oner Submitted to The Spine Journal

8. Conducting a European multi-center trial: first experiences with the new EU Clinical Trials Directive from an academic perspective. D Delawi, WJA Dhert, FC Oner European Spine Journal, 2008 Aug;17(8):p1113-5.

Chapter 1

Introduction, aims and outline of the thesis

Spinal fusion is a surgical technique in which one or more vertebrae of the spine are joined (fused) through the formation of a bone bridge, so that motion can no longer occur between them. It is performed for deformities, instability, infections, fractures and pain from degenerative disorders. The gold standard for achieving a fusion between the adjacent vertebrae is by using autologous bone grafts (autograft) from the iliac crest. During surgery, the bone graft is harvested from the patient's own pelvis and placed around the spine. The bone graft will stimulate the body to form bone tissue, and in several months the vertebrae will grow together — 'fuse' — into a single bone.

Although for many surgeons autologous bone is the gold standard for spinal fusion, this is associated with significant drawbacks. One of the main shortcomings is failure of the fusion, the so-called non-union. This has been reported to occur in more than 30% of the patients.[1–4] The introduction of metal implants has decreased the rate of non-union,[5] but its incidence remains unacceptably high. Another major drawback of bone grafting is the harvesting of bone from the iliac crest, which requires an additional surgical procedure that is time consuming and is associated with complications. In some cases the harvesting procedure can even be more problematic than the primary surgical procedure itself. Major complications, such as pelvic fractures, nerve injuries, vascular injuries and infections, have been reported.[6–11] The most common complication, however, is persistent postoperative pain at the donor site. The exact incidence of chronic donor site pain is unclear, since it ranges in literature from 6% to 39%.[10,12–18] Another concern regarding autologous bone grafts is that the amount available for transplantation can be insufficient. This is especially the case in children, when multiple levels need to be fused, or when autograft has been harvested before. These disadvantages of autologous bone grafting drive the quest for alternative grafts capable of bone regeneration.

The biological processes involved in bone regeneration require three essential elements: osteoconduction, osteoinduction and osteogenesis. Osteoconduction describes the facilitation of a framework, or scaffold, on which osteoblasts from the neighboring bone can spread and form new bone. Thus, the bone formation is conducted through the graft site. Osteoinduction is the initiation of differentiation of susceptible mesenchymal cells towards the osteogenic lineage. A cascade of growth factors signals mediates this process of which the most important belong to the TGF-ß super-family.[19] Osteogenesis refers to the presence of cells that can form bone (i.e. osteoblasts). Autologous bone possesses all the three elements. Although an ideal bone graft should exhibit osteoconductive, osteoinductive and osteogenic properties, it does not imply that all characteristics are mandatory for a bone graft to be effective.

Allograft is currently one of the most frequently used alternatives for autologous bone grafts,[20] because it is readily available and avoids complications associated with the harvest of autologous bone. Besides osteoconductive it is considered to have weak osteoinductive capabilities. As a result of processing to decrease immunogenicity and the risk of disease transmission, it contains almost no viable cells. Allograft is considered less potential in its bone forming capacity than autologous bone.[21] Several clinical trials indicated that allograft alone was not sufficient to achieve an acceptable fusion rate for posterior

spinal fusion in the adult patients.[22–24] Beyond the question of efficacy, the potential risk of disease transmission remains a concern associated with the use of allograft bone.[25] In an attempt to develop a bone graft substitute that overcomes the disadvantages of allograft and autologous bone, while maintaining an acceptable rate of fusion, several strategies to engineer an alternative by using regenerative strategies have been developed.

Regenerative Medicine Strategies

Bone tissue engineering or regenerative medicine strategies have shown to be a promising technology to develop an alternative for autologous bone graft. The concept of these techniques is to develop biologically active implants that restore, maintain or improve tissue function.[26] In general, regenerative therapy strategies are based on synthetic scaffolds that contain or recruit growth factors and/or inducible cells to form bone. Despite the efforts of many scientists and clinicians, the clinical implementation of regenerative therapies is still limited. Nevertheless, a number of bone graft alternatives are currently commercially available for orthopedic use. They vary in composition, mechanism of action, and many other characteristics. The bone graft substitutes can be categorized in the mechanism(s) by which they facilitate bone formation: osteoconduction, osteoinduction, osteogenicity or osteopromotion.[25]

Osteoconductive materials

Osteoconduction is probably the most important characteristic that is likely to be a prerequisite for any bone graft substitute. Examples of osteoconductive materials include most ceramics and coatings of endoprostheses. Also the function of bone from cadaver sources (allograft) largely relies on osteoconduction.

Ceramics

Ceramics are solid, inorganic compounds consisting of metallic and nonmetallic elements held together by ionic or covalent bonds. Since calcium phosphates are the main constituent of the inorganic phase of natural bone, these ceramics have already been investigated for over four decades.[27] The advantages of ceramics are unlimited supply, low costs, ease of sterilization and storage. The most commonly used ceramic scaffolds for spinal fusion are calcium phosphates such as hydroxyapatite, tricalcium phosphate, and combinations of these. Of special interest are the tricalcium phosphate (TCP) ceramics due to their good bioresorbability that allows complete remodeling, in contrast to the non-resorbable hydroxyapatite. Preclinical studies,[28–31] as well as clinical studies,[32–39] have generated encouraging data when using TCP in lumbar posterolateral fusions, but almost exclusively in combination with local bone, bone marrow aspirate and/or bone morphogenetic proteins. The vast majority of ceramic scaffolds are only osteoconductive. However, it was recently shown that ceramics could be endowed with biologically instructive properties by changing basic physicochemical parameters of the materials, such as surface textures.[40] In this way TCP was capable of possessing an intrinsic osteoinductive capacity comparable to the addition of BMPs. In a comparative study osteoinductivity of

calcium phosphate ceramics appeared to be advantageous for bone defect healing when compared to non-osteoinductive phosphate ceramics. [41] To date, no clinical studies have evaluated this new calcium phosphate ceramic in spinal indications.

Osteoinductive growth factors

Bone regeneration based on the delivery of osteoinductive molecules is an emerging field within tissue engineering research that has evolved tremendously over the past decades. It is generally accepted that conclusive evidence for osteoinduction can only be given when implantation of the agent gives bone formation in tissues where bone normally does not grow. Currently, two well-known examples of osteoinductive agents are bone morphogenetic proteins and demineralized bone matrix.

Bone Morphogenetic Proteins

Dr. Marshall Urist discovered almost 5 decades ago that proteins embedded in the bone matrix can initiate a cellular response resulting in new bone formation when implanted at an ectopic site. [42] He later named the active component 'bone morphogenetic protein' (BMP). [43] It took, however, several decades before BMPs could be isolated and produced in large quantities, since it required synergy of the advances made by several distinct disciplines during the last century, including biochemistry, biomaterial science, imaging, and molecular biology. Although BMPs were originally discovered by their ability to induce bone formation, it is currently well known that BMPs affect structures and processes throughout the entire body, ranging from embryonic patterning and development through stem cells, to tissue homeostasis and regeneration. [44]

Today, two BMPs are commercially available for bone regeneration: BMP-2 and BMP-7. BMP-7, which is also known as Osteogenic Protein-1 (OP-1), has received a humanitarian device exemption (HDE) for revision posterolateral lumbar spinal fusion and long bone nonunions in 2004. A HDE is actually intended for a small subset of patients for which the manufacturer's research costs make FDA approval financially unattractive. Therefore it does not require any clinical investigations demonstrating its effectiveness. BMP-2 also has a HDE, for posterolateral lumbar spine non-unions. In addition, it has FDA pre-market approval for use in anterior lumbar interbody fusions, acute open tibial shaft fractures and maxillofacial sinus and alveolar ridge augmentations.

Despite this limited approval, BMP usage has been rapidly incorporated into the standard surgical practice with nearly 40% usage in lumbosacral spinal fusion surgeries in the United States. [45] The scientific basis for the use of BMP-2 in spinal indications has recently been firmly criticized, due to methodological biases, and structural underreporting of adverse events in industry-sponsored publications. [46–50] For OP-1, also no clear evidence is currently available that has indisputably shown its efficacy in spinal applications. More controlled clinical trials for each BMP and for each application need to be performed, not only to define the clinical efficacy, but also to clearly define safety parameters for these highly osteoinductive proteins, before implementing into standard care.

Demineralized Bone Matrix

Demineralized Bone Matrix (DBM) is a family of commercially available products that are created by removing the mineral content of cadaver bone (allograft) by acid extraction. The remaining matrix contains collagen, non-collagenous proteins and a low concentration of growth factors. After demineralization, the matrix is mixed with a variety of carriers designed to provide various handling characteristics and is available in various forms, such as chips, gel, putty or powder. DBM has shown to have osteoinductive properties based on BMP activity, although the concentrations are extremely low compared to the purified BMPs. Additionally, the BMP content varies significantly between different DBM products and even more between batches of the same product.[51] This, in combination with different carriers with distinct osteoconductive capacities, makes DBM a highly variable product despite their common name. In general, DBM is only effective as a bone graft extender for spinal fusions, rather than a stand-alone product.[24]

Cell-based therapies

During the last decade much research focused on developing specific cell-based therapies, in particular involving the multipotent mesenchymal stem cell (MSC). These MSCs exhibit potency to differentiate into cells capable of producing mesenchymal tissues, including bone, cartilage, muscle and adipose tissue. The cells can be harvested from readily available sources including bone marrow and fat tissue. The studied applications of the MSCs range from direct injection of unprocessed blood or bone marrow aspirates, to fabrication of engineered constructs by seeding of natural or synthetic scaffolds with cells.[52] A major drawback of one stage cell-based therapies is that the number of directly obtained MSCs from the donor tissues is low. To increase the concentration of MSCs, several techniques have been developed, especially ex-vivo cell expansion, but many problems limited its clinical application, such as long culture time, high cost and the mixture of human cell culture medium with fetal bovine serum.[53] There are several animal studies showed promising results when using MSCs in combination with ceramics for spinal fusion.[54,55] Also, three clinical trials have shown comparable fusion rates when using MSCs with ceramics to autologous bone graft from the iliac crest.[53,56,57] However, these studies did not investigate the added value of the MSCs. More clinical studies are needed to fully establish the efficacy of these MSCs in terms of successful spinal fusion, including evaluation to a control group without cells.

Osteopromotive agents

Osteopromotive agents are (growth) factors that assist in de novo bone formation, although these agents are not distinctly osteoinductive. Increasingly used osteopromotive agents are platelet-derived products. These preparations have been used since the 1990s to promote bone and soft tissue healing.[58] Platelet rich therapies can be produced by either centrifugation or filtering of autologous whole blood with an anticoagulant to obtain supraphysiological concentration of platelets. This fraction can be used alone as plasma, or in combination with a platelet-activator, such as thrombin

to create a gel. The rationale for using platelets rich therapies lies in the identification of platelets as the main regulators of in the inflammatory phase of tissue repair, and in the essential role they play in the proliferation and differentiation phase.[59] This process is mediated by platelet derived growth factors, including platelet-derived growth factor (PDGF), transforming growth factor-ß (TGF-ß) and vascular endothelial growth factor (VGEF), which have been shown to contribute to bone regeneration and vascular proliferation.[60] Thus, the possibility of delivering these growth factors within a bone defect is behind the theory of the use of platelet rich strategies for bone regeneration.

The platelet rich therapies for bone regeneration are only suitable to enhance the bone forming capacities in combination with other bone grafts, such as ceramic or local bone. To date, several clinical trials have been conducted with platelet derived products, but firm conclusions are difficult to make due to conflicting results, which may be caused by the lack of standardized preparation protocols and differences in application techniques.[61]

Aim of this thesis

The central aim of this thesis is to study the potential of several promising bone grafts substitutes in comparison to iliac crest autograft for spinal fusion. The emphasis will be on posterolateral fusion, since this is one of the most commonly performed techniques, but also one of the most challenging indications for bone grafts, due to the large distance that needs to bridged, limited contact surface and the unfavorable biomechanical environment. This thesis includes several preclinical and clinical studies including a randomized clinical trial.

Outline

We started with critically investigating two assumptions that are generally made when discussing the use of autograft: 1) the morbidity of bone graft harvesting and 2) the relevance of viable cells within this graft. Although many severe and major complications are reported from harvesting bone from iliac crest,[6,7,11,12,62–64] the most common complication is enduring pain at the donor site. As mentioned before, the reported incidence of donor site pain varies between 6% to 39%.[10,12,13,15,17,18,63] The close proximity of the primary surgery to the iliac crest, however, could interfere with the reported incidence. In **Chapter 2** the incidence of donor site pain was compared between patients who underwent fusion of different spinal levels to evaluate this effect and to determine the 'true' importance of donor site pain after posterior iliac crest bone harvesting. In **Chapter 3** we investigate the relevance of viable osteogenic cells in autologous bone grafts. This is important as viable cells are a prominent difference between autologous bone graft and most conventional alternatives, which may be a reason for its superiority. On the other hand it is unlikely that these cells survive after transplantation.[65,66] The bone forming capacity between viable and devitalized autologous bone grafts was evaluated in a chamber model mounted on the transverse processes of a goat.

In the continuing chapters several bone grafts or enhancers were compared to iliac crest autograft. In **Chapter 4** the effect of adding platelet-leukocyte gel to three bone grafts (autologous bone graft, biphasic calcium phosphate and trabecular metal) was evaluated in the same transverse process cassette model that represents the initial phase of bone formation for spinal fusions. The next objective was to determine if a new putatively bioactive tricalcium phosphate was a suitable bone graft substitute for spinal fusions in a large animal model. It was compared to the currently most used grafts: iliac crest autograft and allograft. In **Chapter 5** we present the results of an instrumented posterolateral fusion study in goats.

The final part of this thesis focuses on bone morphogenetic proteins (BMPs). In **Chapter 6** we describe how the sequential converging of the technologies of biochemistry, biomaterial science, imaging, and molecular biology finally resulted in the development of a new regenerative treatment (the use of BMPs) in orthopaedics. In contrast to the decades it took from the discovery of BMPs to becoming commercially available, these proteins are rapidly incorporated in the clinical routine with limited, if any, evidence for most indications. In this thesis, we conducted a European multi-center study comparing OP-1 (BMP-7) to iliac crest autograft in instrumented posterolateral fusions, which is one of the most applied indications of BMPs. We started with a pilot phase to obtain information on safety and feasibility of which the results are shown in **Chapter 7**. After this pilot, we continued the whole study that is presented in **Chapter 8**. During the course of this clinical study, the European Union adopted a new Clinical Trials Directive (2001/20/EC) as a framework for good management in trials of medicine. The goal of this directive was simplifying and harmonizing the administrative provisions governing clinical trials in EU countries. In **Chapter 9**, we discuss the practical consequences of this Clinical Trials Directive. Especially with respect to our situation during the conduction of a European multi-center study.

We conclude this thesis with a general summery, which addresses the previously discussed items in Chapter 10, and finish with a general discussion and future perspectives in **Chapter 11**.

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Chapter 2

The real incidence of donor site morbidity after bone graft harvesting

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Introduction

Autologous bone graft is the gold standard for various reconstructive orthopaedic procedures, including spinal fusion. Although many bone substitutes are nowadays available, autologous bone has the advantage of being osteoinductive, osteoconductive, and potentially osteogenic. Furthermore, autologous bone grafts raise no concerns regarding immunogenicity, histocompatibility, disease transmission or high costs. The iliac crest is the most common donor site for obtaining autologous bone grafts due to the easy accessibility to large quantities of corticocancellous bone. However, the surgical removal of bone from the iliac crest requires an additional surgical procedure with a distinct set of potential complications. In some cases the grafting procedure can even be more problematic than the primary surgical procedure itself.

Although many severe and major complications are reported,[1–7] the most common complication is enduring postoperative pain at the donor site. The reported incidence of chronic donor site pain after posterior iliac crest bone grafting procedures ranges form 6% to 39%.[4,8–13] It should, however, be realized that, the incidence of donor site pain after bone graft harvesting from the posterior iliac crest is mainly reported from studies in patients who underwent low lumbar or lumbosacral surgery. The close proximity of the primary surgery to the iliac crest could interfere with the reported incidence of donor site pain since patients may have difficulties in differentiating between residual low back pain and pain originating from the iliac crest. This may lead to an overestimation of the real incidence of donor site pain.

The objective of this study was to compare the incidence of donor site attributed pain after posterior iliac crest bone graft harvesting, between patients who underwent fusion for traumatic fractures of different spinal levels. We postulate that patients with fusion of low lumbar levels would have a higher incidence of donor site attributed pain when compared with patients who underwent fusion of higher spinal levels.

Materials and Methods

A single-center, retrospective cohort study on the incidence of posterior iliac crest donor site morbidity in patients who underwent instrumented posterolateral spinal fusion for traumatic spinal fractures between 1994 and 2002 was performed. The study population was divided into two groups. The first group consisted of patients with a fusion between T2 and L2 (high fusion group). The second group consisted of patients of whom the fusion extended to L3 or more caudally (low fusion group). Patients were asked to complete an in-house created questionnaire regarding their iliac crest morbidity as explained in Table 1. Additionally, patient demographics, cause of trauma, Injury Severity Score (ISS),[14] fused spinal levels, complications associated with the harvesting of the bone grafts and any additional trauma were obtained form the medical records. The neurological status was evaluated with the use of American Spinal Injury Association's (ASIA) impairment scale.[15]

Table 1 Questionnaire

1. Do you currently have pain at the iliac wing?
   - □ No
   - □ Yes

   Please only fill in the next two questions if you currently have pain at the iliac wing. If you do not have any pain, please proceed to question 4.

2. How would you grade the pain that you have at the iliac wing?
   - □ Mild Pain
   - □ Moderate Pain
   - □ Severe Pain

3. How severe is you pain today. Please place a vertical mark somewhere on the line below to indicate how severe your pain is today. The left side of the line represents no pain and the right site represents the worst pain you can imagine.

   No Pain ——————————— Worst Pain

4. Do you have a numb sensation near the scar on your pelvis?
   - □ No
   - □ Yes

5. How satisfied are you with the cosmetic result of the procedure performed at the iliac wing?
   - □ Satisfied
   - □ Neutral
   - □ Dissatisfied

6. Do you have any limitations in your daily activities due the procedure performed at the iliac wing?
   - □ No Limitations
   - □ Some Limitations
   - □ Severe Limitations

7. In general, how aggravating do you find the procedure performed at your iliac wing?
   - □ Not Aggravating
   - □ Quite Aggravating
   - □ Very Aggravating

Inclusion and exclusion criteria

All patients submitted to our hospital since 1994, with traumatic thoracolumbar or lumbar spinal fractures, who underwent posterolateral fusion with pedicle screw fixation with autologous bone graft from the posterior iliac crest were included in the study. The minimal follow-up was two years. Patients with an additional pelvic and/or sacrum fracture were excluded from the study since differentiation from the iliac crest pain could be difficult. Also patients with substantial neurological deficits, defined as an ASIA score of A and B, were excluded. In assessing the functional limitations due to bone graft harvesting procedure, only patients without any persistent neurological deficits, defined as an ASIA score of E, were included.

Surgical technique

All surgeries were performed by, or under superior vision of, two orthopaedic surgeons (F.C.O. and A.J.V.) in a standardized way. There were no differences in the contribution of high or low levels fusion between the two surgeons.

The autologous bone grafts were harvested through a posterior approach using a separate oblique incision parallel to the superior cluneal nerves and perpendicular to the posterior iliac crest and consisted of unicorticale corticocancellous bone grafts. Notably, all bone grafting procedures were unilateral and no bicortical or tricortical iliac crest grafts were included. The gluteus maximus origin was identified and elevated, displaying the outer wall of the ilium. Subsequently, strips of corticocancellous bone graft were harvested. Once the outer table of the ilium was broached with osteotomes, corticocancellous bone was harvested in strips using Capener gouges. The gluteus maximus was reattached to the fascia with absorbable sutures and a drain was placed subcutaneously. No substance was used to fill in the defect in the iliac crest.

Outcome measurements

The primary outcome of this study was the incidence of donor site attributed pain in patients with high and low spinal fusions. All identified patients received an in-house created questionnaire, including several semiquantitative multiple-choice questions regarding their donor site attributed pain, sensory disturbances, functional limitations, and cosmetic appearance (Table 1). In patients who indicated to have iliac crest related pain, an extra multiple choice question regarding the intensity of the pain was used. In addition, the intensity of the residual iliac crest pain was also scored based on a 10-point visual analog scale (VAS; range, 0-10). A VAS score of 0 was defined as no pain, and a score of 10 was defined as the worst pain imagined by the patient. Notably, the patients were clearly instructed on paper that the questions concerned their iliac crest-related morbidity and not any other possible trauma related pathology. In addition to the questionnaires, all medical records were reviewed for the occurrence of complications associated with the bone graft harvesting procedure.

Patients

A total of 136 patients were identified by the search of the medical records. Of these patients, 19 (14%) were excluded due to persistent sensory neurological deficits. Additionally, 10 patients (7%) were excluded due to concomitant pelvic and/or sacrum fractures, and 2 patients died (1%) of unrelated causes.

Of the 105 remaining patients, 71 (68%) responded to the questionnaire. The response rate was not significantly different in both groups; 69% in the high fusions *versus* 66% in the low fusion group. In 58 patients (82%), assessment of functional limitations was performed. The percentage of patients in which assessment of functional limitation could be performed was also not significantly different between the high and low fusion group; 84% *versus* 77% respectively.

Statistical analysis

All data were collected and recorded using Filemaker Pro version 7.0. SPSS version 12.0.1 software was then used to conduct statistical analyses. Frequency and descriptive analyses were conducted on all data sets. Patient characteristics and results of the questionnaires were compared between patients with high and low fusions. For continuous variables exhibiting normal distribution, analyses were performed using an independent sample *t* test. In the event that normality was not the case, a Mann-Witney *U* analysis was used. For categorical variables, a Fisher exact test was used. A Pearson correlation test was used to analyze the association between the VAS score and time after surgery. The threshold for statistical significance was established at $p \leq 0.05$.

Results

Of the 71 patients in this study, 49 (69%) were men and 22 (31%) were women. The average age was 47.6 years. The mean follow-up period was 7.3 years (range, 2.3-11.6 years). Patient demographics, number of fused levels, follow-up period, ISS, and cause of trauma were not different between the 2 groups (Table 2).

Table 2 Patient Characteristics

	Total Group	High Fusion	Low Fusion
N	71	49 (69%)	22 (31%)
Age ± SD (y)	47.6 ± 12.8	46.6 ± 12.3	49.5 ± 14.1
Follow-up (y)			
Mean ± SD	7.3 ± 2.4	7.2 ± 2.5	7.6 ± 2.4
Range	2.3 - 11.6	2.3 - 11.5	3.0 - 11.6
Sex			
Male	49 (69%)	34 (69%)	15 (68%)
Female	22 (31%)	15 (31%)	7 (32%)
Levels Fused			
Mean ± SD	2.4 ± 1.0	2.5 ± 1.1	2.3 ± 0.7
Range	1.0 - 6.0	1.0 - 6.0	1.0 - 4.0
No Neurological Impairment	81.7% (58)	83.7% (41)	77.3% (17)
ISS ± SD	14.6 ± 7.9	15.8 ± 7.7	14.06 ± 7.9

The results of the questionnaires are summarized in Table 3. In patients with high fusions, the donor site pain attributed pain was significantly lower when compared with patients with low fusions, 14.3% *versus* 40.9%, respectively ($p < 0.03$). In addition, patients with high fusions found the bone harvesting procedure in general less aggravating. Of the patients who have indicated to have residual iliac crest pain, the mean VAS score was also significantly lower in patients with high fusions compared with patients who underwent fusion of low levels (1.6 ± 1.3 and 4.9 ± 1.8, respectively) (Table 4). However, there was no correlation between donor site pain and time after surgery (correlation coefficient = -0.09, $p = 0.75$) (Figure 1).

There were no significant differences in sensory disturbances, functional limitations, and cosmetic appearance between the two groups. Finally, the medical records revealed no other complications directly related to bone graft harvesting.

Table 3 Results of the Questionnaires

	Total Group (n)	High Fusion (n)	Low Fusion (n)	p-value
Donor Site Pain				
No Pain	77.5% (55)	85.7% (42)	59.1% (13)	p = 0.03
Pain	22.5% (16)	14.3% (7)	40.9% (9)	
Numbness				
No	63.4% (45)	61.2% (30)	68.2% (15)	p = 0.61
Yes	36.6% (26)	38.8% (19)	31.8 (7%)	
Functional Limitations				
No Limitations	79.7% (47)	85.7% (36)	64.7% (11)	p = 0.16
Some Limitations	16.9% (10)	11.9% (5)	29.4% (5)	
Severe Limitations	3.4% (2)	2.4% (1)	5.9% (1)	
Cosmetic Result				
Satisfied	56.3% (40)	59.2% (29)	50% (11)	p = 0.43
Neutral	29.6% (21)	24.5% (12)	40.9% (9)	
Dissatisfied	14.1% (10)	16.3% (8)	9.1% (2)	
In General				
Not Aggravating	54.9% (39)		36.4% (8)	p = 0.04
Quite Aggravating	43.7% (31)		59.1% (13)	
Very Aggravating	1.4% (1)		4.5% (1)	

Table 4 Quantification of the Donor Site Pain

	Total Group (n)	High Fusion (n)	Low Fusion (n)	p-value
Donor Site Pain				
Mild Pain	56.3% (9)	71.4% (5)	44.4% (4)	p = 0.63
Moderate Pain	31.3% (5)	14.3% (1)	44.4% (4)	
Severe Pain	12.5% (2)	14.3(1)	11.1% (1)	
VAS ± SD	3.2 ± 2.3	1.6 ± 1.3	4.9 ± 1.8	p = 0.01

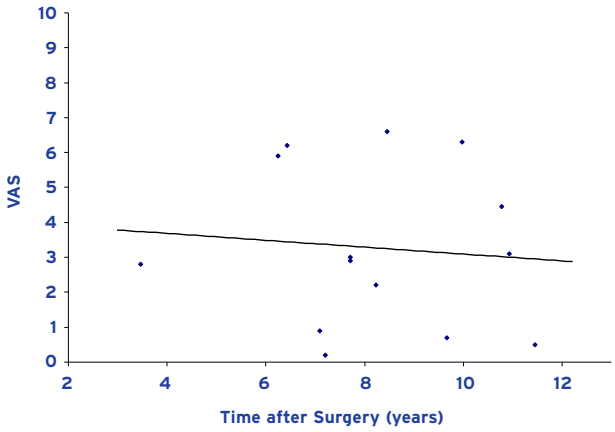

Figure 1 The VAS score plotted against time from surgery: The reported donor site pain is chronic and does not decrease in time.

Discussion

The results from the present study clearly demonstrate that, in patients with a posterior fusion after spine trauma, iliac crest attributed pain is more prominent in the group of whom the fusion level(s) included L3 or extended below this level compared with patients of whom the fusion level was cranial to L3. Additionally, these patients have more severe pain as represented by the higher VAS score. These finding suggests that, in lower lumbar fusions, iliac crest pain cannot be reliably differentiated from pain due to the main procedure. This is in line with previous reports that stated that patients who considered the operation to have relieved their back pain suffered from less donor site pain than those who were dissatisfied with the clinical outcome. [9,12]

The reported incidence of donor site pain after posterior iliac crest bone harvesting shows a broad variation, ranging from 6% and 39%. [4,8–13] The incidence is, in contrast to bone harvesting from the anterior iliac crest, only reported in patients who underwent spine surgery. We have shown that the proximity of the primary surgery to the iliac crest could be one of the factors accounting for this broad variation. Another possible confounding factor could be the study population used to evaluate the donor site pain. A previous report has shown that patients who underwent spinal surgery for painful lumbosacral disorders have twice the incidence of donor site pain compared with the lumbosacral trauma group, [8] One of the possible causes of the higher incidence might be the different coping strategies of these patients or the bias of the surgeons to explain the failure of the primary procedure by complications of bone harvesting.

We postulate that spinal trauma patients who undergo fusion cranial to level L3 are probably a suitable population to evaluate the true incidence of donor site pain after posterior iliac crest bone harvesting, while results on iliac crest donor site pain from patients that underwent a fusion below this level should be interpreted with caution. Interference of pain related to the primary surgical site (low level lumbar fusion) with pain related to the iliac crest donor site could occur.

In spinal trauma patients who underwent fusion of high levels, we found that 14% of the patients had donor site pain several years after the surgery. The majority (71%) of these patients graded the pain as mild. Additionally, none of the patient records revealed any complication directly related to the bone grafting procedure. In general, iliac crest pain does not seem to be a major problem, even in patients reporting discomfort.

Injury to the superior cluneal nerves, which provides sensation in the region of the posterior iliac crest and the cephalad portion of the buttock, is one of the postulated mechanisms that may account for donor site pain after bone graft harvesting from the iliac crest. A modified incision for the harvesting of bone grafts from the posterior iliac crest, parallel to the cluneal nerves and perpendicular to the iliac crest, which minimizes injury to this nerve, is associated with less donor site morbidity. [16] However, since the superior cluneal nerves arise from the first, second, and third lumbar dorsal rami, it is possible that surgery of these levels might also cause injury to the superior cluneal nerve. As a result, surgery of high lumbar levels could cause pain in the donor site region, which is not

related to the bone grafting procedure. This should be the topic of further investigation. There are some limitations of our study. First, this is a retrospective study and no volumetric data of the amount of bone graft are available. However, the surgical technique was performed in a standardized way under supervision of two surgeons. Furthermore, there was no work up bias since there were no differences in the contribution of high or low levels fusion between the two surgeons. Another potential limitation is the use of a non-validated questionnaire. Since the questionnaire only concerned descriptive data, the effect of this limitation is expected to be minimal.

Despite the limitation of this study, according to the authors, the results clearly show that patients with low back surgery have a significant higher incidence of donor site attributed pain compared with patients with surgery more distant from the iliac crest.

In conclusion, during the last decades, extensive research has focused on eliminating the potential complication associated with harvesting of autologous bone graft from the iliac crest. However, the reported incidence of donor site pain after posterior iliac crest bone grafting might be overestimated, due to interference of the primary surgery.

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Chapter 3

The relevance of viable cells in autologous bone graft

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Introduction

Bone is probably the most frequently auto-transplanted viable tissue.[1] For decades, the autologous bone graft has been successfully applied to establish bony unions and to restore defects. Posterolateral spinal fusion, a common procedure in spinal surgery, especially requires large quantities of bone graft to create a bone bridge between two or more aligned vertebrae. Typically one or both iliac wings are used as a donor site for the graft material, which inevitably causes donor-side morbidity, like mild pain, and occasionally even more serious complications.[2,3] Besides donor-site-related disadvantages, the amount of autologous bone is limited and the procedure is time consuming. Alternatives such as allogeneic bone are therefore often applied, however its use is also associated with important drawbacks, such as chance of disease transmission and immunogenic response. Additionally, the effectiveness of allogeneic bone in challenging environments, such as posterolateral fusions, has not indisputably been demonstrated.[4] Tissue engineering of bone is a recent promising technique that applies the principles of engineering and life sciences.[5] In general, tissue engineering strategies are based on bioactive synthetic scaffolds that contain or recruit growth factors and/or inducible cells to form bone. This mechanism is to some extent similar to the way by which bone grafts are expected to function.[1,6] The role of these individual components during graft-enhanced bone formation, however, is still largely unknown. Especially the most prominent feature of the autograft -the presence of viable osteogenic cells- is of dubious importance, because only some histological studies are present that reported cell death in most of the grafts of clinical size (i.e. larger than 0.5ml).[6,7] Biologically, it is also unlikely that many cells will survive, as in the first weeks of implantation the graft is deprived of vascularization. Nevertheless, in transplants of small bone chips, the cells have shown the ability to survive and to form bone.[8-11] Also, in intramuscular, i.e., ectopically transplanted clinically sized autologous bone grafts, we have shown strong indications for surviving of the cells with subsequent osteogenesis.[12] In a mouse femoral defect model, it was shown that besides providing a source of osteogenic cells, viable grafts enhanced vascularization and osteoclastic resorption.[13] This confirms our earlier observations that clinically sized viable cortical autografts where resorbed much faster than similar dead autografts.[12] Therefore, the initial presence of viable cells inside clinically sized autografts may be relevant, not only because they participate in bone formation, but also due to their stimulatory effect on physiologic remodeling of the graft.

To investigate the hypothesis that viable cells enhance bone formation in autografts, we used our previously described transverse process model,[14,15] which focuses on the initial bone formation from the transverse process in posterolateral spinal fusion.[16] Viable and devitalized cancellous bone autografts were compared. In addition, a condition without grafting was included to account for previously described spontaneous bone formation after decortication.[17,18]

Materials and Methods

Experimental design

The Dutch Animal Care and Use Committee approval was obtained for 13 adult Dutch milk goats. The goats received a total of eight two-chamber cassettes that were mounted on the bilateral lumbar transverse processes L1-L4. For the current study, one, two or three chambers were used per goat that were filled with viable or devitalized autograft, or left empty according the design of Table 1. After 12 weeks, samples were retrieved and analyzed for bone formation.

Table 1 Presence of the study conditions per goat. AG, autografts; NSAID, nonsteroidal anti-inflammatory drug.

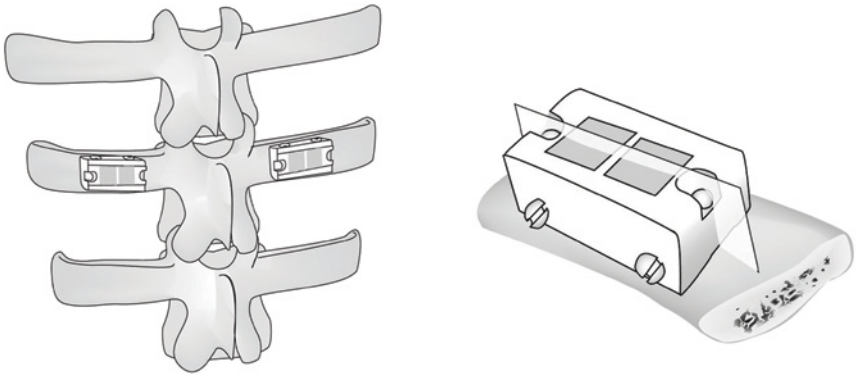
	Viable AG	Devitalized AG	Empty	Remark
Goats 1-6	Yes	Yes	No	Nr. 3 treated with NSAID
Goats 7-9	Yes	Yes	Yes	Nr. 9 deceased
Goats 10-13	No	No	Yes	

Animals and implantation

Surgical procedures were performed under standard conditions. After shaving and disinfection of the dorsal thoracolumbar area, a midline skin incision was made. The paraspinal muscles were separated longitudinally to expose the lumbar transverse processes, which were thoroughly decorticated using an angled bone rasp. Polymeric cassettes as described previously were then mounted on the processes L1-L4.[14,15] Each cassette consisted of two 8mm high x 7mm long x 6.5mm wide chambers, separated by a 0.5mm thick Teflon® sheet (Figure 1). The chambers were open to the decorticated underlying bone and overlying paraspinal muscles, and were filled with one of the graft conditions according a randomized scheme. Six goats received both a viable and a devitalized bone graft, in three goats a condition without grafting was additionally included (three chambers were used) and the last three goats received only an empty chamber (Table 1). This resulted in n=9 (paired) for vital and devitalized graft and n=6 (unpaired) for the empty condition. In addition to the conditions presented here, several biomaterials, that did not contain cells or factors that could influence neighboring conditions, were investigated in the other chambers of the cassettes that were mounted on other lumbar transverse processes.[19] Cancellous iliac bone graft was obtained from both iliac wings with a trephine under constant cooling, and after morcellizing and cleaning divided over two aliquots of 1ml. To devitalize 1ml of autograft, a previously validated protocol was used.[12] In brief, the graft was frozen three times in liquid nitrogen and thawed with warm saline. In contrast to other methods, like gamma irradiation, this method has minimal effect on the osteoinductive properties of the bone graft.[20] After filling the chambers with the graft material, finger pressure was applied on the graft to ensure direct contact with the underlying bone. The muscles were then closed over

the cassettes and the fascia sutured. The skin was closed in two layers. Pain relief was given by Durogesic (fentanyl transdermaI patches, Janssen-Cilag, Belgium). To monitor the dynamics of bone growth, the goats received fluorochrome labels at 3 weeks (Calceine green, 10 mg/kg, I.V., Sigma, The Netherlands), 6 weeks (Oxytetracycline, 32 mg/kg I.M., Mycofarm, The Netherlands) and 9 weeks (Xylenol orange, 80 mg/kg, I.V., Sigma) as described earlier.[21,22] At 12 weeks, the animals were euthanized by an overdose of pentobarbital (Organon, The Netherlands). The transverse processes with the cassettes were sawed off the vertebrae and the medial sides were marked.

Figure 1 The model. (Left) Cartoon of the position of the cassettes on the lumbar transverse processes. (Right) Detail of the 8mm high cassette with two chambers separated by a Teflon® sheet. The plane for histomorphometry is indicated.



Histological processing and histomorphometry

Explanted samples were fixated in a solution of 4% glutaraldehyde and 5% paraformaldehyde, dehydrated by ethanol series and embedded in polymethylmethacrylate. Two transverse, centrally located sections (Figure 1), were cut from each sample using a sawing microtome (Leica, Nussloch, Germany). The first section remained unstained for epifluorescence microscopy, and the second section was stained with 1% methylene blue and 0.3% basic fuchsin after etching with HCl/EtOH mixture. All samples were qualitatively analyzed using a light/fluorescence microscope (Leica DMR 2500, Nussloch, Germany), equipped with a triple filter block (BGR, dichroic mirror 415, 510 and 590 nm). For histomorphometry, high-resolution digital photographs (300 dpi) of the stained sections were made. The methods used for histomorphometrical analysis have been described in detail previously.[12] A program was developed to quantify two different parameters concerning bone formation: (1) The percentage of bone occupying the available area (area%), and (2) maximal bone height measured from the transverse process/implant interface (bone height). In addition to these parameters, the maximum height (=distance to the transverse process) of each fluorochrome label was measured to investigate the dynamics of bone formation.[21]

Statistics

Prior to the study, it was determined, based on previous data,[12,23] that a sample size of n=8 was required to find a 40% difference, which was considered relevant, with a power of 80%. The effect of no grafting was expected to be minimal, therefore only six samples where included. SPSS version 12.01 software was used to evaluate the normality of the data distribution and to perform two-tailed Student's t-tests for paired comparisons between viable and devitalized grafts and for unpaired comparisons between empty chambers and grafted conditions. Significance was assumed when $p < 0.05$.

Results

Surgery

One day after surgery, one goat with a viable and a nonviable graft developed an inflammatory hoof disease (Laminitis). Another goat with all three conditions developed this condition six weeks post operatively. Treatment with a single dose of a non-steroidal anti-inflammatory drug (NSAID, Diclofenac®) could rescue the first goat; the other had to be terminated. Although NSAIDs are well known for disturbance of the prostaglandin pathway and associated bone formation, we decided not to exclude this goat from the study because this effect is only described for longer application periods.[24] Furthermore, the comparisons between the two graft conditions were paired.

Histology and histomorphometry

Upon explantation, all cassettes were firmly attached to the underlying bone. There were no signs of adverse tissue reactions. In most of the chambers, well-vascularized new cancellous bone with fat marrow was present; cartilage or dead bone remnants, as indicated by empty osteocyt lacunae, were never observed in any of the conditions (Figure 2). Apparently all bone graft had been resorbed or remodeled. The appearance of the bone and soft tissues that filled the chambers was similar in all conditions. In general, it appeared that bone had migrated from the transverse process up toward the overlying muscle, as the amount of bone decreased with height. The empty chambers showed new bone in four of five chambers, with new bone reaching the maximum height in one. The grafted chambers always showed new bone formation. In three of eight viable graft samples, abundant bone was also present in contact to the overlying muscle, suggesting bone had also initiated at this location. In the devitalized grafts, this submuscular bone formation was never observed.

Analysis of the growth dynamics by fluorescence microscopy confirmed that bone was migrating upwards, as the early 3-week label was never found beyond a distance of 1.8 mm from the transverse process, with subsequent labels further away. Only in the three viable graft samples with bone in the upper part of the chamber, this submuscular area appeared to be another location of early osteogenesis, containing the green 3-week label (Figure 2e,f). Interestingly, the growth dynamics of the five vital graft samples without bone in the upper area and the devitalized grafts were quite similar, suggesting that viability did not influence the process of osteoconduction in this model (Figure 3a). Histomorphometry showed an area% bone in the empty chambers of 8.9 ± 5.5 (mean ± SD), while the maximum height at explantation was 4.5 ± 2.9mm. These were not different from the grafted conditions ($p > 0.1$). In the viable graft, the area% bone of 12.8 ± 2.9% appeared higher due to the samples with bone in the submuscular region, however, this was not significant when compared to the 9.5 ± 5.3% in the devitalized condition (p=0.09). Also, the maximum bone height reached at 12 weeks (6.0 ± 1.5 mm for viable graft *versus* 4.4 ± 1.3 mm for devitalized graft) was not statistically different (p = 0.2) (Figure 3b).

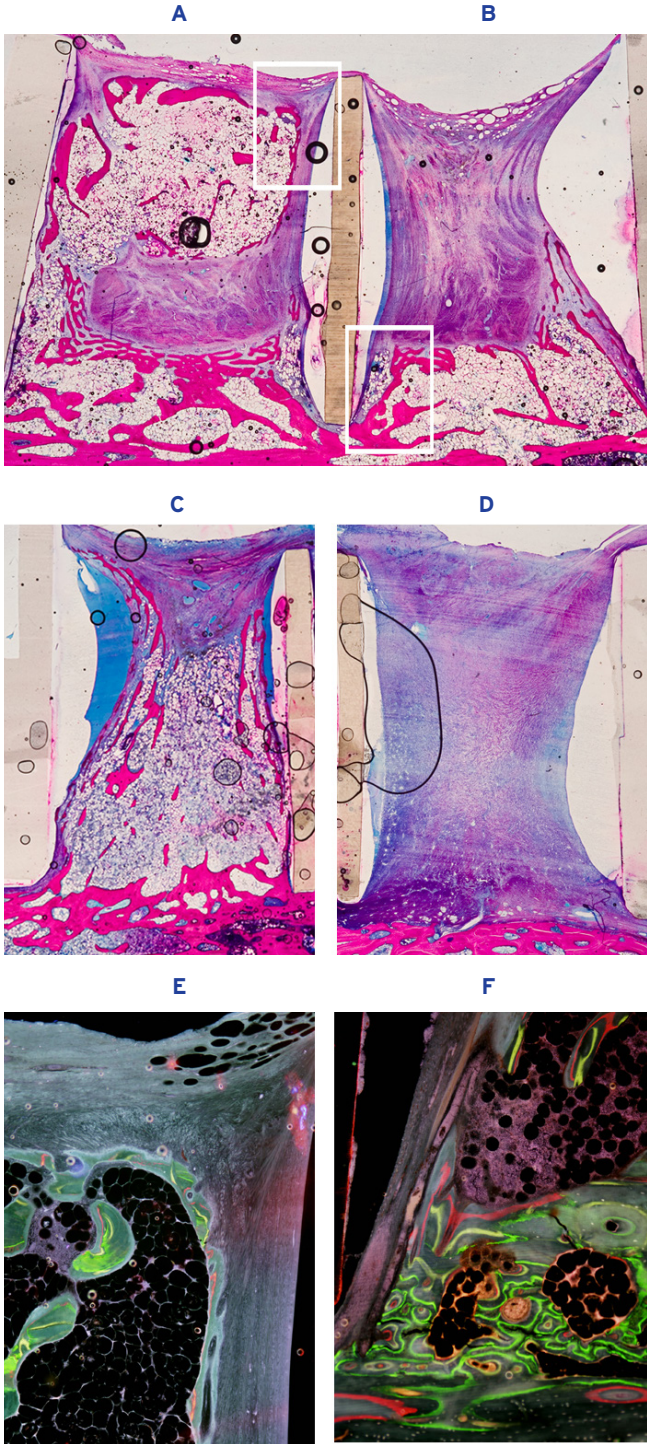
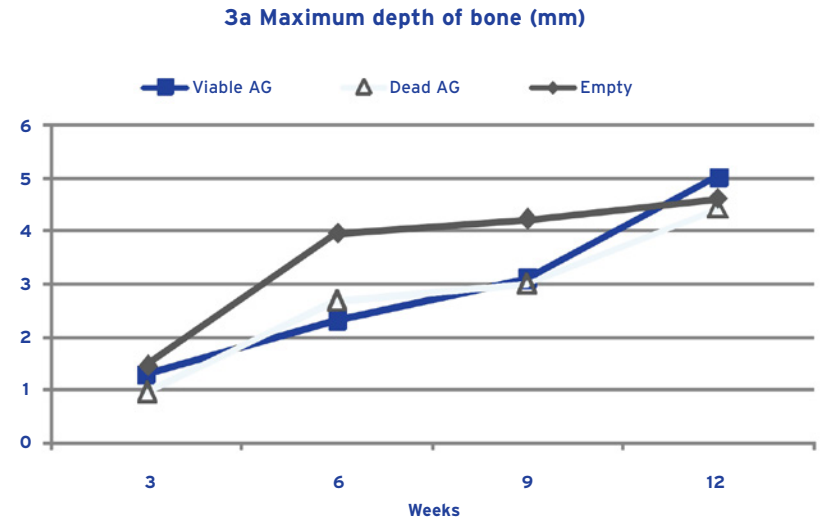
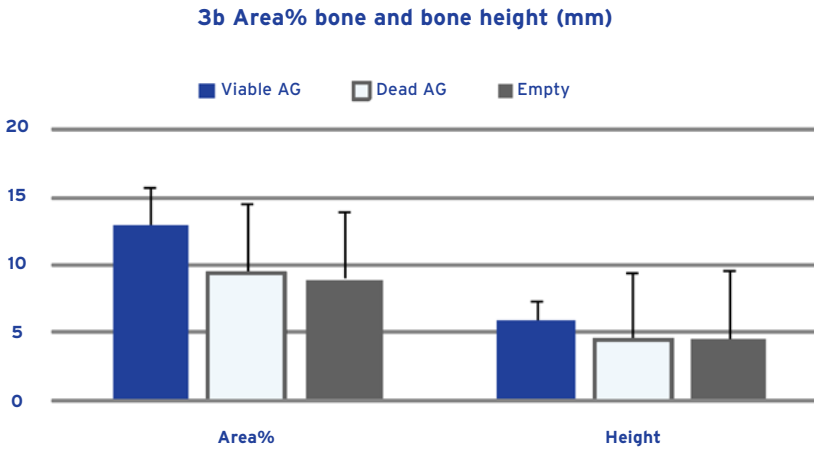


Figure 2 Histology at 12 weeks. Low magnification image of a cassette originally filled with (a) vital autologous bone, showing exclusively new cancellous bone (pink) also in the submuscular upper area and (b) devitalized autologous bone with exclusively new cancellous bone only in the lower part. Soft tissue is purple. (c, d) Chambers that were left empty occasionally showed bone reaching the maximum height and occasionally no bone formation at all. (e) Fluorescence microscopy image of early osteogenesis in the upper area of viable graft indicated by the rectangle in a. Note the presence of the green 3-week label. (f) Fluorescence microscopy image of the lower part of devitalized AG, indicated by the rectangle in (b). Note the absence of the green label more upward from the transverse process.

Figure 3 Fluorescense and histomorphometry. (a) Graph showing the dynamics of bone ingrowth from the transverse processes towards the top of the chamber. The three viable graft samples that showed osteogenesis in the upper area are excluded, to focus solely on the osteoconductive process. Note the similarity between viable and devitalized autograft. (b) Graph showing the area% bone on the left and the reached bone height on the right. There were no significant differences.





Discussion

Until today, the autologous bone graft has been the gold standard for grafting procedures in bone surgery.[1,6,25] Surprisingly, little is known about the mechanism behind the clinical success of this graft, and especially the role of viable osteogenic cells within the graft is controversial. With the emergence of bone tissue engineering as an alternative to the autologous bone graft, knowledge of the exact role of these cells has become crucial.[26] Previously, we found an obvious effect of viability in terms of de novo bone formation in clinically sized vital and devitalized bone grafts implanted ectopically.[12] In the current study, we investigated the effect of graft viability in a more clinically relevant transverse process model. As some bone formation in response to decortication was anticipated, we included a condition without grafting (empty). Bone formation in these empty chambers appeared considerable; in one of the five samples new bone even reached the overlying muscle. This result is possibly caused by the specific nature of the model used, which does not easily allow infiltration of surrounding soft tissue; therefore relatively slow bone ingrowth is not prevented by soft tissue that occupies the area. This indicates the limitations of this model: Although it allows investigation of local effects, it does not represent autologous bone grafting in general, where often larger and more exposed defects are treated. Furthermore, the chamber cannot be regarded as a critical-sized defect, making this transverse process model only applicable for comparative studies.

The comparison of vital versus devitalized bone grafts showed no differences with respect to the absence of any dead bone remnants, vascularity and microscopic appearance of viable new bone. Also, the rate of osteoconduction from the underlying transverse process into the graft, as determined with fluorochrome labeling (figure 3a), appeared similar. However, there was an obvious qualitative effect of graft viability, as demonstrated by the fluorochrome labels. Viable grafts had the unique potential to generate bone distant from the transverse processes, as evidenced by the early 3-week and subsequent labels found in the submuscular bone. This finding is in agreement with previous observations in the same model, where bone formation in this submuscular area was significantly more in constructs of ceramic scaffolds loaded with bone marrow-derived stromal cells, as compared to ceramic implants alone.[15] Although this advantage of graft viability appears to be limited to the well-nourished peripheral implant areas, such as the submuscular region, it is likely to be relevant in posterolateral spinal fusion, where nonunions often occur away from the transverse processes.

An interesting recent finding in this respect is that the advantage of viable grafts is not necessarily dependent on viable cells. In a mouse femoral defect model, Schwarz et al.[13] showed that frozen allografts could be engineered to stimulate bone formation like viable grafts, not only by adding bone morphogenetic protein (BMP)-producing mesenchymal stem cells,[27] but also by only adhering viral vectors to the allograft surface for targeted delivery of genes that stimulated osteoinduction, vascularization and osteoclastic

resorption.[28-30] This alternative approach of "reviving" the (dead) allograft by stimulating the host instead of providing viable cells may be more applicable in the much larger grafts as used in the clinical situation, since it will circumvent the problem of cell survival after transplantation.

In conclusion, this study identifies the potential advantage of bone graft viability, however this benefit appears to be limited to the well-nourished periphery of the graft and the clinical relevance remains to be investigated.

Acknowledgements

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Chapter 4

Effect of platelet gel on bone regeneration in three bone grafts

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Introduction

Bone grafts are widely used in orthopaedic surgery, maxillofacial surgery, and neurosurgery to manage fracture nonunions, spinal fusion, and reconstructive surgery.[1-4] The traditional approach for bone grafting uses autologous-derived bone.[5] However, the availability of autologous bone is restricted and harvesting is often associated with morbidity.[6,7] Therefore, bone graft substitutes are considered to provide a substitute for autograft bone. The success of bone grafting procedures depends, among others, on the bone healing time for graft host integration.[8] This involves a variety of biological actions, including an adequate blood supply and availability of an osteoconductive matrix.[9] Furthermore, platelet growth factors play an important role in this process by providing signaling for osteogenesis and osteoinduction, through an osteogenic cell response to these signals.[10] At present, platelet leukocyte gel (PLG) can be prepared from autologous freshly drawn whole blood, which is subsequently sequestered in platelet poor plasma (PPP), platelet-leukocyte rich plasma (P-LRP), and erythrocyte concentrate with miniaturized centrifuges.[11,12] The P-LRP is composed of a high concentration of platelets and leukocytes, which is mixed with thrombin to create PLG.[13,14] PLG can be applied to wound tissues, autograft bone, or bone substitutes. The rationale for using PLG is to enhance soft tissue healing or to improve bone growth and vascular proliferation because of the presence of high concentrations of platelet growth factors.[15,16] Mixing PLG with bone will result in the recruitment of mesenchymal stem cells by mitogenic activity, which at wound sites has the potential to differentiate into osteoblasts and contribute to bone regeneration.[17] Specifically, platelet-derived growth factor (PDGF) has mitogenic properties acting as a chemotactic agent. At higher concentrations, PDGF may also increase the proliferation of cells and promote the formation of the extracellular matrix.[18] Transforming growth factor beta (TGF-ß), present in bone and platelets, is an important bone stimulatory growth factor, and it has been suggested to be one of the local regulators of bone formation and resorption.[19] Furthermore, during the different stages of fracture healing, platelets act as an exogenous source of growth factors that stimulate osteogenesis.[20,21] Therefore, the underlying principle of PLG application is to mimic and accelerate the natural healing process. However, the effects following the use of PLG growth factors have been dissimilar, and a large variety of P-LRP production devices have being used in various studies.[22-24] The aim of this study was to investigate a potential *in vivo* clinical effect of PLG on bone growth, using autogenous bone and two different bone substitutes in a goat transverse process implant model, with a new commercial available point-of-care miniaturized centrifuge to produce PLG.

Materials and Methods

Animals

The animal Institutional Review Board of the University of Utrecht approved the study protocol. Adult Dutch female milk goats (24-27 months) were acquired and allowed to acclimate for at least 3 weeks. They stayed at all times in a vivarium.

Experimental design

Ten adult goats were used for this experiment. In each goat, two 3-compartment polyacetal cages were bilaterally implanted on the transverse processes of the L4 vertebrae. All cages contained autograft bone (AB), porous biphasic calcium phosphate (BCP, IsoTis Orthobiologics, Bilthoven, The Netherlands) and porous trabecular metal (TM, Zimmer Inc, Warsaw, IN, US), in a randomized order. There are no reports on toxicity of both bone substitutes against animal cells.

One cage was treated with PLG, and the cage positioned on the contralateral side served as a control, with no PLG treatment. The animals were sacrificed after nine weeks. The implant sites were processed for (fluorescence) histology and histomorphometry (bone area and bone contact length) to determine the effect of PLG on each of the three bone substitutes.

Anesthesia

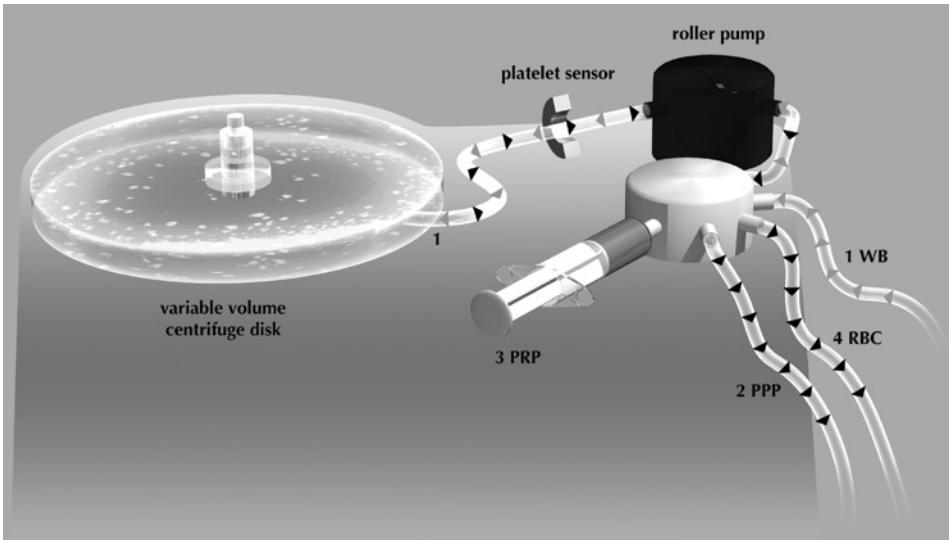
The procedures were performed under general anesthesia using an isoflurane in air gas mixture (Abbott Laboratories, AST Pharma, Oudewater, The Netherlands), preceded by dexmedetomidine sedation (Pfizer, Capelle aan de IJssel, The Netherlands).

Platelet-leukocyte rich plasma preparation method

Whole blood was gained with of a 17 G intravenous infusion catheter, inserted into the jugular vein. Two 60-mL syringes were prefilled with 7 mL of anticoagulant citrate dextrose A solution. Fifty-three ml of whole blood was slowly withdrawn via the infusion line in each syringe. After the syringes were filled, they were inverted five times to ensure proper mixing with the anticoagulant to avoid blood clotting.

The preparation of P-LRP was performed using the Angel Whole Blood Processing System™ (AWBPS; Sorin Group, Mirandola, Italy), approved for clinical use. The AWBPS is a semi-automated tabletop centrifuge system using a flat-disc, separating whole blood volumes, ranging from 60 to 180 ml, into three blood components. The predonated blood was injected in the blood collection reservoir of the AWBPS. Following centrifugation at 3200 rpm, at 1,200 x *g* for 19 minutes, the PPP was automatically removed. Subsequently, P-LRP was pumped in a syringe, and the erythrocyte concentrate was collected in a retransfusion bag (Figure 1). P-LRP was mixed with 10% calcium chloride and bovine thrombin (500 U/mL; Jones Pharma Inc, St Louis, MO, US) in a 10:1 ratio to create PLG. The PPP volume and erythrocyte concentrate were retransfused to the animals.

Figure 1 Schematic representation of the platelet rich plasma preparation method. (1) Whole blood (WB) is injected by means of the roller pump in the Angel™ whole blood processing system, blood is pumped in the disk and sequestered; (2) platelet poor plasma (PPP) is removed from the disk followed by the collection of; (3) platelet-leukocyte rich plasma (P-LRP) and; (4) red blood cells (RBC).



Transforming growth factor-β1 determination

In all animals, TGF-ß1 concentrations in the circulating whole blood and in a separate 1 mL sample of PLG were measured with a commercially available enzyme-linked immunosorbent assay kit (ELISA) (R&D Systems, Minneapolis MN, US), and validated for measuring TGF-ß (Quantikine TFG-ß), according to the manufacturer's instructions. The ELISA procedures were programmed in an automated analyzer (Coda Automated EIA™ analyzer; Bio-Rad Laboratories, Hercules CA, US). Samples were measured in duplicate and in appropriate dilutions prepared as required for the respective calibration curves. Repeat analysis was performed when differences between duplicates were larger than 10%.

Spinal cages and bone substitutes

Polyacetal cages were placed in the goat lumbar spine transverse processes. This comparative model was previously described in more detail.[25,26] Each cage consisted of two sidewalls, two end pieces, four stainless steel machine screws for cage assembly, and two self-tapping bone screws to place and fix the cages to the decorticated transverse processes. Each cage consisted of three sections, separated by tightly fitted Teflon® sheets. Besides the AB, the remaining sections were filled with BCP and TM. The BCP material had an 80/20 weight percent ratio of hydroxyapatite/beta-tricalcium phosphate, respectively. The BCP was ~55% porous, including 10–20% microporosity, with a pore size of 200–800 μm. The TM material, a trabecular metal composed of a carbon substrate that has elemental tantalum deposited on the surface, has 70-80% porosity with a 120-150 μm pore size. The

sections were open to both the underlying bone and overlying soft tissues and had cross sections of 0.8 by 5.0 mm and were 8.0 mm in length. The entire cage components, including BCP and TM, were sterilized as individual pieces by autoclave.

In the treatment group, the prepared bone chips, BCP, and TM were placed in a cup containing P-LRP during the fixation of the cage. Shortly before the cage was filled, 3 mL of P-LRP was activated with 0.3 mL of calcified bovine thrombin (100 mg/mL). Two milliliters of PLG was injected into the cage and covered the transverse processes before the three sections were filled with the materials. After filling the cage, 1.3 mL of PLG was evenly delivered on top of each of the three cage sections before wound closure.

Surgical technique

After shaving and disinfecting the dorsal thoracolumbar region, a midline incision from T5 until T10, which extended along the right iliac wing, was made to expose the paraspinal muscles and iliac wing. This incision supported implantation of intramuscular and iliac implants that are not discussed in the this manuscript. Bilateral incisions were made though the paraspinal muscles to expose both transverse processes of the L4 vertebrae. These processes were decorticated with an angled bone rasp until a flat, bleeding surface was obtained. Two spinal cages were aseptically assembled for each animal with six grafts arranged according to a randomized complete block design. Autograft bone, for one of the three treatments in each cage, was obtained as tricortical graft from the right iliac wing. The collected bone was fragmented in small spongious and cortical bone chips prior to cage implantation. The cages were screwed to each L4 transverse process (Figure 2). Slight finger pressure was applied to the top of the blocks of the cassette to ensure direct contact of all grafts with the underlying bone. The muscle fascia, subcutaneous tissues, and skin were subsequently closed in layers. Postoperative pain relief was provided by buprenorphine (Schering-Plough, Maarssen, The Netherlands).

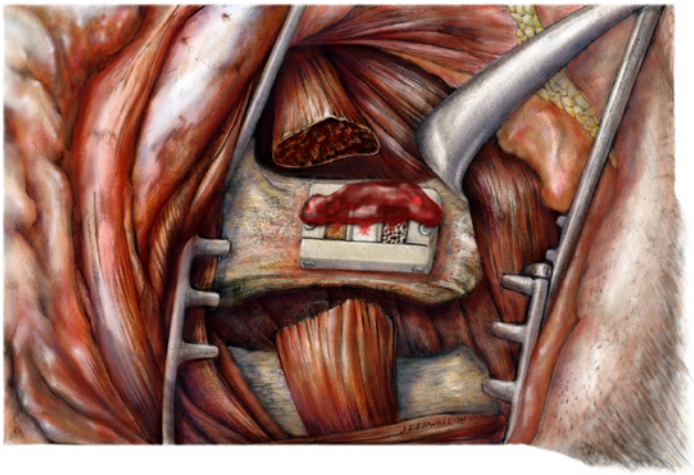


Figure 2 Illustration showing a cage, with the three sections, screwed to the decorticated L4 transverse processes, with platelet-leukocyte gel applied to it. (Note: the vertical transverse muscle is partially eliminated for a better visualization of the cage position).

Fluorochrome labeling

Sequential fluorochrome markers were administrated at two weeks (Calcein Green, 10 mg/kg intravenously, Sigma, Zwijndrecht, The Netherlands); three weeks (Oxytetracyclin, Engemycine, 32 mg/kg intramuscularly, Mycofarm, De Bilt, The Netherlands) and five weeks (Xylenol Orange, 80 mg/kg intravenously, Sigma, Zwijndrecht, The Netherlands).

Histological processing and histomorphometric analysis

At nine weeks, the animals were sacrificed with an overdose of pentobarbital (Organon, Oss, The Netherlands). The implants were retrieved by removing the entire transverse processes of L4. The explanted samples were fixed in a solution of 4% paraformaldehyde, dehydrated by ethanol series, and embedded in polymethylmethacrylate during a two-week period. Two centrally located 10 μm thick sections were cut from each sample using a microtome (Leica, Nussloch, Germany). The first section remained unstained for epifluorescence microscopy, and the second section was stained with methylene blue and basic fuchsine. Tissue response, bone formation, and the fluorochrome markers were evaluated using a light/fluorescence microscope (E600, Nikon, Japan) equipped with a quadruple filter block (XF57, dichroic mirror 400, 485, 558 and 640 nm, Omega Optics, The Netherlands). High-resolution digital scans of the stained sections were made for histomorphometry using a photographic film scanner (Dimage Scan Elite 5400, Minolta, Japan). Subsequently, the scans were pseudocolored, bone with yellow and scaffold with green, using the Adobe Photoshop 7.0 on a commercial PC. Histomorphometry was performed using KS400 software (version 3, Zeiss, Nussloch, Germany).

A custom computer macro was developed to measure in each channel the bone height, the bone area, and the length of direct contact between bone and scaffold in the BCP and TM channel (bone contact length). This allowed calculating the percent bone in the available section (bone area %) and the percent of bone apposition (contact %). Because AB does not have a scaffold area, we determined the effect of PLG on the AB growth by measuring bone height and bone area in square millimeters in the appropriate cage space. The contact % was calculated because new bone only forms by apposition on the scaffold surface.[26]

Statistical analysis

Statistical analysis utilized SAS statistical software (SAS Institute, Cary, NC; USA, 2003). Data were expressed as mean ± one standard deviation of the mean. Each of 10 animals had 3 chambers (groups), each with PLG treatment and control. There were 58 observations made due to technical analytical problems with one chamber. The group values represent the findings in ten animals. Statistically significant differences between groups were determined using analysis of variance and Tukey's student range test on each principal effect means to account for multiple comparisons. In the case where the group sizes were different, the Tukey-Kramer test was used. To verify that the unbalanced cells (nine observations in two groups rather than ten) did not affect the results, general linear models were calculated. For all statistical tests, a p-value ≤ 0.05 was significant.

Results

In vitro results

The TGF-ß1 concentration of the PLG, 108,789 pg/mL ± 31,133, was significantly increased in all animals when compared to the circulating blood levels 16,192 ± 4,088 pg/mL ($p < 0.001$).

In vivo results

All animals recovered well from surgery, without complications, and survived the follow-up period without difficulties. All cages were firmly attached to the underlying transverse processes when they were removed from the animals. A typical example of a cross section from a retrieved cage used for histomorphometric analysis is shown in Figure 3.

Histology

Histological observations showed no signs of infections or adverse tissue reactions. Stained sections revealed bone growth in BCP and AB sections of the control cages. Nevertheless, the control TM sections showed poor bone formation, with no observable effect of the applied PLG. However, in the BCP and AB sections of the PLG treated cages, more bone integration was noted compared to the nontreated cages (Figure 4).

Figure 3 Image of a mid section of PLG treated cage screwed on the transverse processes. From left to right between the screws of the polyacetal end pieces: (A) bicalcium phosphate; (B) autogenous bone; (C) trabecular metal.

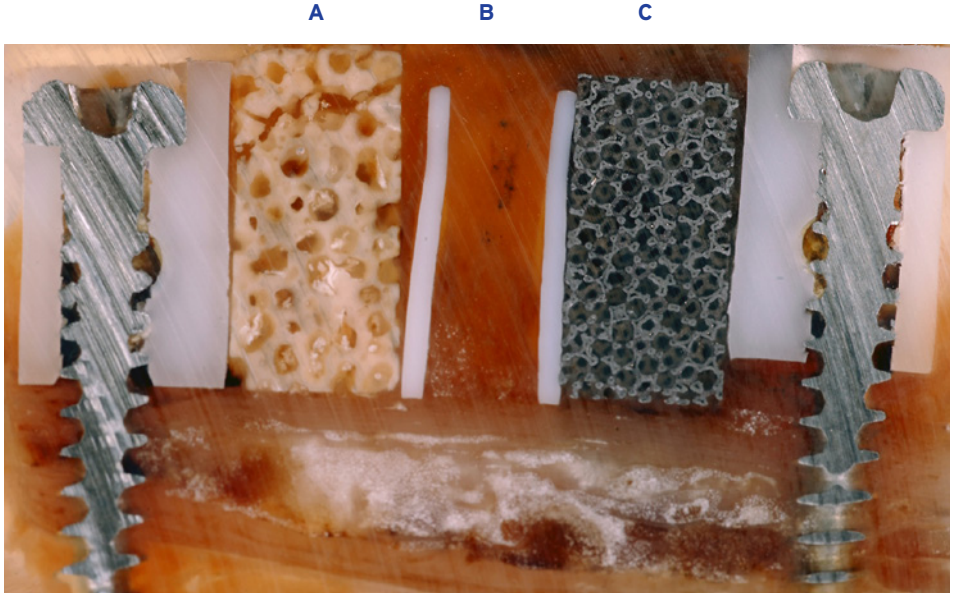
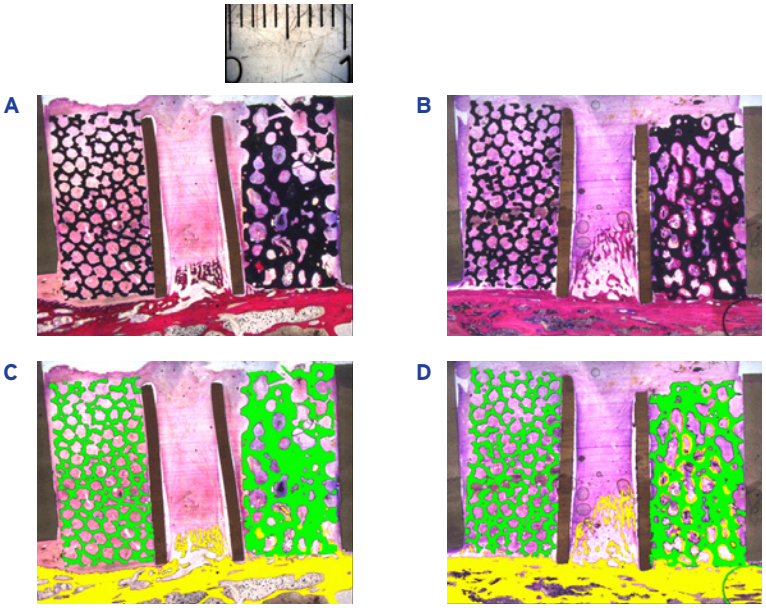


Figure 4 Histology. A low magnification image of a mid-section through a control cage (A) and a platelet-leukocyte gel treated cage (B) from the same animal. All three sections, separated by Teflon sheets, can be seen. In (C) and (D) a pseudocolored version of images (A) and (B), respectively (green = scaffold, yellow = bone). The difference in pore size can be observed with a preference of bone for apposition in the larger pores. (Bar = 10 mm)



Fluorescent microscopy

The three markers for fluorescent microscopy confirmed that bone grew from the underlying transverse processes toward the upper part of the cage during the nine weeks follow-up period in the PLG treated cages. The first, yellow, label (two weeks) was only present in the lower part and the last label (5 weeks) in the upper part of the section. The application of PLG resulted in more bone growth after 5 weeks than in the control cages. A composed image of for autologous bone growth is given in figure 5.

Histomorphometric analysis

Histomorphometric data of the control cages showed a difference in bone apposition (contact %) between BCP and TM scaffolds, 8.3 ± 6.2 % and 0.73 ± 0.79 %, respectively ($p < 0.001$) (Table 1). Concerning the application of PLG, bone apposition in the BCP sections was greater when compared to control sections ($p < 0.001$), whereas no effect of PLG was seen for the TM sections ($p = 0.34$). The percent of bone present in the nontreated BCP sections (bone area %) was 8.0 ± 4.1 % and in the TM sections 3.2 ± 1.9 %. A significant effect of PLG in bone area % was seen in the BCP section (12.8 ± 3.1 %, $p < 0.001$) when compared with the TM sections (3.4 ± 2.0 %, $p = 0.37$) The bone height and bone area were significantly greater when PLG was used in the BCP and AB sections, whereas no effect was present in the TM sections. Analysis revealed that the potential bone contact length of the TM scaffold was greater than for the BCP in the treated and nontreated cages ($p < 0.001$).

Figure 5 Fluorescent microscopy. Visualization of a composed image of an autogenous bone filled section after platelet-leukocyte gel application. The green (2 weeks), yellow (3 weeks) and orange (5 weeks) labels are present in the underlying transverse processes and the cage area. (T-P: transverse processes).

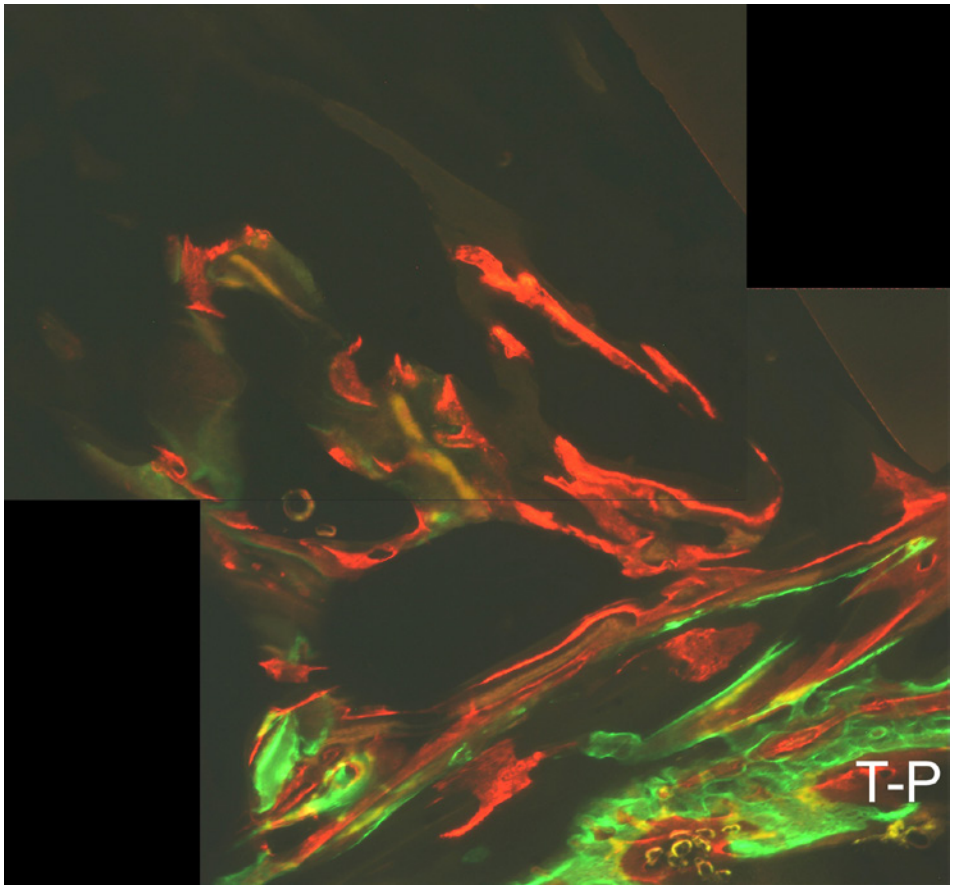


Table 1 Effect of PLG on bone growth parameters for autologous bone (AB), bicalcium phosphate (BCP), and trabecular metal (TM).
Data represent mean values and ± standard deviation.
[a]p < 0.05, compared to control section.
[b]p < 0.001, compared to control section.
[c]p < 0.001, compared to the TM scaffold treated with PLG.

	BCP	BCP + PLG	TM	TM + PLG	AB	AB + PLG
Bone apposition (contact %)	8.3 ± 6.2	14.9 ± 6.6[b,c]	0.73 ± 0.79	0.41 ± 0.6	N/A	N/A
Bone area (%)	8.0 ± 4.1	12.8 ± 3.1[b,c]	3.2 ± 1.9	3.4 ± 2.0	N/A	N/A
Bone height (mm)	5.2 ± 2.2	7.0 ± 1.6[a,c]	0.97 ± 0.7	0.84 ± 0.4	3.5 ± 1.0	5.0 ± 1.5[a,c]
Bone area (mm^2)	1.7 ± 0.9	2.7 ± 0.8[a,c]	0.74 ± 0.5	0.73 ± 0.4	2.0 ± 0.83	3.3 ± 1.3[a,c]
Bone contact length (mm)	133 ± 17	132 ± 22	182 ± 9b	183 ± 5	N/A	N/A

Discussion

In this study, we demonstrated that the application of platelet-leukocyte gel promoted new bone formation when autogenous bone and BCP was used in a goat spinal transverse processes implant model. Adding growth factors to bone and other scaffolds improves bone regeneration time.[27,28] The platelet growth factor PDGF-AB plays an important role in inducing proliferation of undifferentiated mesenchymal cells. PDGF activity is modulated by interactions with several other growth factors, such as TGF-ß and other pro-inflammatory cytokines (e.g. interleukin), and tumor necrosis factor.[29] This suggests that PDGF is an important mediator for bone healing and remodeling. It also enhances bone regeneration in conjunction with other growth factors, but it is unlikely that PDGF provides entirely osteogenic properties by itself. The TGF-ß measurements referred to in this article are the TGF-ß1 and TGF-ß2 proteins, which are the common proteins and generic growth factors involved with bone regeneration and connective tissue repair.[30,31] In addition, they have the ability to stimulate osteoblasts deposition of the collagen matrix involved in wound healing and of bone growth.[32]

It is presumed that PDGF, TGF-ß, and epidermal growth factor are released from platelet clots at the time of fracture.[33] In addition, osteoblasts also secrete PDGF, TGF-ß, and other growth factors which are also found in platelets.[34] It is therefore a reasonable hypothesis that the exogenous application of PLG with the subsequent release of high concentrations platelet growth factors, might lead to an improved, and faster bone growth. Human and animal studies have demonstrated a significant increase in PDGF, TGF-ß, and vascular endothelial growth factor (VEGF) concentrations in activated PLG when compared with circulating blood levels.[15,16] In this study, we only measured TGF-ß because there are no reliable PDGF and VGEF assays available to measure these growth factor concentrations in goat blood. The PLG had a five-fold increase in TGF-ß concentrations when compared with the circulating blood concentration. This observation has been confirmed in previous reports from Anitua and Weibrich.[16,17] Unfortunately, we were not able to measure platelet counts because goat platelets and erythrocytes have similar sizes and are therefore difficult to distinguish by the blood analyzer. However, comparative analysis between human and goat blood demonstrates similar results with regard to their specific function.[35] In our study, the effect of PLG treated cages compared to non-PLG cages could be assessed. Both cages were implanted in the same animal, and the position of the scaffolds inside each cage was randomly selected. Similar studies suggested that this transverse process goat model is reproducible, and it provides results for evaluating bone growth by histomorphometric analysis and fluorochrome labeling.[25,26] The objective in this model was not to identify optimal scaffold characteristics, but merely to study the effect of PLG on bone growth when autologous bone and two bone substitutes were used. PLG in combination with different bone materials has been used in various clinical and animal studies, showing that PLG may or may not improve bone formation and bone maturation.[36,37] Analysis of these conflicting data revealed that no, or negative effects of PLG, could be contributed to small study sizes, the lack of standardized PLG preparation protocols, the lack of a consensus

on the definition of autologous PLG, and differences in application techniques.[38] However, our data on bone growth with autologous bone scaffolds are in accordance with the results from a goat study of Fennis *et al,*[39] who used platelet concentrates in combination with particulate cancellous bone grafts during mandibular reconstructions.

We showed that adding PLG resulted in significantly more bone growth from the transverse processes in AB and BCP sections, compared to the same sections in the contralateral, non-PLG treated, transverse processes. Cross talk between treatment and control sites was not likely in this model. In general, in the AB and BCP sections bone growth were far better than the TM scaffolds. The lack of bone growth in the TM scaffolds is an observation that is in contrast with the results as described by Bobyn and Hacking.[40,41] Their studies consisted of a canine and dog model, respectively. An explanation for the dissimilar results on bone ingrowth with tantalum might be due to the differences in pore size and the potential bone contact area of the TM and BCP scaffolds. Macro- and micro porosities, the chemical composition of scaffolds and other factors as scaffold surface characteristics for bone cell attachment might influence the local micro-environment for bone growth.[25,42] Furthermore, no data are available on the affinity or toxicity of the TM against goat cells. In an other goat model, Sidhu et al., showed similar results concerning bone ingrowth when porous tantalum was used.[43]

In conclusion, this study proves that autologous prepared platelet-leukocyte gel contains a high concentration of TGF-ß, and its application to autologous bone and BCP produced a quantifiable improved bone growth in comparison with nontreated grafts. Augmenting bone defects by exogenous topical application of autologous derived platelet growth factors is a challenge, with a large potential for a variety of clinical indications. Future research should be directed to settle standards for PLG preparation methods, application techniques, and establish guidelines for clinical implantation of platelet growth factor gel therapies. Furthermore, diffusion of platelet growth factors into autologous bone and bone substitutes should be studied to understand bone ingrowth rate and differences in bone growth.

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Chapter 5

TCP compared to allograft and autograft in posterolateral fusion

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Introduction

Each year over 2.2 million bone grafting procedures are performed worldwide, making bone the second most common transplantation tissue, with blood being most common.[1,2] The most common application of bone grafts is spinal fusion surgery, especially posterolateral fusions. The current gold standard is to use autologous bone harvested from the iliac crest to create a bony bridge between the vertebrae of the affected segments. This iliac crest autograft provides all three mechanisms associated with bone regeneration: osteoconduction, osteoinduction, and osteogenic cells.[3] Harvesting of bone graft from the iliac crest, however, requires an extra surgical procedure in healthy bone, which is associated with additional morbidity, including donor site pain, infections and neurovascular damage.[4–7] To circumvent the disadvantages of iliac crest autograft the quest for an alternative bone graft substitute remains. An ideal bone graft should exhibit osteoconductive, osteogenic and osteoinductive properties; be able to degrade; provide a favorable environment for invading blood vessels and bone forming cells; and should be biomechanical stabile. This does not imply, however, that all characteristics are mandatory in each indication for a bone graft substitute to be effective.

Allograft from bone banks is a frequently chosen alternative,[8] which eliminates donor site morbidity and is available in large quantities. Allografts are osteoconductive, but they are considered to have weak osteoinductive capabilities and contain no viable cells for osteogenesis.[1] Despite extensive use, allograft is generally considered less effective than autologous bone graft,[9] which may be a result of the absence of viable cells or the host immune response.[10–12] Finally, the risk of disease transmission continues to be questioned.[13–15] Therefore, the need for an effective bone graft alternative remains.

Bone tissue engineering or regenerative medicine strategies have shown to be a promising technology to develop such an alternative. The concept of these techniques is to develop biologically active implants that restore, maintain or improve tissue function.[16] A well-known example of clinical application of this technology is the use of bone morphogenetic proteins (BMPs). For spinal fusions, BMP application has increased exponentially in the United States the last decade.[17,18] Recently, however, serious concerns have been expressed regarding underreporting of complications and product-related adverse events,[19,20] which may be related to the high doses of recombinant proteins that are administered.

The potential of synthetic bone grafts, such as calcium phosphate ceramics, has been investigated for over four decades.[21] These materials have the advantage of unlimited supply, low costs, and ease of sterilization and storage. Of special interest are the recently introduced tricalcium phosphate (TCP) ceramics, due to their good bioabsorbability compared with hydroxyapatite (HA) and biphasic calcium phosphate (BCP), and thereby not interfering with physiological bone remodeling. Preclinical studies[22–25] and clinical studies,[26–33] have generated encouraging data when using TCP in lumbar posterolateral fusions, but almost exclusively in combination with local bone, bone marrow aspirate and/or BMPs. Recently, it was shown that ceramics can be endowed with biologically instruc-

tive properties by changing basic physicochemical parameters of the materials, such as surface textures.[34] In this way TCP was capable of possessing an intrinsic osteoinductive capacity comparable to autograft or BMPs. In a comparative study, osteoinductivity of calcium phosphate ceramics appeared to be advantageous for bone defect healing when compared with non-osteoinductive phosphate ceramics.[35]

The purpose of this study was to determine if this TCP, alone or in combination with local autograft, is a suitable bone graft substitute for instrumented posterolateral fusion in a large animal model compared with the currently most used grafts; iliac crest autograft and allograft. Fusion was quantitatively and qualitatively assessed using computed tomography (CT), and histological evaluations.

Material and Methods

Experimental design

After approval by the animal care committee, nine Dutch milk goats (60-70 kg, age 23-28 months) underwent a two-level (L2-L3 and L4-L5) pedicle screw instrumented posterolateral fusion. We choose this model based on our experience with goats[36,37] and its resemblance to the human vertebral size. Each side of the spine was randomized into one type of graft (10mL), making a total of four grafts per goat. The groups were as defined below:

- Group 1: autograft from the iliac crest;
- Group 2: fresh-frozen allograft;
- Group 3: TCP with local autograft;
- Group 4: TCP alone.

All animals were sacrificed 16 weeks after the surgery. At necropsy, the specific spinal levels were harvested *en bloc* and cleaned of soft tissue, and the instrumentation was removed. The primary outcome was the presence/absence of fusion based on CT scans. In addition, the volume of the newly formed bone was semi-automatically calculated using custom software program. Finally, the spinal segments were processed for histological analysis.

Surgical technique

The procedures were performed under general inhalation anesthesia of an isoflurane in air gas mixture (Abbott Laboratories, AST Pharma) preceded by dexmedotomidine sedation (Pfizer). After shaving and disinfection of the dorsal thoracolumbar area, a midline incision between T12-L5 was made to expose the muscle fascia. The paraspinal muscles were subperiosteally stripped from the spinous processes and retracted laterally. The laminae, posterolateral aspect of the pars, facet joints and transverse processes were denuded of all soft tissue and thoroughly decorticated with a rasp. The pedicle screws were inserted after probing the pedicles and interconnected with rods (BWM-system, Stryker Howmedica Osteonics). Iliac crest bone graft was obtained using the same midline incision and by dissecting a subcutaneous flap free from the underlying lumbodorsal fascia until the iliac crest. An incision was made parallel to the iliac crest and the musculature was stripped off the outer surface of the ilium so that a large enough graft could be obtained. Using a rongeur, 10mL of tricortical bone was harvested. From the spinous processes of the level receiving TCP, 5mL of local autograft was obtained using a rongeur. In a randomized fashion, the experimental grafts were placed at one side of the spine in the decorticated lateral gutter against the lamina and the dorsal base of transverse process. The muscle fascia, subcutaneous tissues, and skin were subsequently closed in layers. Postoperative pain relief was provided by Buprenorphin (Schering-Plough). After 16 weeks, the animals were killed using an overdose of pentobarbital (Organon).

Bone grafts

A total of 10 mL of graft was used for each experimental condition. The amount of graft was determined by filling a syringe with graft material until 10mL of graft was obtained with careful hand-pressing. Care was taken not to crush or break the material. The iliac crest bone graft and local autograft was cleaned from soft tissue and morselized into corticocancellous granules of 1-3mm. For the TCP/local autograft group, 5 mL of local autograft from the spinous processes was morselized to granules of 1-3mm and uniformly mixed with 5mL of TCP. Allograft was obtained from the femoral heads of nine Dutch milks goats from a previous experiment, which could not have affected the bone quality, [38] under sterile conditions in the operation room. On harvesting of each of the femoral heads, swabs from the surface were taken and tested for contamination. Subsequently, the femoral heads were frozen to -80°C. If the allograft specimens were clear of bacterial contamination, the allograft was thawed at room temperature in its sterile container prior to surgery. Under sterile conditions, remnants of the femoral neck were resected to leave only the sphere of the femoral head. With the use of a rongeur the allograft was morselized into corticocancellous granules (1-3mm).

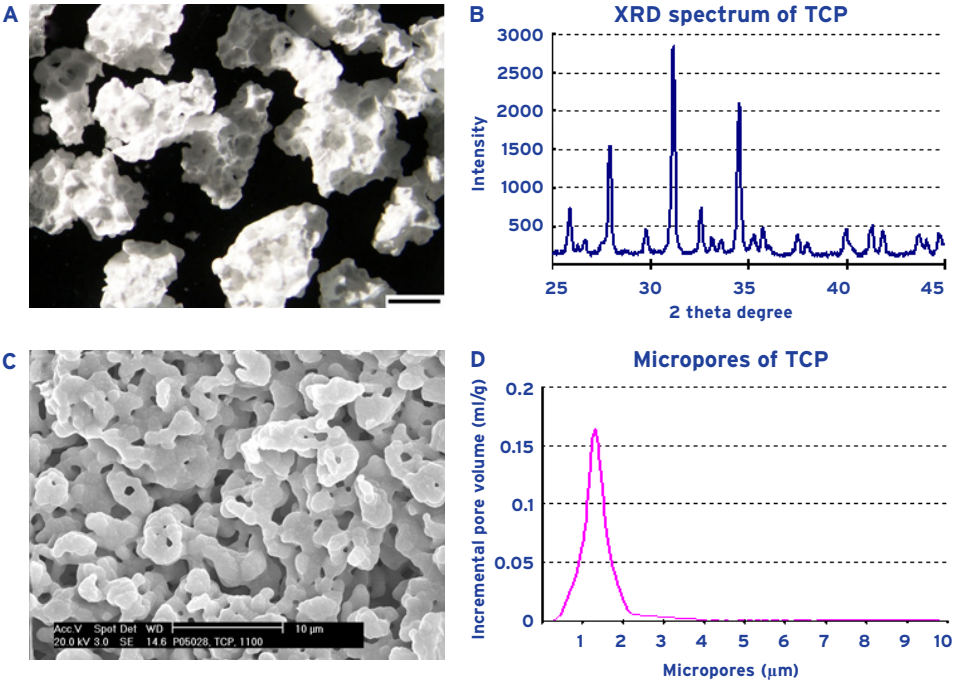
TCP granules

Calcium phosphate ceramics were produced from TCP powder (Plasma Biotal) with the H_2O_2 foaming method, as previously described. [39] In brief, the green bodies formed with diluted H_2O_2 were sintered at 1100°C for 8 hours. The resulting blocks were crushed and sieved to select the specified granules (1-2 mm) (Figure 1A). X-ray diffraction (XRD) showed that the ceramic contained phase-pure ß-TCP with peaks according to the Joint Committee on Powder Diffraction Standards (JCPD card) (Figure 1B). The material showed an interconnected porosity of around 80%, including abundant micropores as shown with scanning electron microscopy (Figure 1C). The interconnected porosity of the material was measured with mercury intrusion (AutoPore IV 9500, Micromeritics GmbH). The distribution of the micropores was homogeneous with a size range of 0.4-2.2µm (average at 1.2µm) (Figure 1D). The specific surface area was 1.3485 m^2/g measured with mercury intrusion (Micrometrics Instrument Incorporation). The ceramic particles were steam sterilized at 121°C for 30 minutes before use.

Computed tomography

After harvesting of the spinal segments and removal of the instrumentation, CT scans with sagittal and coronal reconstruction were used to evaluate the presence/absence of fusion. The CT imaging protocol consisted of 0.6-mm thick and 0.3-mm overlapping axial slices that were taken without bone filter. Scans were made using a Philips Tomoscan AVE (Philips CT Secura, Philips Medical Systems). The window and level settings were set to optimize trabecular bone detail. Two observers (D.D. and F.C.O) reviewed all CT scans in a blinded fashion. In case of conflicting findings consensus had to be reached. A modified classification system of Christensen *et al.* was used to determine the fusion rate. [40] Each side was judged separately and categorized in:

Figure 1 Characterization of the tricalcium phosphate ceramic. (A) Microscopic image of the TCP granules (1-2mm). (B) XRD analysis showing the composition of the material (C) SEM images depicting the microstructure of the TCP. (D) Incremental pore volume shows that the micropores were homogeneous with a size range of 0.4-2.2μm.

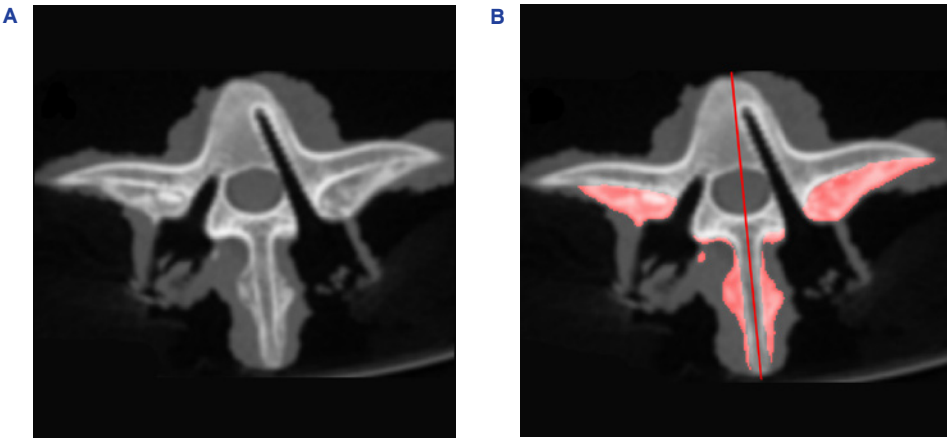


- "Fusion" was defined as a continuous bony bridge from the base of the pedicle or transverse processes from the one vertebra to the other. If the fusion was doubtful in any way, the case was not classified as "fused";
- "Doubtful fusion" indicated suboptimal quality of the bone bridging or some doubt ful discontinuity;
- "Nonunion" indicated definite discontinuity the fusion mass.

Quantification of bone volume

Original software developed at the Image Sciences Institute of the University Medical Center Utrecht was used to quantify the fusion volume. This was done by intensity-based selection of all bone on blurred CT images, and subtracting the vertebrae by manually defining the borders (Figure 2). This was done for all axial slides of each fused level, which was limited to both transverse processes and the intertransverse space. A line in the middle of, and parallel to, the spinous process was used to divide the spine into a left and right side. The selected area of all images was summed to obtain the total fusion volume per level and side of the spine. TCP particle remnants could not be detected with the CT scan and were considered negligible. The selection of new bone was done by a single investigator (D.D.) blinded to the treatment group.

Figure 2 Image of an axial CT slide. (A) Blurred image on which newly formed bone is visible against the transverse processes and spinous process. The anatomic border of the vertebra can be clearly distinguished from the new bone. Note the position of the removed pedicle screws. (B) Semi-automatically selection of the newly formed bone (pink). Red line divides vertebrae into a left and right side. By selecting the newly formed bone on all axial slices, the volume of newly formed bone was calculated of each spinal segment.



Histologic processing

After the CT scans, the spinal segments were split in the sagittal plane in the middle of, and parallel to, the spinous processes. Each side of the spine was then fixed in 4% formalin, dehydrated by ethanol series and embedded in polymethylmethacrylate (MMA). Sections (20-30 μm thick) were sawed in the axial plane at the middle of the segments using the Leica® SP1600 Saw Microtome system (Leica). The axial sections were stained with methylene blue and basic fuchsine and evaluated by regular light microscopy (Olympus-BX51). The purpose of the histology was to assess: (1) the overall morphology of *de novo* bone; (2) the amount of residual TCP; (3) the maturity of the bone; (4) the presence of any other cell type in the fusion site. Additionally, histomorphometric analysis on high-resolution digital scans was performed to quantify the amount of residual TCP. This was done by pseudo-coloring the TCP remnants and the total area of newly formed bone using Abode Photoshop CS5. The area% of TCP remnants was calculated by dividing the number of pixels.

Statistical analysis

SPSS version 14.0.0 software (SPSS Incl.) was used to conduct statistical analyses. Frequency and descriptive analyses were conducted on all data sets. Data were expressed as mean ± standard deviation. Comparison of radiological fusion rates between the treatment groups was done by a two-tailed Fisher-Exact test. A two-tailed paired *t*-test was used to assess any differences between the volume measurements between the TCP groups and the control groups. The threshold for statistical significance was set at $p < 0.05$.

Results

All animals recovered well without neurological deficiencies from surgery and survived the follow-up period without difficulties. At necropsy, one goat showed signs of surgical infection at both levels and was excluded from further analysis. No instrumentation failure (i.e., breaking of the rod) or loosing of screws was observed.

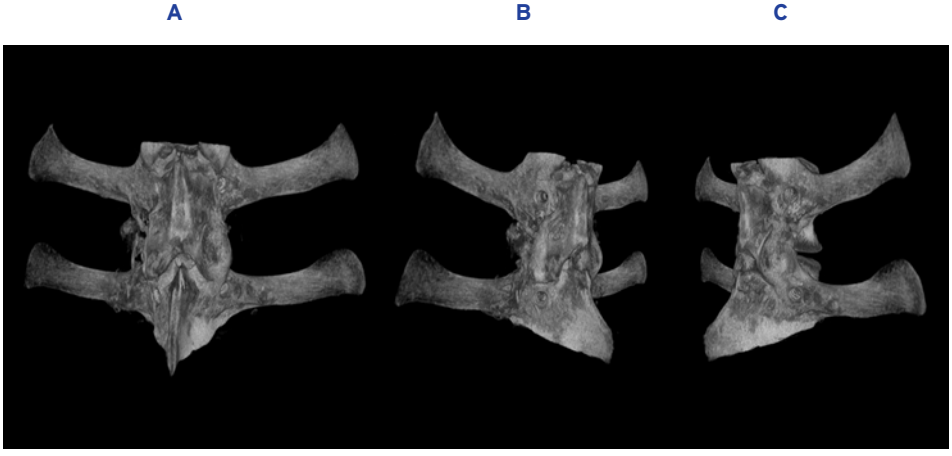
Radiological fusion

The fusion rates were 4/8 in the TCP group and 5/8 in the TCP/local autograft group, compared to 3/8 in the autograft group and 4/8 in the fresh-frozen allograft group. No statistical differences were found between the treatment groups (p=0.26). The exact classification per treatment is summarized in Table 1. The newly formed bone was mainly located medial to the connecting rod, appositioned on the lamina (Figure 3). Notably, in all groups the graft material positioned lateral to the rod had not resulted in new bone formation.

Table 1 Fusion grading by CT. No statistical differences in fusion rates between treatment groups (p = 0.26).

Group	Fusion	Doubtful Fusion	Non-union
Autograft	3/8 (38%)	5/8 (63%)	0/8 (0%)
Allograft	4/8 (50%)	4/8 (50%)	0/8 (0%)
TCP	4/8 (50%)	2/8 (25%)	2/8 (25%)
TCP / Local Autograft	5/8 (63%)	1/8 (13%)	2/8 (25%)

Figure 3 3D reconstruction of CT images of the TCP groups. (A) Posterior view. Left side received TCP combined with local autograft and right side received only TCP. New formed bone is present against the lamina, medial to the connecting rod (removed before scanning), and bridges the adjecent spinal levels. (B) Oblique view of left side (TCP/local autograft) (C) Oblique view of the right side (TCP alone).



Volume measurements

The fusion volume was 6.1 ± 1.2 mL in the TCP group and 6.0 ± 1.2 mL in the TCP/local autograft group, compared with 7.8 ± 1.8 mL in the autograft group and 8.9 ± 4.4 mL in the allograft group. There were statistical differences between the autograft group and TCP group (p=0.04), and also between autograft and TCP/local autograft group (p=0.01). In addition, significant more volume was present in the allograft group compared to the TCP/local autograft group (p=0.05). The volumes per treatment group are shown in Figure 4. The CT method was limited in that it could not differentiate between new bone formation and residual graft materials, which limited the evaluation to only an assessment of total fusion mass volume and not specifically new bone formation.

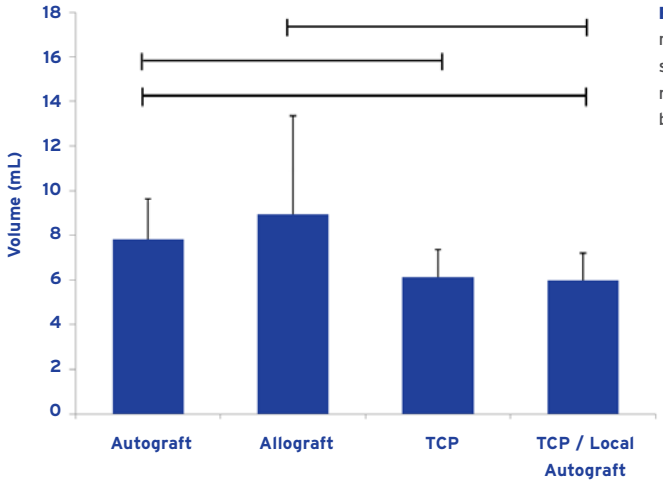


Figure 4 Volume of the fusion mass from CT. Error bars represent standard deviation. Horizontal bars represent significant differences between treatment groups (p<0.05)

Histological evaluation

Histological analysis confirmed that most new bone was formed at the medial site of the connecting rod, against the lamina. The newly formed bone was mainly lamellar and no differences were seen between the treatment groups (Figure 5). On the soft tissue side, a layer of fibrous tissue was observed between the fusion mass and the adjacent muscle. Tissue responses surrounding the implants were similar in all groups. The vast majority of TCP was resorbed and no differences were seen between the TCP group and TCP/local autograft group. Almost all unabsorbed TCP remnants were osseointegrated in newly formed bone islands and only few small fragments were seen within the fibrous tissues. Osteoclasts were surrounding the embedded TCP remnants indicating that these were subject to bone remodeling (Figure 6). Histomorphometric analysis showed that the TCP remnants constituted 1.40 ± 1.01 % of the total area of newly formed bone for both TCP groups, which was comparable between the TCP alone (1.56 ± 1.21%) and TCP/local autograft group (1.30 ± 1.03 %).

Figure 5 Overview of histological axial sections in the middle of the both spinal segments in one goat. The upper slides (A/B) represent level L2-3, whereas the lower slides (C/D) represent level L4-5. Each side of the spine received one type of graft: (A) TCP alone (B) TCP/local autograft (C) Iliac crest autograft (D) Fresh-frozen allograft. Please note that the bone is mainly formed against the lamina and is similar located in all groups. Minimal TCP remnants are present (arrow) * indicates the spinal canal. # represents posterior part of vertebral corpus. ¶ represent the lamina. δ is located in the (removed) connecting rod.

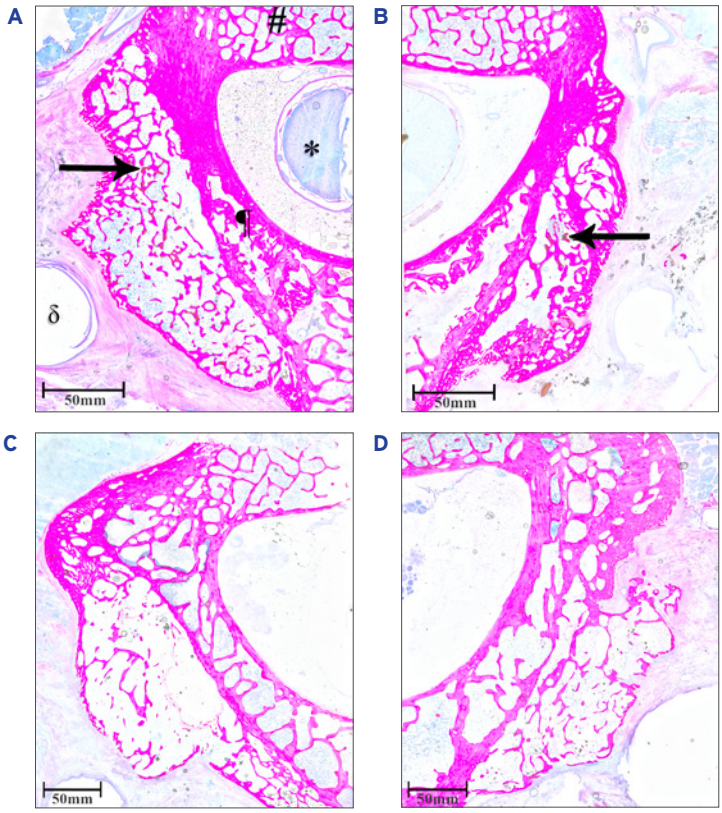
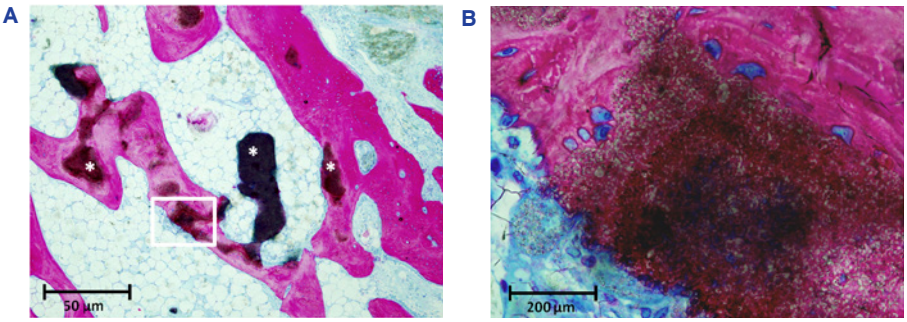


Figure 6 Histological view of TCP. (A) TCP remnants (indicated by *) are incorporated in bone without any foreign body reaction. The box represents the area of figuwre B. (B) Detailed view of TCP remnant incorporated in bone and surrounded by osteoclastic cells.



Discussion

Posterolateral fusion is generally believed to be one of the most challenging indications for bone grafts due to the large distance that needs to be bridged, limited contact surface, and the unfavorable biomechanical environment due to the lack of compression forces. The primary outcome in terms of efficacy in this study was based on the ability of achieving bony fusion on CT-scans. This study clearly shows that TCP, alone or in combination with local autograft, was capable of achieving fusion similar to allograft and autograft in instrumented posterolateral fusion in a large animal model. Additionally, no adverse tissue responses were seen and almost all TCP resorbed within 4 months.

Ceramics in posterior fusion are mainly used as bone graft extenders rather than bone graft substitutes as these types of materials are generally considered to be only a template for osteoconduction. Recently, however, the possibilities to improve the bone-forming capacities of ceramics have been recognized and are increasingly investigated. A successful strategy appears to be the addition of trace elements, like silicon.[41,42] In a study that evaluated silicon substituted calcium phosphates, the investigators found promising results in an instrumented sheep posterolateral fusion model.[43] However, it is not precisely clear whether the trace element itself or the resulting microstructure is responsible for the apparent improved performance.[44,45] The design of the material that was used in the present study was a first generation of ceramic materials with a modified microstructure that can render the material with bone stimulatory properties.[34]

Despite similar fusion rates between all groups, the measured fusion volume was lower in the TCP groups when compared with iliac crest bone graft or allograft. Potentially, this difference could be due to a less effective bone forming capacity of TCP compared to the controls; however, a limitation of our CT volume measurements is the inability to differentiate between newly formed bone and remnants of graft material. Histological analyses showed that almost all TCP resorbed, but the amount of unabsorbed allograft/autograft remains unclear and may cause overestimation of the newly formed bone volume. Another aspect that should be taken into consideration is that the differences could be based on slower bone formation in the TCP group, which may lead to similar volumes at longer follow up. In any case these findings strongly support further evaluation of the current TCP graft.

Fresh-frozen allograft performed comparable to autograft in our animal model. This is surprising since in clinical studies the results of allograft in posterior fusions are generally poor.[9] This is especially the case for freeze-dried allograft which was not capable of achieving any fusion in a comparative study, where fresh-frozen allograft did achieve fusion, although less than iliac crest autograft.[46] Unfortunately, the efficacy of fresh-frozen allograft has never been reported in other large animal models evaluating posterolateral spinal fusion.

There are several limitations to our study. The relatively small number of animals in our study limits statistical power, especially for categorical variables such as fusion. This was partly compensated for by evaluating all conditions in the same animal, and thereby allowing paired measurements. It should also be emphasized that the aim of the

study was to evaluate the potential of TCP and not to determine exact differences with autograft/allograft. Another limitation is that we did not include a group who received no graft, so the effect of decortication alone was not determined. However, this was recently investigated by our group in a separate study, where only minimal bone formation was seen and fusion in only one of four segments without any graft.[47] Finally, the interpretation of internal control studies should be viewed with caution. The effect of grafts placed on the contralateral side of the spine is unknown. This is especially the case for the potential immunological response to allograft. The mechanical effect of fusion at the contralateral side, however, is negligible due to the use of rigid instrumentation.

Despite these limitations, the results of the present study show that the investigated TCP is a promising bone graft alternative circumventing the disadvantages of autograft and allograft. The efficacy can be further optimized by studies addressing volume, microporosity and grain size. Finally, clinical trials need to determine its applicability as a stand-alone alternative to autograft.

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Chapter 6

BMPs in spine surgery through converging technologies

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Introduction

The basic approaches in orthopaedic treatment have evolved during the last 150 years. As described by Thomas A. Einhorn in 1998, the evolution of orthopaedic surgery can be divided into three discrete ages.[1] First, there is The Age of Resection. Due to the limited surgical possibilities, treatment was often restricted to amputations of injured limbs and excision of damaged tissue. Although this was quite effective for certain disorders, it was also clearly a very primitive and disabling treatment. After several decades, surgeons started emphasising on the immobilisation of joints and the cutting and realignment of bones. With this, orthopaedic surgery had entered the second age of development, The Age of Reconstruction.

In the early 1960s, important steps were made in joint replacement, especially by a very innovative surgeon, Sir John Charnley.[2] Some of his ideas were so bold and creative that he was seriously questioned by many of his colleagues. He aggressively pursued and tried to solve the problems he encountered. The efforts of Charnley and others resulted in the introduction of joint replacement in orthopaedic surgery. With this therapy, the damaged joints of patients, most often knees or hips, are replaced by artificial joints. During the last decades of the 20th century, joint replacements became a more and more popular medical intervention, and orthopaedic surgery has now entered the third age, The Age of Replacement. Joint replacements are currently one of the most successful treatments in surgical practice. However, there are some drawbacks. Joint replacements are mainly used in patients with damaged joint cartilage. This cartilage normally covers the bone ends of a joint and will allow virtually frictionless and pain-free movement. If this cartilage is damaged, the joints will become painful. Since articular cartilage does not regenerate by itself in the body, joint replacement therapy can be an option, but artificial joints unfortunately have no unlimited life span. A substantial percentage of the prosthesis will wear out, and the orthopaedic surgeon will have to remove the previously implanted joint. Replacement of a prosthesis is a challenge for both the patient and the orthopaedic surgeon. Such a revision requires the removal of the previous prosthesis, in many cases the cement in which the prosthesis was fixated, the surrounding tissue and dead bone, before a new prosthesis can be inserted. In addition to the complexity of the surgery, the outcome of revisions is often inferior to the results of primary joint replacement as well.[3]

It was anticipated that by the beginning of this millennium we would have entered the fourth age of development, The Age of Regeneration. Regenerative therapies have the potential to change the approach of the treatment by being used to repair specific tissues, like cartilage and bone. The treatment of cartilage damage can include the regrowth of cartilage instead of replacing the natural joint with an artificial one. The use of bone is necessary to restore bone defects or to fuse bone parts with each other. Despite the efforts of many scientists and clinicians, the clinical implementation of regenerative therapies is still limited. Spinal fusion is one of the few applications where a regenerative therapy is currently being investigated clinically, and this is the topic that will receive further attention in the present chapter.

Spinal fusion surgery

One of the causes of low back pain is abnormal or excessive motion of the spine caused by degeneration of the intervertebral disc (Figure 1) or instability due to other degenerative causes. A treatment option for a carefully selected group of patients is spinal fusion. Spinal fusion is a surgical technique in which one or more of the vertebrae of the spine are joined (fused) through the formation of a bone bridge, so that motion cannot longer occur between them. The rationale for spinal fusion is based upon a successful use of surgical immobilisation (arthrodesis) of painful joints. The gold standard for achieving a fusion between the adjacent vertebrae is by using autogenous bone grafts (autograft). During surgery, the bone graft will be harvested from the patient's own pelvis and placed around the spine. The bone graft will stimulate the body to form bone tissue, and in several months the vertebrae will grow together — 'fuse' — into one long bone.

Figure 1 A degenerated intervertebral disc: The disc has lost height and protrudes. This can cause back pain due to instability of the vertebral joint.

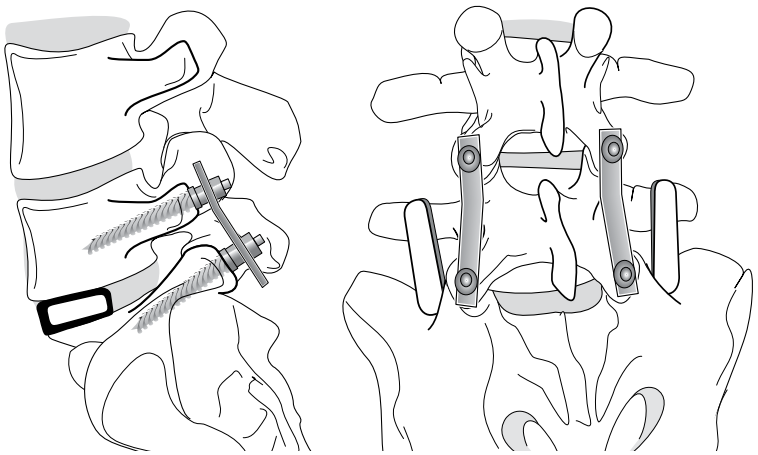
An autogenous bone graft stimulates bone formation, because it contains living cells, a structural framework into which bone can grow, and several growth factors.[4,5] The living cells are required for bone formation, since only living cells can make new bone tissue. The success of any bone grafting procedure depends on having enough potential bone-forming cells in the area. In some situations, the healthy tissues around the graft site will contain a sufficient number of such cells. In many settings, however, the number of such cells in the surrounding tissues may be limited. Bone grafts can provide the required bone-forming cells or the precursor cells that can differentiate into bone-forming cells. In addition, autogenous bone grafts also provide a framework on which the new bone can grow. Bone-forming cells generally function much better when they have a scaffold or matrix to attach to. Thus, the bone healing response is conducted through the

graft site. Finally, autogenous bone grafts contain several growth factors, including bone morphogenetic proteins (BMPs) and transforming growth factor (TGF). Growth factors are vital to bone formation as they are part of the messaging or communication system, which tells cells what to do – grow, or become a dedicated bone-forming cell. In this way growth factors can initiate, or induce, bone growth. These three properties of autogenous bone grafts contribute to the successful use of autogenous bone grafts in procedures requiring new bone formation, such as spinal fusions.

In addition to the use of autogenous bone grafts, the chances of achieving a successful fusion are enhanced when motion is prevented or minimised.[6] Motion can be prevented by using hard plastic braces, or by internally immobilising the vertebrae with metal implants Typically, these implants include rods, hooks, plates, screws, and more recently — threaded interbody cages (Figure 2). The role of the metal implants is to correct deformity and to provide additional spinal stability while helping the fusion set-up. If bone fusion does not occur, the metal construct will fatigue and break. Thus, the metal implants are only an adjunct to successful fusion — they immobilise the spine while the body forms new solid bone. These metal implants may substantially increase the chance of successful fusion but are also associated with some risks, such as injury to nerves or blood vessels.

Although for many surgeons spinal fusion using autogenous bone in combination with instrumentation is the gold standard, it is associated with significant complications. Failure to achieve such a fusion has been reported to occur in more than 30% of the patients.[7–10] The introduction of metal implants has decreased the rate of non-union,[6] but its incidence

Figure 2 Instrumented spinal fusion with the use of metal implants: The left drawing shows an intervertebral cage to correct the loss of height. Pedicle screws were placed at the back of the spine and connected by a plate. The right drawing shows a bone graft in situ between the two vertebrae. The bone graft will accelerate the formation of bone and in time the two vertebrae will unite (fuse).



remains unacceptably high. In addition, the operative removal of bone from the iliac crest requires an additional surgical procedure with a distinct set of potential complications. In some cases the grafting procedure can even be more problematic than the primary surgical procedure itself. Major complications, such as pelvic fractures, nerve injuries, vascular injuries and infections, have also been reported.[11–16] Although many severe and major complications are reported, the most common complication is persistent postoperative pain at the donor site. The reported incidence of chronic donor site pain after bone-grafting procedures from the pelvis ranges from 6% to 39%.[15,17–22] Another concern regarding autogenous bone grafts is that the amount available for transplantation can be insufficient. This is especially the case in children or when multiple levels need to be fused.

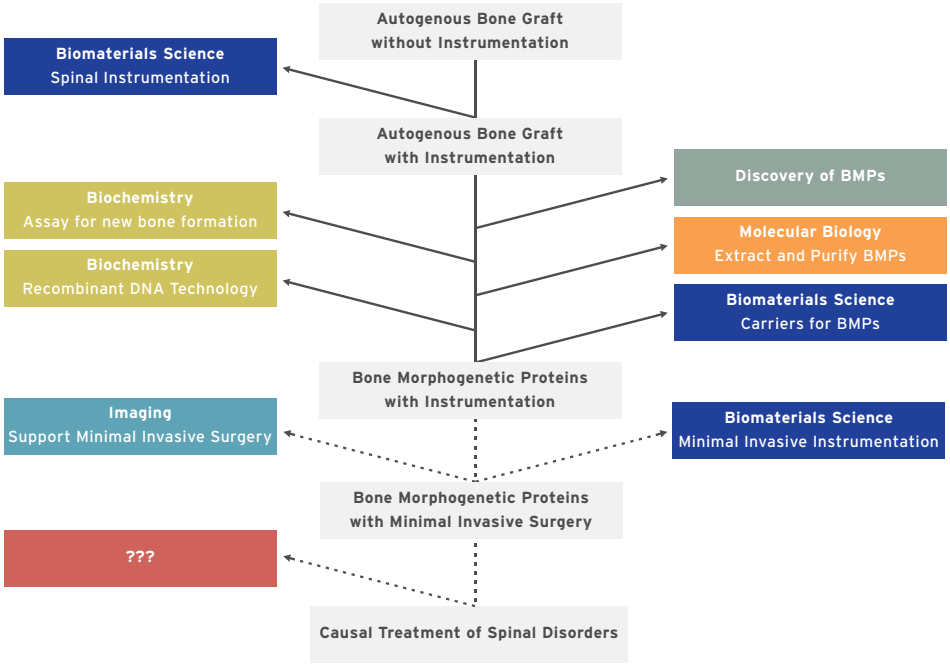
In an attempt to augment the limited quantity of autogenous bone that is accessible to the surgeon and to minimise the morbidity of grafting procedures, while maintaining an acceptable rate of fusion, several other strategies have been developed. One of the potential strategies is the use of growth factors, such as bone morphogenetic proteins (BMPs). BMPs are by nature present in bone. Nowadays, several human studies have reported the safe and effective use of BMPs as a replacement for autogenous bone grafts in spinal fusions.

BMPs for spinal fusion: a novel therapy through converging technologies

Since Albee[23] and Hibbs[24] first described their series of spinal fusions in 1911, various technical advances have occurred and evolved leading to the introduction of spinal fusions with BMPs. The implementation of BMPs in the clinic could be achieved by combining the advances made by several distinct disciplines during the last century. Albee and Hibbs were not only the first to perform spinal fusions but also the first surgeons to employ autogenous bone grafts for the purpose of immobilisation of a joint. On the basis of the work of Albee and Hibbs, spinal fusion utilising autogenous bone became surgical standard procedures. Below, we will describe how the sequential converging of the technologies of biochemistry, biomaterial science, imaging and molecular biology has finally resulted in the development of a new, revolutionary regenerative treatment in orthopaedics. This is summarized in Figure 3.

When spinal fusion was introduced, it consisted only of placing autogenous bone graft around the spine without the use of any additional metal implants. In the late 1950s, metal implants for immobilising the spine were introduced as a means of creating a faster and better fixation. In the following years, the use of metal implants for spinal fusion became more common, as surgeons saw the benefits for their patients. However, it was found that the devices applied were less than ideal in establishing rigidity and that the spinal forces were much greater than anticipated. As insights in biomaterials science progressed and metallurgy evolved, the metal implants were progressively strengthened. In the ensuing years, the implants were refined, and by the last decade of the twentieth century, instrumentation technologies had become common place.

Figure 3 BMPs for spinal fusion: a novel therapy through converging technologies. Spinal fusion started with the use of autogenous bone grafts without metal implants (instrumentation). During the last century, the influence of several distinct disciplines has led to the progression of spinal fusion techniques. Converging the knowledge of these disciplines has led to the introduction of BMPs for spinal fusion. In the near future, BMPs can be combined with minimal invasive surgery. In the long run, by further convergence of future advances, a more causal treatment of spinal degeneration can be achieved.



The seminal discovery of BMPs was made by Urist in 1965.[25] Urist was director of the bone research laboratory at the University of California, Los Angeles School of Medicine, and was a practicing orthopaedic surgeon. Bone consists of cells and a surrounding substance between these cells, the bone matrix. Urist showed that extracts of this matrix have the possibility to induce new bone formation when injected outside bone tissue. His research, however, was hampered by the fact that he was unable to isolate the agent that was responsible for the forming of the new bone, although he named the active component 'bone morphogenetic protein' or 'osteogenic protein'. The reason for this was that he did not have a test to measure bone formation.[26] In addition, it was not conclusively determined that this protein was responsible for the formation of new bone rather than any other component of the bone matrix.

He and others struggled with this for nearly 20 years. Only after the progress in biochemistry, Reddi and Sampath developed a reproducible test for measuring new bone formation. With the aid of this test they were able to show that when the protein component was dissociated from the bone matrix, the remaining matrix in itself did not induce new

bone formation.[27] However, if the protein was returned to the matrix, the latter turned out to be as effective as the original matrix in inducing bone formation. This conclusively proved that the protein contained within the extract was responsible for bone formation.

After identifying the active protein, the scientists needed to extract and purify it from the matrix. This required new molecular biological techniques. Due to technological progress in this discipline, it became possible to extract and purify these bone-inductive proteins from the matrix, which resulted in the identification of many types of bone morphogenetic proteins. Nowadays, over 20 different BMPs have been identified.

The next step was to find a way to produce these BMPs in large quantities. As bone contains only very small amounts of naturally occurring BMP, it would require hundreds of kilograms of donor bone in order to obtain enough BMPs to be clinically useful. Therefore, the next challenge to the scientists was to find a way to synthetically produce BMPs. This became possible through further progression of recombinant DNA technology. Through this progress in biochemistry, segments of foreign DNA are transferred into another cell, and thus the substance for which they code may be produced. With this incorporated DNA the cells become 'factories' for the production of the protein. By using this technique, natural human BMPs can be produced in large quantities.

The final step before these proteins can be used in a clinical setting required the innovations made by biomaterial science during the last decades. This implied the identification of a suitable carrier to ensure a specific release of the protein in time. BMPs are water-soluble and are relatively low–molecular weight proteins that diffuse easily when administered at a surgical site. It is therefore necessary to contain the BMPs. In addition, bone-forming cells generally work better when a strong framework is available. In other to meet these two requirements, various carriers have been investigated both in the laboratory and in patients.

Combining the progress made by different specialties within life sciences resulted in the first clinical study by Johnson and associates with purified human BMP in 1992.[28] At present, BMPs have been investigated as an alternative to bone autograft in a variety of clinical situations, including spinal fusions, the internal fixation of fractures, treatment of bone defects, and reconstruction of maxillofacial conditions.

Current status of BMPs in spine

Soon after Sampath and Reddi invented a test to measure new bone formation and showed that the BMPs were responsible for the induction of bone formation, the development race was on. Two possible BMPs, identified in the bone extract, were 'chased' by two biotech firms, Genetic Institute and Creative BioMolecules.[26] Genetic Institute hired Reddi as a consultant, and Sampath joined Creative BioMolecules. The objectives of these firms were to identify the genes and complementary proteins that were responsible for the bone formation and to obtain the rights to this potentially lucrative market. In order to identify the responsible genes, the first step was to purify the protein, i.e. separate it from other proteins

in the bone mixture. When they succeeded in purifying enough BMPs, they needed to determine the basic structural building units (amino acids) of the protein, the protein sequence. After having determined the partial protein sequence, both companies discovered that it contained a specific contribution of amino acids.[26] That pattern is characteristic of proteins belonging to the transforming growth factor-ß (TGF-ß) superfamily of regulatory proteins. This clue allowed the groups to fill in the blanks of the incomplete protein sequence with sequences from other members of the TGF-ß superfamily and then to develop gene probes. Using the gene probes as bait, the two groups went fishing among a library of human fragments. Both companies found the complementary genes and proteins for various BMPs and applied for patents simultaneously. The determination of who owns the right to which genes and proteins was resolved by the court. Creative BioMolecules obtained the rights to produce BMP-7 (= Osteogenic Protein-1 (OP-1)) and associated with Stryker Corporation (Kalamazoo, Michigan). Genetics Institute started collaborating with Medtronic so as to produce BMP-2.

At present, these two recombinant human BMPs (rhBMPs) are commercially available. In the United States, rhBMP-2 is approved for long-bone fractures and for anterior spinal fusions. RhBMP-7 has a Humanitarian Device Exemption for recalcitrant long-bone non-unions and revision postero-lateral lumbar fusions. This Humanitarian Device Exemption is available to those devices intended for fewer than 4,000 patients per year. While the largest clinical trials and randomised studies demonstrate that rhBMP can be an alternative to an autologous bone graft for interbody spinal fusion,[29,30] treatment of tibial fractures[31] and fracture non-unions,[32] these results cannot be extrapolated to other clinical indications, because of the variations in carrier and delivery systems. To date, there are inadequate data to document efficacy in other clinical applications, and therefore other clinical trials need to be performed before expansion of the labelled indications.

How bone morphogenetic proteins work

Bone is unique compared to all the tissues in vertebral organisms. When injured, it heals by forming new bone. By contrast, most other tissues, such as the heart muscle, kidney and brain, heal by replacement of connective tissue rather than the original tissue. BMP is a protein existing by nature in our bodies and stimulates bone formation. The protein is essential for the healing of broken bones. BMPs are osteoinductive as they are able to stimulate new bone formation.[25] The principle of their working mechanisms is the initiation of a complex multistage cascade of events in promoting bone formation (Figure 4).[33]

Under the influence of BMPs, undifferentiated stem cells are attracted from the surrounding tissue.

After being recruited from the surrounding tissue, some of the stem cells multiply, while others differentiate into specialised cells that are necessary for bone formation, bone-forming cells or blood vessel cells.[33] Due to the ability of BMPs to transform cells from primitive undifferentiated stem cells into specialised ones, they are known as morphogens (from the Greek shape, and creation). The bone formed under influence of BMPs is biochemically

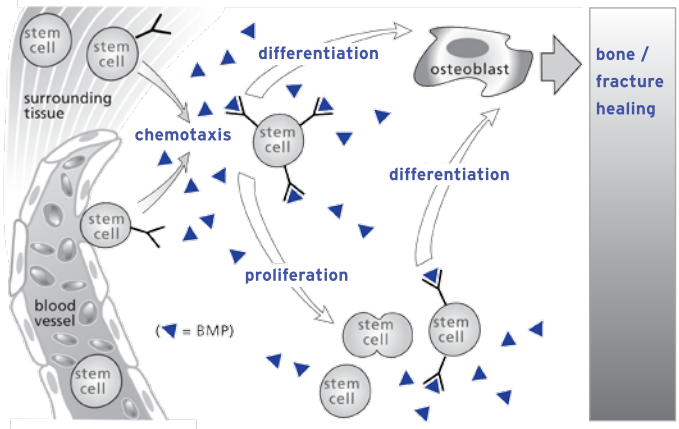


Figure 4 Schematic depiction (simplified) of the working mechanism of BMPs. Under influence of BMPs undifferentiated stem cells are attracted from the surrounding tissue and blood vessels (chemotaxis). After being recruited, some of the stem cells multiply (proliferate), while others differentiate into bone-forming cells (osteoblasts). These osteoblasts start forming bone.

and microscopically the same as normal bone. A significant amount of the research into BMPs has been performed to elucidate the effects of individual BMPs at a cellular level, but up to now, unfortunately, the exact cellular mechanism is still unclear.

Fundamental science versus application

It is more than 4 decades ago since Marshall Urist made the seminal discovery that bone-derived proteins can induce bone formation, but so far BMPs did not have a major impact on orthopaedic surgery. Controlled clinical trials for each BMP, for each anticipated application/location need to be performed, not only to maximise clinical efficacy, but also to clearly define safety parameters for these highly osteoinductive compounds. One of the limiting factors is that the exact cellular mechanism targeted by BMPs, and the complete working mechanisms are not yet revealed. As mentioned before, laboratory studies have shown that under the influence of BMPs stem cells multiply and differentiate towards bone-forming cells. However, we do not know exactly what happens within the cells. With BMPs, we switch on the process of bone formation without actually knowing how to turn it off. In theory this could lead to excessive bone formation and even to an uncontrolled division of cells, although in practice this has not been observed. The question is, when do we know enough in order to allow clinical application, and which uncertainties can we accept in relation to the advantages obtained by this new therapy? Of course, in order to make progress in medicine, new potential therapies should be evaluated in human clinical studies, and here the difficulty remains to decide when it is safe to use it in a human clinical study. An example of a practical approach is total hip replacement. As mentioned in the introduction, modern artificial joints owe much to the work of Sir John Charnley. He pursued and tried to solve the problems encountered by replacing not only the femoral head (the ball) but also the acetabulum (hip socket). Charnley himself did suffer 'trials and tribulations' when developing his hip replacement, but he never gave up.[34] Finally, in November 1962, the Charnley hip replacement became practical reality and it has become the gold standard for this form of treatment. Several decades had passed, when biomaterials science and

biomechanics supplied more fundamental insights into and explanations of the working mechanism of the total joint prosthesis. We should, of course, first thoroughly explore the safety and efficacy in experimental models, but in order to make progress we should also dare to make the step towards clinical studies.

Cost-effectiveness

A major limitation of BMPs is their high price. Due to the ageing population and longer life expectations of the population in Western industrialised countries, it has become increasingly important to evaluate the economic impact of new therapies. The use of new technologies is generally presumed to be more expensive. Measuring the cost-effectiveness of a treatment is a tool that has been used as a decision-making aid for optimal societal resource allocation.

Only one published cost-effectiveness analysis of BMPs for spinal applications is currently available, in which an economical analysis of BMP-2 (InFUSE, Medtronic Sofamor Danek) versus autogenous bone graft was performed in patients who underwent one-level fusions with the use of cages.[35] The authors suggested in a preliminary analysis that the price of BMP-2 is likely to be entirely compensated by reductions in the use of other medical resources. In other words, BMP-2 appears to be cost-neutral in this application. The cost reduction when using BMPs instead of autogenous bone grafts was largely attributed in this study to the prevention of pain and complications associated with autogenous bone harvest. Less obvious costs include decreased blood loss and obviated treatment of donor site complications, as well as potentially decreased transfusion requirements and shortened hospital length of stay. Additionally, by eliminating the need to harvest the autograft, anaesthesia time and surgeons' fees could compensate for the high price of BMPs. Last but not least, a reduction of the costs associated with fusion failures largely contributed to the compensation of the price of BMPs. However, current literature has not indisputable shown that the use of BMPs provides this kind of reduction in non-unions, which could compensate for the high price of the product.

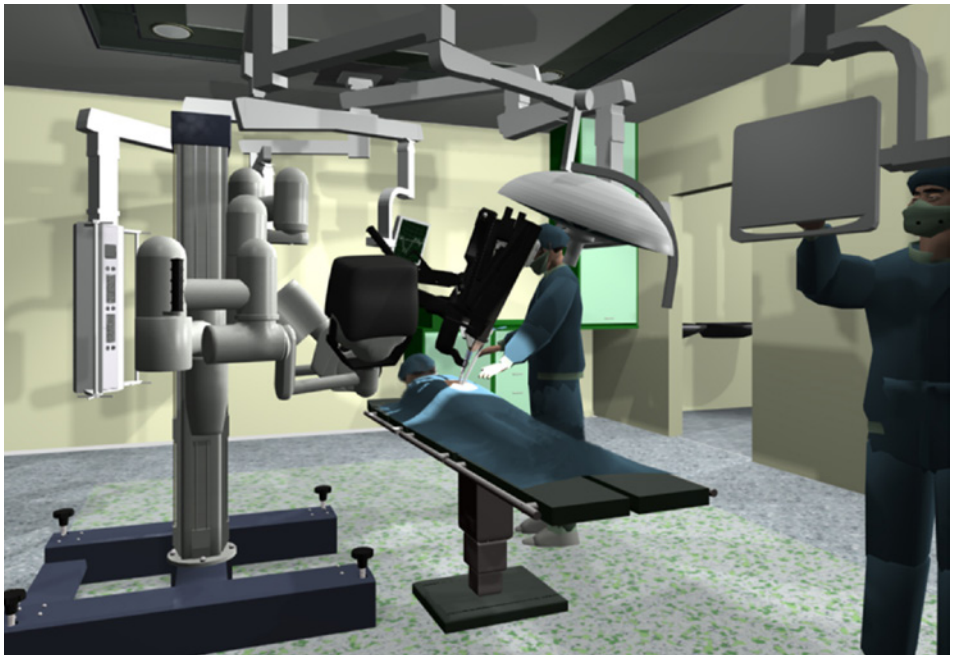
Although future research is needed to evaluate the cost-effectiveness of BMPs in the spine, it is possible that additional costs of BMPs may not be fully compensated. It is possible that the only advantage of BMPs will be elimination of chronic donor site pain and perhaps a slightly better fusion success, which is particularly relevant for patients 'at risk' (e.g. heavy smokers, diabetes etc.), or in combination with new surgical techniques to achieve fusion by less invasive methods. Currently, the economical analyses do not take into account the quality-of-life impact of pain. A difficult ethical question that needs to be considered is the appreciation of the iliac crest pain in term of costs. In other words: What is pain avoidance worth? A more general discussion that needs to be addressed is whether these innovations have a place in a community of which the healthcare is not going to be affordable within a couple of decades due to the ageing population and longer life expectation. Should we only use regenerative medicine in certain indications? If so, will certain patients have more 'rights' to the new treatment, e.g. those patients 'at risk', the young productive sector of the population, or will this be equally accessible to everyone undergoing a spine fusion?

Future treatments options with BMPs

Clinical application of osteoinductive factors is still in its infancy. Current studies are mainly focussed on avoiding morbidity associated with the harvesting of autogenous bone grafts from the pelvis. A greater advantage of BMPs can be established by using these inductive proteins in combination with a minimal invasive medical procedure, also known as 'keyhole' or percutaneous surgery. This procedure is carried out by entering the body through the skin, but with the smallest damage possible to these structures. Special medical equipment may be used, such as fibre optic cables, miniature video cameras and special surgical instruments handled via tubes inserted into the body through small openings in its surface (Figure 5).

Minimally invasive surgery results in less operative trauma for the patient. It is also less expensive, reduces hospitalisation time, causes less pain and scarring, and reduces the incidence of complications related to the surgical trauma. Without the use of BMPs, spinal fusions using a minimal invasive technique will still require an additional procedure to harvest the autogenous bone graft. This procedure will limit the advantages of the minimal invasive surgery, since an additional 'open' procedure is required. Nowadays, the first studies evaluating the combination of minimal invasive surgery with BMPs are being published and they show promising results.[36,37] Minimal invasive surgery in combination with BMPs will presumably be introduced in orthopaedic practice in the near future.

Figure 5 The operation room of the future will contain special medical equipment, making minimal invasive surgery possible. This equipment may include as fibre optic cables, miniature video cameras and special surgical instruments handled via tubes inserted into the body through small openings in its surface.



In order to optimise the bone-forming capacities of BMPs, a combination with other growth factors and scaffolds should be considered. The synergistic effect of several factors stimulating bone-formation will result in higher fusion rates and a more successful treatment. By increasing the success of the treatment, the cost-effectiveness will increase. Also less BMPs may be required, which leads to a lower price of the bone graft substitute.

Future for regenerative medicine in the spine

Although the use of BMPs to establish a fusion between two vertebrae can increase the success rate of the treatment, spinal fusion remains a 'salvage' treatment. The patient has back pain due to degeneration of the spine and the therapy consists of immobilisation. When we look at the ages of development in orthopaedic surgery, spinal surgery has mainly remained in the Age of Reconstruction.

Ever since artificial hips and knees were introduced in the 1960s, scientists have also explored the idea of prosthetic replacements for damaged or degenerated intervertebral discs. Currently, several intervertebral disc prostheses are commercially available. One major intended benefit of artificial discs over spinal fusion is that potentially it does not change the biomechanics of the spine. Spinal fusion causes a decreased motion at one or more levels of the spine and will cause more stress to be transferred via the adjacent levels. This increased stress can create new problems at the other spinal levels. The artificial disc will facilitate the spine to maintain its normal range of motion and thereby reduces the risk of degeneration in adjacent segments. However, there are also drawbacks for the use of artificial discs. As mentioned before, intervertebral disc degeneration is a common cause of back pain in younger adults (age 30 to 50) and the demands for artificial discs will be great. Therefore, a substantial percentage of the patients will need revision surgery. Since this surgery is nearby vascular structures and scar tissue from the original surgery, revision procedures for artificial discs are complex and can be dangerous. In addition, the long-term outcomes of artificial discs are not well-known as yet.

In an attempt to improve the disadvantages of the current surgical treatment options for disc degeneration, spinal fusion or disc replacement, a more causal treatment of degenerative disc disorders is desired. Current treatment of disc degeneration aims at relieving the consequences of disc degeneration rather than at focusing on the cause of the disease. A potentially promising technique that is currently under the attention of several groups is to restore the intervertebral disc by regenerative strategies. The extensive research performed on BMPs in the last decades has given scientists more insight in regenerating mechanisms of bone and cartilage. We do not yet know all of the growth factors that are involved in disc cell regeneration, but it appears that BMPs are a key component. In the long run, this may lead to new therapies which can stop and maybe partially reverse the degenerative process in the spine. New therapies could focus on stimulating the synthesis of the disc by injecting stem cells or by injecting growth factors. As compared with the commonly used 'salvage' treatments, this causal approach of disc degeneration has many advantages: It requires no surgery other than the injection of specific agents into the disc and therefore limits the risk for surgical complications, whereas the

demand for hospitalisation and rehabilitation is expected to considerably decrease. Furthermore, long-term complications of fusion or prosthetic placement will be absent, as the proposed technology aims at restoration of original function and mobility.

Another important aspect that needs to be solved is the diagnosis of low back pain due to disc degeneration. Over the last 15 years, advances in biomedical imaging have resulted in the widespread use of magnetic resonance imaging as an evaluative tool in the diagnosis of patients with back pain. Degenerative changes seen on the MRI could account for the chronic back pain disc. However, there is no 1:1 correlation of disc degeneration to pain. As we age, the disc normally also undergoes degenerative changes. The MRI is a relatively sensitive test for the detection of degenerative changes within the intervertebral disc, but it is incapable of providing a pain association. In order to prevent procedures from being performed for the wrong reasons and to improve diagnostic accuracy, a reliable diagnostic tool for painful disc degeneration is required.

As discussed above, the implementation of these new technologies will likely be accompanied with higher costs. In order to keep health care affordable in the upcoming decades, cost effectiveness will play an increasingly important role in determining whether a new therapy will be implemented in clinical practice. This seems to clash with the rising quality-of-life demands of society. For instance, patients with low back pain wish to be able to continue practicing their sports. As a result of the emphasis on cost effectiveness, these people will not receive treatment as it is calculated that the costs do not warrant the benefit to their quality of life.

Furthermore, when determining the cost effectiveness of a new treatment, we should realise that it is generally presumed that all new technologies are more expensive in the beginning. However, the costs can decrease after the initial development costs are compensated. Additionally, after the implementation of the new treatment on a broader scale, the reduction of the production costs will also count for a decrease in the price of the new treatment. This can eventually lead to a cost effective treatment, while initially the treatment was companied with higher costs.

In addition to the focus on cost-effectiveness of new treatments, it is also important to realise that innovations often give insight in the mechanism of diseases and can lead to other treatment options. The current application of BMPs for spinal fusion could be more expensive than the regular treatment with autogenous bone grafts. However, after the safety and efficacy of BMPs in spinal fusion has been shown, it is feasible that BMPs will be used in combination with minimal invasive medical procedures. Minimal invasive surgery is associated with less complications, shortened hospitalisation and less work absenteeism, thus leading to a reduction of health care utilisation.

Finally, BMPs have given insight in the mechanism of diseases, like intervertebral disc degeneration. This brings a causal treatment option for patients with low back pain a bit closer. Since low back pain is associated with high costs of health care utilisation, a new treatment will reduce the impact of this major health and socioeconomic problem.

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Chapter 7

Study of OP-1 in posterolateral fusion: a European multi-center trial

I. Pilot study on safety and feasibility

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Introduction

Instrumented posterolateral fusion of the lumbar spine is a common procedure for a variety of spinal disorders. The conventional technique for achieving posterolateral fusion involves placing iliac crest bone graft between the decorticated surfaces of lamina, facet joints and transverse processes. However, the removal of bone from the iliac crest requires an additional surgical procedure with a distinct set of potential complications[1-3] and is limited by the available bone quantity. In an attempt to address the disadvantages of autologous bone graft, extensive research has focused on the development of alternatives.

The seminal discovery by Urist[4] that bone-derived proteins can induce bone formation launched the promising strategy of bone regeneration using bone morphogenetic proteins (BMPs). Extensive data have shown that molecules belonging to the BMP family can initiate the cascade of bone formation, including the migration of pluripotent mesenchymal stem cells and their differentiation into osteoblasts.[5] Currently, two BMPs (Osteogenic Protein-1 [OP-1] and BMP-2) are commercially available and have been evaluated in a variety of clinical situations, including spinal fusion.[6-10] Although most studies have generated encouraging data, controlled clinical trials for each BMP and for each anticipated application need to be performed, not only to define the clinical efficacy, but also to clearly define safety parameters for these highly osteoinductive compounds.

In a prospective European multi-center study, we evaluated the use of OP-1 combined with locally obtained bone from the laminectomy, as a replacement of iliac crest autograft in single-level instrumented posterolateral fusions in patients with isthmic or degenerative spondylolisthesis with central or foraminal stenosis. This paper describes the safety and feasibility of the use of OP-1 for this indication, based on the 1-year results of the first 36 patients comprising the pilot group of the study.

Material and Methods

Study design:

In a prospective, randomized, multi-center study, 36 patients who required one-level instrumented posterolateral fusion of the lumbar spine for central or foraminal stenosis by isthmic or degenerative spondylolisthesis were enrolled in 5 participating pilot centers. The study was performed according to the principles of the Declaration of Helsinki and Good Clinical Practice. There were two treatment groups in a 1:1 ratio, making a total of 18 patients per treatment group. One group received OP-1 combined with locally obtained bone form the laminectomy (OP-1 group) and the other group received autologous bone graft obtained from the iliac crest combined with locally obtained bone (autograft group). The patients were observed before surgery and at 6 weeks, 3 months, 6 months and 1 year after surgery. The primary outcome was the presence or absence of radiological fusion (computed tomography scans) after a one-year follow-up. The clinical outcome was measured using the Oswestry Disability Index (ODI).[11] Additionally, the safety of OP-1 was evaluated by comparing the frequency and severity of adverse events that occurred between both populations.

Inclusion and exclusion criteria:

After approval from the Institutional Review Board, patients qualifying for decompression and fusion of one spinal level (L3-S1) with the use of autograft were recruited through the medical institutions of the participating investigators. All patients had a degenerative or isthmic spondylolisthesis with symptoms of neurological compression caused by central or foraminal stenosis. The exact inclusion and exclusion criteria are summarized in Table 1.

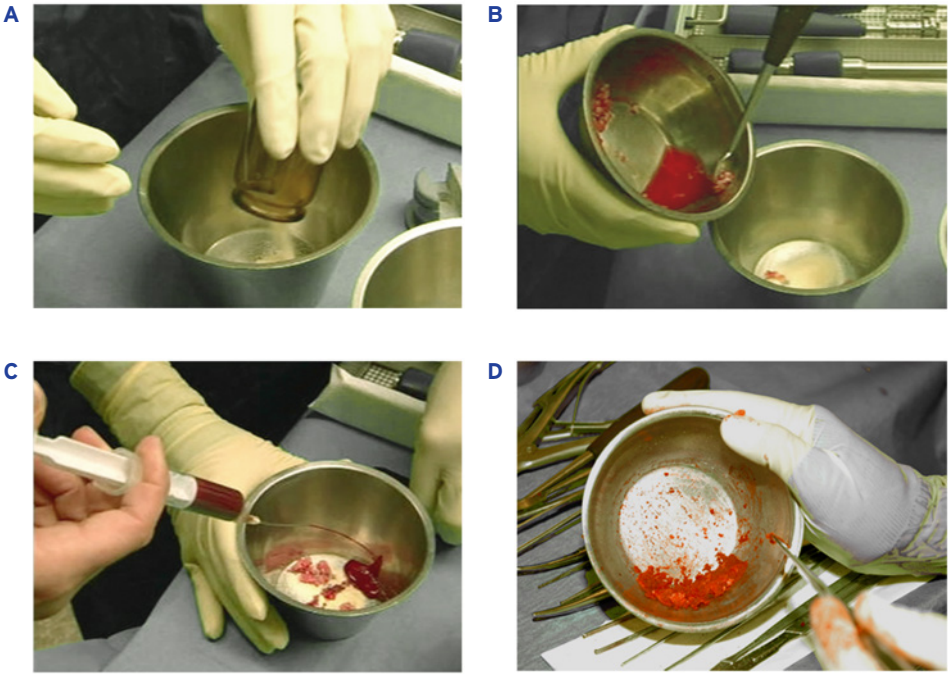
Table 1 Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
· Degenerative or Isthmic Spondylolisthesis (Grade I and II) with central or foraminal stenosis	· Gross instability that requires multiple levels fusion
· Eligible for decompression and single-level fusion (L3-S1)	· Severe osteoporotic / osteopenic patients
· Symptoms of radiculopathy and/or neurogenic claudication	· Suspicion of active spinal or systemic infections
· A preoperative Oswestry Disability Index > 30	· Women who were pregnant or who planned to become pregnant
· Non-responsive to at least 6 months of non-opera tive treatment	· A known sensitivity to collagen
· No previous fusion attempt(s) to the affected level	· Morbidly obese patients
· Skeletally Mature	· Patients who have in the last year been prescribed systemic corticosteroids
	· Known to require additional surgery to the lumbar spinal region within six months

Bone graft substitute

One unit Osigraft® (Stryker Biotech, Hopkinton, MA, US) containing 3.5 mg lyophilized rhOP-1 in 1g of collagen type I carrier was used per side of spine, for a total of 2 units. Each unit was prepared in a separate bowl (Figure 1). The bony parts collected from the decompression (local autograft) were morselized using a bone nibbler, and divided into two equal proportions. At least 2.5 mL of fresh unheparinized blood was added to the each bowl. The local autograft was uniformly mixed with the Osigraft®. In order to improve the handling characteristics, the mixture of OP-1 and local autograft was allowed to clot for a minimum of fifteen minutes before implantation.

Figure 1 Preparation of OP-1: Each unit OP-1 (3.5mg) was prepared in a separate bowl (A).The local autograft, obtained from the decompression, was combined with the OP-1 (B). At least 2.5 ml of unheparinized fresh blood was added per bowl of Osigraft® (C). The mixture was left to clot for at least 15 minutes in order to improve the handling (D).



Surgical technique

All instrumented posterolateral lumbar fusions were strictly standardized and identical for the two groups with the exception of the bone grafting technique. Each of the patients underwent surgery using general anesthesia. Prophylactic cephalosporine were given for 24 hours starting at least 15 minutes before the incision. A posterior midline incision was made with subsequent dissection of the paraspinal muscles down to the transverse processes of the affected levels. Decortication was performed all through the posterolateral

lumbar area from the transverse processes to over the posterior aspect of facet joint. Decompression was achieved by performing a bilateral laminectomy or partial laminectomy and medial facetectomies, as necessary to completely decompress the neural elements. All patients received posterolateral spinal fusion with pedicle screw instrumentation (Xia Spinal System; Stryker Spine, Allendale, NJ, US). In case the patient was randomized in the control group, bone graft was obtained using the surgeons preferred technique at a single site of the iliac crest. Preferably, the initial midline incision was used to harvest the autograft and no additional incision was made. Care was taken not to deviate from the normal autograft amount taken in a typical one-level fusion.

Before placing the graft material, careful haemostasis secured a dry fusion bed, which is required for adequate containment op the OP-1. The appropriate graft material, either OP-1 with local autograft or morselized iliac crest autograft with local autograft, was placed in the lateral gutters on the decorticated bony surfaces of the transverse processes, pedicles, and along the pars interarticularis. No irrigation of the wound was performed after the placement of the bone graft. Closure was in three layers, and no deep drain was used.

After the surgery, patients were prohibited to use nonsteroidal anti-inflammatory drugs for at least 6 weeks, and thrombosis prophylaxis was given according to the local used treatment standard. A brace or orthosis was given for at least 8 weeks after the surgery, to protect the spine from excessive movements.

Randomization

All participants were enrolled between July 2004 and June 2005. They were first seen in the practice of their surgeon, who evaluated eligibility for enrolment based on inclusion and exclusion criteria. A computer-generated randomization code was produced according to the "random-permuted-block" by a researcher not affiliated with the trial using SYSTAT for windows (SYSTAT Inc., Evanston, Illinois, US). By using this randomization scheme, each participating center included an equal number of patients per treatment group. To prevent any potential bias, the surgeons were blinded to treatment group as long as possible. That means that the decompression and placement of the screws were performed before the envelope containing the randomization of the patient was opened and the surgeon received the result of the randomization.

Radiographic outcome measurements

The computed tomography (CT) scans were reviewed by a spinal surgeon (F.C.O.) and a senior radiology resident (H.Q.U.) blinded to the treatment group and the institute where the procedure was performed. A third observer, a spinal surgeon (N.V.), was used to adjudicate conflicting findings. In the exceptional case that all three observers classified the fusion differently, the patient was classified as "Doubtful fusion". A detailed classification system based on the classification system described by Christensen *et al*,[12] was used to determine the fusion rate. This classification consisted of the following three categories:

1. "Fusion" defined as a continuous bony bridge from the base of the pedicle and transverse processes from one vertebra to the other, at a minimum of one side of the spine, in absence of any secondary signs of nonunion, such as fracture or loosening of the screws. If the fusion was doubtful in any way, the patient was not classified as fused.
2. "Doubtful fusion" indicated suboptimal quality of the bone bridging or some doubtful discontinuity, including fusion mass possible hidden behind instru mentation, at a minimum of one side of the spine, in absence of "fusion" on the other side.
3. "Nonunion" indicated definite discontinuity or lack of the fusion mass at both sides of the spine.

Clinical outcome measurements

Clinical assessments were completed before surgery and at 6 weeks and 3, 6 and 12 months after the surgery. The outcome analyses were supported using the Visual Analog Scale (VAS) and the ODI, version 1.0 (ODI).

The Oswestry score was obtained from both groups. The ODI is a validated and standardized instrument commonly used for outcomes in spinal pathology.[11] This evaluation questionnaire is scored from 0% (no disability) to 100% (total disability) and is related to subject perception of the effect of his or her current low back pain on activities of daily living. One question specifically rates the intensity of pain.

In the arm receiving iliac crest autograft an additional semi-quantitative multiple-choice question was asked regarding their donor site pain. Additionally, the intensity of the postoperative iliac crest site pain was scored based on a 10-point VAS (range, 0-10). A VAS score of 0 was defined as no pain, and a score of 10 was defined as the worst pain imagined by the patient. Notably, the patients were clearly instructed on paper that the questions concerned iliac crest related morbidity and not the low back pain.

Safety evaluation

The safety of OP-1 was evaluated by documenting details and severity of adverse event that occurred within the study population. An adverse event included any untoward medical occurrence in a patient, regardless of the nature of the event or its severity, which does not necessarily have a causal relationship with the treatment. The nature and frequency of the occurred adverse events were compared between both groups.

Statistical analysis

All data were collected and recorded using Filemaker Pro version 7.0v1 (FileMaker Inc., Santa Clara, California, US). SPSS version 14.0.0 software (SPSS incl., Chicago, Illinois, US) was then used to conduct statistical analyses. Frequency and descriptive analyses were conducted on all data sets. Comparison of categorical variables between the treatment groups was analyzed using a Fisher exact test. For comparison of age and preoperative Oswestry scores between both groups, an independent sample *t* test was used. A Mann-

Witney nonparametric test was used to analyse differences in Body Mass Index (BMI), blood loss, operative time and length of stay, which is appropriate for variables that are not normally distributed. A repeated measurement analysis of variance was used to assess any significant differences of the Oswestry score after surgery and differences between both treatment groups. The threshold for statistical significance was established at $p < 0.05$. All values are given as mean ± standard deviation.

Results

Patients follow-up

In a total of 36 included patients, four protocol violations occurred, and two patients did not complete the 12-month follow-up period. Two protocol violations (autograft group) concerned a different surgical procedure than specified in the protocol: one patient had a fracture of the pedicle during surgery, necessitating a two-level fusion and one patient received only local autograft as grafting material. These patients were subsequently excluded from any statistical analysis. The two other protocol violations (OP-1 group) concerned preoperative Oswestry scores less than the minimum required score of 30. Because the primary outcome was based on radiological fusion rates, these patients were not excluded from the study. Of the patients that did not complete the follow-up period, one patient was lost to follow-up (OP-1 group) and one patient (OP-1 group) had a concurrent medical condition (primary brain tumor) diagnosed after the surgery. The patients who failed to have finished their 12-month visit were included in the statistical analysis until their last follow-up visit, at 6 weeks and 6 months, respectively.

Patients demographics

The OP-1 group included 8 women (44%) and 10 men (56%), with an average age of 53 ± 18 years. The origin of the instability was degenerative spondylolisthesis in 10 patients (56%) and isthmic spondylolisthesis in 8 patients (44%). The autograft group consisted of 10 women (63%) and 6 men (38%), with an average age of 55 ± 13 years. The origin of the instability was degenerative spondylolisthesis in 11 patients (69%) and isthmic spondylolisthesis in 5 patients (31%). There were no significant differences in baseline characteristics between both groups, expect for the distribution of the fused spinal levels. Complete patient demographics are shown in Table 2.

Table 2 Patients demographic and clinical details. BMI = Body Mass Index. * p > 0.05.

	OP-1 Group (n=18)	Autograft Group (n=16)	p-value
Age (y)	53 ± 18	55 ± 13	0.77
BMI (kg/m^2)	26 ± 4	27 ± 3	0.89
Gender			0.29
Male	10 (56%)	6 (38%)	
Female	8 (44%)	10 (63%)	
Smoker	8(44%)	4 (25%)	0.24
Origin of Instability			0.43
Degenerative Spondylolisthesis	10 (56%)	11 (69%)	
Isthmic Spondylolisthesis	8 (44%)	5 (31%)	
Level Fused*			0.01
L3-L4	4 (22%)	2 (13%)	
L4-L5	5 (28%)	12 (75%)	
L5-L6	0 (0%)	1 (6%)	
L5-S1	10 (50%)	1 (6%)	
Preoperative ODI	44 ± 15	53 ± 13	0.07

Surgery

The surgical data are summarized in Table 3. There were no significant differences in blood loss, surgery time, and length of hospitalization between both groups.

Radiographic fusion rates

The fusion rates were not statically different between the treatment groups (p=0.95). Ten of 16 (63%) patients were classified as definitely fused in the OP-1 group, compared with 10 of 15 (67%) in the autograft group. The complete fusion rates are summarized in Table 4. An example of fusion of a patient that received OP-1 is shown in Figure 2.

Table 3 Surgical data.

	OP-1 Group (n=18)	Autograft Group (n=16)	p-value
Surgery Time (minutes)	178 ± 73	178 ± 47	0.54
Blood Loss (cc)	422 ± 265	373 ± 301	0.50
Hospitalization (days)	10.5 ± 4.9	10.9 ± 6.4	0.93

Table 4 Radiological fusion rates based on the CT-scan at one year follow-up. No significant differences in fusion rates between both groups (p = 0.95)

	OP-1 Group (n=16)	Autograft Group (n=15)
Fused	10 (63%)	10 (67%)
Doubtful Fused	4 (25%)	3 (20%)
Non-union	2 (13%)	2 (13%)

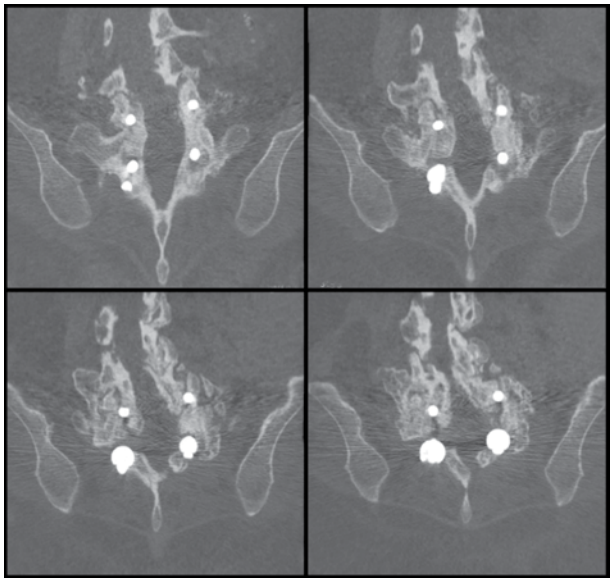
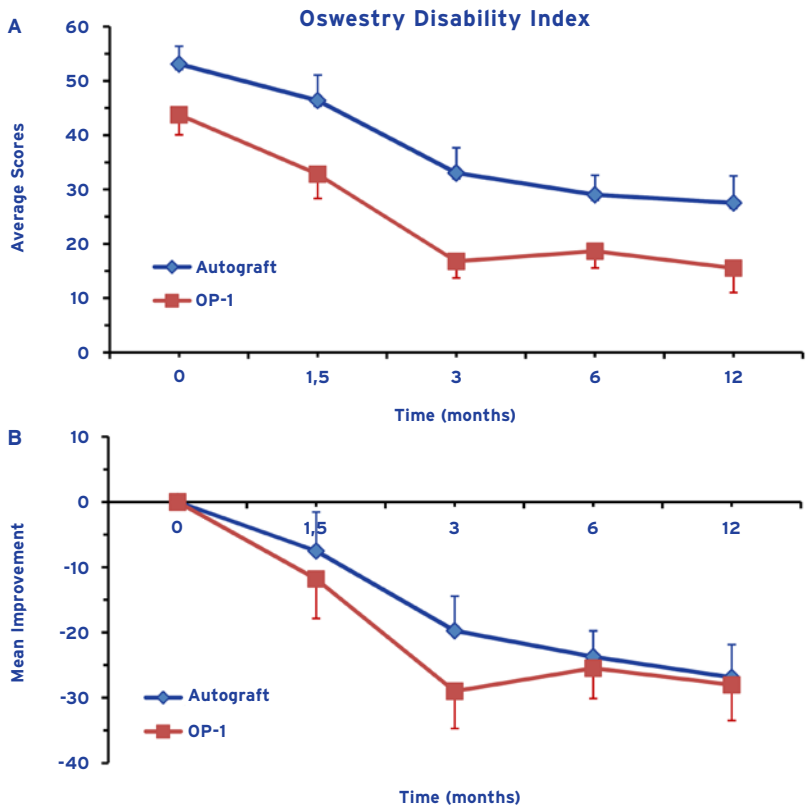


Figure 2 Coronal reconstruction of a CT-scan of a patient with OP-1 demonstrating a solid intertransverse bony fusion at one-year follow-up.

Clinical follow-up

Oswestry scores improved after the surgery as compared to the preoperative scores ($p > 0.001$) (Figure 3A). Because there was a slight, but not significant, difference in the pre-operative values between the two groups, the average improvement in the Oswestry Score was calculated at each time-interval (Figure 3B). There were no significant differences in the mean Oswestry scores between the study group and control group at any time point ($p = 0.56$).

Figure 3 The average Oswestry scores (A) and mean improvement (B) are shown per time-point. A lower score indicates less disability. Oswestry scores decreased compared to preoperative values ($p>0.001$). There was no significant difference in the improvement of the Oswestry scores after the surgery between the study group and control group ($p=0.52$). Error bars present the standard error of the mean.



Pain at the donor site of the bone graft

Pain at the donor site was only measured in the control group. In two of the 16 patients (13%), a separate incision was made to harvest the iliac crest bone graft. The classification of the donor site pain per time point is summarized in Table 5. One year after the surgery, 64% of the patients classified their donor site pain at least as mild. The average donor site pain at one-year follow-up was graded as 2.7 ± 2.8 using the VAS (Table 6). No complication directly related to the bone graft harvesting procedure occurred.

Adverse events and complications

Adverse events were experienced by 17 of the 34 patients (50%) (Table 7). This was not significantly different between treatment groups (p = 0.43). The adverse events were typical for the complications expected with for instrumented posterolateral fusion with decompression. In one patient in the OP-1 group, a primary brain tumor was diagnosed 11 months after the surgery. Histological examination revealed a grade IV glioblastoma. Because the patient refused any additional medical care and has voluntarily withdrawn from the study, the further progress of the disease is unknown.

Table 5 Qualification of the donor site pain in the autograft group.

	None (%)	Mild (%)	Moderate (%)	Severe (%)
6 weeks	23	46	31	0
3 Months	33	42	25	0
6 Months	21	21	50	7
12 Months	36	43	14	7

Table 6 VAS of donor site pain.

	VAS (mean ± SD)
6 weeks	3.0 ± 2.8
3 Months	1.7 ± 1.7
6 Months	3.8 ± 3.5
12 Months	2.7 ± 2.8

Table 7 Reported number of adverse events per treatment group. No statistical differences in complication rates (p = 0.48).

	Number of Subject with Complications	
	OP-1 Group (n=18)	Autograft Group (n=16)
Cardio/vascular	1	1
Respiratory	1	0
Malignancy	1	0
Dural Tear	1	1
Surgical Infections	1	1
Hematoma	2	0
Neural Injury	1	1
Instrumentation Failure	0	1
Herniation	1	0
Excessive leg pain	1	2
Total	10/18 (56%)	7/16 (44%)

Discussion

This is the first study comparing OP-1 with iliac crest autograft in one-level lumbar spine instrumented posterolateral fusion. The preliminary results indicate that OP-1 combined with local autograft is a safe and effective alternative for autologous bone graft from the iliac crest. Using strict criteria, fusion rates of 63% were found in the OP-1 group, which was not statistically different from iliac crest autograft. The main advantages of using of OP-1 instead of autologous bone graft are that it prevents the morbidity associated with the grafting procedure and that the fusion procedure is not limited by the quantity of autologous bone that is available from the iliac crest(s).

To date, few randomized prospective trials using OP-1 in spinal fusions have been reported that have generated encouraging data.[7-10] It remains, however, difficult to compare the studies due to differences in indication, use of spinal instrumentation, carriers, and outcome parameters.

In the present study we enrolled patients with degenerative as well as isthmic spondylolisthesis requiring one-level instrumented posterolateral fusion. The results can, therefore, be applied to a large patient group with low-grade spondylolisthesis requiring fusion surgery. It was possible to include both indications, because the primary outcome parameter of the study was radiographic fusion and not clinical outcome. Radiological fusion is the most appropriate assessment of the clinical efficacy of an osteoinductive factor,[13] since their main working mechanism is bone formation. This is particularly the case when the primary determinant of clinical outcome is not directly related to the success or failure of arthrodesis. In contrast to most studies, all included patients underwent fusion with the use of spinal instrumentation. Although the efficacy of spinal instrumentation remains controversial, several clinical studies documented the positive effects of the rigid environment for success of fusion.[14-17] Finally, the autologous bone obtained from the decompression was not discharged, but combined with the OP-1. We believe that combining local bone with OP-1 would be a realistic scenario for anyone using BMPs, because the availability of some local autograft is inherent to the surgical technique used for this indication. To date, no sound evidence is available proving that local bone alone is sufficient. One study retrospectively compared local bone to iliac crest bone.[18] The authors concluded that the use of local bone graft alone achieved a similar fusion rate in single-level fusion, but a much smaller fusion rate in multilevel fusion compared with the iliac crest autograft group. However, this concerned a retrospective study in which there was a substantial selection bias. Since the patients were not randomized, the surgeon decided during surgery which graft should be used. This was probably based on the amount and quality of local bone, the degree of slip, nicotine use, which definitely affect the success rate. Another limitation of this study was that the fusion rate was evaluated by conventional radiograms alone.

In this study, no product-related adverse events occurred. In one of the patients receiving OP-1, a glioblastoma was diagnosed 11 months after the surgery. To our knowledge, there are no previous reports relating application of BMPs to the occurrence of glioblas-

toma. The extremely limited and short-lived systemic bioavailability of OP-1,[19] together with the lack of association of BMPs with any type of tumorigenicity,[20] make it unlikely that OP-1 has been responsible for initiation or progression of the glioblastoma. On the contrary, a recent study has shown that BMPs trigger a significant reduction in the stem-like, tumour-initiating precursors of human glioblastomas.[21,22]

Although the results of this preliminary analysis are promising, there are several limitations to this study. Determining the fusion success without an open exploration remains a significant challenge. Even though computed tomographic scanning present the lowest percentage of inaccuracy,[23] more accurate noninvasive methods are desired. Despite the randomization, there was a significant difference in the distribution of the fused levels between both groups. In the OP-1 group the majority of the patient received a L5-S1 fusion, whereas in the control group L4-L5 was predominantly fused. There are, to the authors' knowledge, no published studies, which have evaluated the differences in fusion rates between level L4-L5 and L5-S1 in patients who underwent instrumented posterolateral fusion. Because our study involved only pedicle screw-based instrumented fusion, the influence of the biomechanical differences between the different levels are expected to be minimal. However, the distance between the transverse processes, and thereby the distance which needs to be bridged, might be less in the L5-S1 level when compared to the L4-L5 level. Although we do not expect this to be of major influence on the fusion rates, this difference should be taken into account.

Another limitation is that the study was not blinded to the surgeon or the patient because this was not possible due to the nature of the surgery. To prevent any potential bias in surgical technique between the treatment groups, the randomization was revealed at the end of the surgery, just before the graft was needed. Additionally, the lack of blinding was compensated by using blinded observers to assess the fusion outcome. Finally, the relative small sample size in this pilot study may have limited our ability to measure statistical differences between the two treatment groups.

In conclusion, the results of the present study demonstrate that OP-1 in a collagen carrier, combined with local autograft, is a safe and effective alternative for iliac crest autograft in instrumented posterolateral fusions. A radiological definitive fusion rate of 63% was observed using OP-1 and no product related adverse events occurred. One significant advantage of OP-1 is that is avoids morbidity associated with the harvesting of autogenous bone grafts from the pelvis. However, larger clinical trials are required to further define the efficacy of OP-1 as replacement of iliac crest autograft in instrumented posterolateral fusions.

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Chapter 8

Study of OP-1 in posterolateral fusion: a European multi-center trial

II. Pivotal study on safety and efficacy

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Introduction

Spinal fusion surgery is frequently performed for several indications [1]. The current practice is to use autologous bone from iliac crest to create a bony bridge between spinal segments. Harvesting of bone graft requires an additional surgical procedure, associated with donor site pain, infections, and neurovascular damage. [2–5]

Bone regeneration based on bioactive molecules is a potential alternative to autologous bone. The field was launched by Urist, who identified proteins from the bone matrix responsible for ectopic bone induction, which he called bone morphogenetic proteins (BMPs). [6] Currently, two BMPs are commercially available: BMP-2 (InFuse®, Medtronic Sofamor Danek, Memphis, TN, US), and BMP-7 (Osigraft®/OP-1 Putty®, Stryker Biotech, Hopkinton, MA, US), also known as Osteogenic Protein-1 (OP-1). BMP-2 has a FDA-approval for spinal indication, restricted to anterior lumbar interbody fusion with a specific cage,[7] and OP-1 has only a Humanitarian Device Exemption approval for revision of posterolateral lumbar fusion in compromised patients.[8] Despite the limited approval, BMP usage has been rapidly incorporated into the standard surgical practice with nearly 40% usage in lumbosacral spinal fusion surgeries in the United States. [9]

The scientific basis for the use of BMP-2 in spinal indications has recently been firmly criticized, due to methodological biases, and structural underreporting of adverse events in industry-sponsored publications. [10–14] For OP-1, also no clear evidence is currently available that has indisputably shown its efficacy in spinal applications.

In the present non-inferiority study, we evaluated OP-1 compared to autologous bone in single-level instrumented lumbar posterolateral fusion. In case of non-inferiority of OP-1, this could be considered a valuable alternative circumventing graft related morbidity.

Material and Methods

Study design

This study was an investigator-initiated trial with an unconditional grant from the manufacturer of OP-1. A randomized, non-inferiority trial in patients requiring single-level fusion of the lumbar spine, with nine participating centers in four European countries was conducted. We reported previously the pilot results on safety, and feasibility. [15] The study was performed according to the principles of the Declaration of Helsinki and Good Clinical Practice [16] with two treatment groups in a 1:1 ratio, receiving OP-1 combined with local bone (OP-1 group), or autologous bone graft from iliac crest combined with local bone (autograft group). Patients were examined preoperatively, and six weeks, three months, six months, and one year after surgery. The primary outcome was defined as a combination of clinical outcome and radiological fusion. The safety of OP-1 was evaluated by comparing the adverse events that occurred in both groups.

Patients with degenerative, or isthmic spondylolisthesis with symptoms of neurological compression caused by central, or foraminal stenosis qualifying for decompression, and instrumented fusion of a single-level between L3 and S1 were recruited in the clinic of the participating surgeons (inclusion and exclusion criteria: Table 1).

Randomization

A computer-generated randomization scheme, coding for either the OP-1, or the autograft treatment was produced according to the "random-permuted-block" by an independent researcher using SYSTAT for Windows (SYSTAT Inc., Evanston, Illinois, US). Each center received codes for an equal number of patients per treatment group. Randomization codes were stored in sealed opaque envelopes. The surgeons were blinded to the treatment group until decompression, and placement of the pedicle screws were completed.

Table 1 Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
· Degenerative or Isthmic Spondylolis thesis (Grade I and II) with central or foraminal stenosis	· Gross instability that requires multiple levels fusion
· Eligible for decompression and single-level fusion (L3-S1)	· Severe osteoporotic / osteopenic patients
· Symptoms of radiculopathy and/or neurogenic claudication	· Suspicion of active spinal or systemic infections
· Preoperative Oswestry Disability Index > 30	· Women who were pregnant or who planned to become pregnant
· Non-responsive to at least 6 months of non-operative treatment	· A known sensitivity to collagen
· No previous fusion attempt(s) to the affected level	· Morbidly obese patients
· Skeletally Mature	· Use of systemic corticosteroids during the previous year
· Informed Consent	· Known to require additional surgery to the lumbar spinal region within six months

Surgical technique

Surgical procedures were standardized during consensus meetings on the technique. Patients received prophylactic antibiotics. A posterior midline approach was used. Transverse processes, and facet joints were decorticated at the beginning of the procedure in order to obtain hemostasis before implantation. Decompression was achieved by (partial) laminectomy, and/or medial facetectomies. The same pedicle screw/rod instrumentation (Xia Spinal System; Stryker Spine, Allendale, NJ, US) was used for fixation.

In the Osigraft group, one unit Osigraft® containing 3.5mg lyophilized recombinant human OP-1 in a 1g of collagen type I carrier, was used for each side. Each unit was prepared in a separate bowl. Local bone from decompression was morselized, and divided over the two bowls. After adding 2.5ml of fresh unheparinized blood this was mixed with the Osigraft®. To improve handling, the mixture was allowed to clot for a minimum of 15 minutes before implantation. In the autograft group, bone was harvested from iliac crest, morselized, and mixed with local autograft.

After securing a dry fusion bed the graft was placed on the decorticated bony surfaces, and the wound was closed without a deep drain. No NSAIDs were prescribed for six weeks. Thrombosis prophylaxis was given according to the local protocols. A brace was used for eight weeks.

Radiographic measurements

CT-scans with multiplanar reconstructions obtained one year postoperatively were reviewed by a spine surgeon (F.C.O.), and a radiologist (H.Q.U.) blinded to the treatment and the institute. A third observer (N.V.), was used to adjudicate conflicting findings. In case all three observers classified the fusion differently, the case was classified as 'Doubtful Fusion'. A classification system based on the Christensen score, [17] was used:

'Fusion': a continuous bony bridge from the base of the pedicle and transverse processes from one vertebra to the other, at least on one side, in the absence of any secondary signs of nonunion, such as fracture or loosening of the screws or rods. If the fusion was doubtful in any way, the patient was not classified as fused;

'Doubtful fusion': suboptimal quality of bone bridging or some doubtful discontinuity including fusion mass possible hidden behind instrumentation, at a minimum of one side of the spine, in the absence of "fusion" on the other side;

'Nonunion': definite discontinuity or lack of fusion mass at both sides of the spine.

Clinical measurements

Clinical assessments were obtained preoperatively, and postoperatively at six weeks, and three, six, and twelve months using the Oswestry Disability Index, version 1.0 (ODI). [18] The patients were not blinded to the treatment group, due to the occasional extra wound of the iliac crest harvesting. Data on surgical time, hospital days and blood loss were collected.

Primary end point

The primary end point, overall success, was based on the 12-month clinical, and radiological results. Patients were classified as success in case of:

- radiographic fusion on the CT-scan;
- improvement in ODI of ³20% from baseline;
- no deterioration in neurological status;
- no second surgical intervention to promote fusion;
- no serious product-related adverse event.

Safety evaluation

The safety of OP-1 was evaluated by documenting details and severity of all adverse events. An adverse event included any untoward medical occurrence, regardless of the nature of the event or its severity, which does not necessarily have a causal relationship with the treatment. Additionally, each adverse event was evaluated for a relation with the OP-1 treatment. The occurrence of re-operations was also compared.

Statistical analysis

Baseline characteristics were assessed by comparing means or percentages. Differences in confounding baseline characteristics were addressed by the appropriate additional analyses.

The non-inferiority margin of 15% was determined by setting it against the advantage of avoiding potential complications related to bone harvesting which is reported to between 8% to 41%.[3–5] The null hypothesis was that the degree of inferiority of OP-1 to iliac crest autograft, based on overall success, was greater than the non-inferiority margin of 15% (H_0: {success autograft} − {success OP-1} ≥ 15%). Non-inferiority was tested against the upper limit of a two-sided 90% confidence interval, corresponding to a 5% test.

The sample size was determined on a success rate of 80% for the iliac crest autograft, based upon literature data.[19–22] To obtain a power of 80% with an alpha of 0.10, 65 patients were required per group using a 1:1 randomization ratio.

As re-operations were considered failures in the primary overall outcome measurement, intention-to-treat was similar to per-protocol analysis. Patients with missing data at one-year were excluded from the primary analysis.

The intention-to-treat principle was applied for secondary outcome measurements. Differences between OP-1 and autograft were assessed for all subcomponents of overall success using a two-sided Fisher exact test. For surgical time, hospital days, and blood loss an independent sample *t*-test was used. Repeated measurement analysis was used to assess any significant differences of Oswestry scores between both groups. In case of missing Oswestry scores, the last value was carried forward.

Data was collected by the University Medical Center Utrecht, and processed using FileMaker Pro version 7.0v1 (FileMaker Inc., Santa Clara, California, US), and analyzed with SPSS 17.0 (SPSS incl., Chicago, Illinois, US). Analysis of risk differences was performed with STATA IC 11 (StataCorp LP, College Station, Texas, US). All values are given as mean ± standard deviation.

Results

Between July 2004 and February 2008, a total of 134 patients were included (flow chart: Figure 1). Five patients were excluded due to protocol violations concerning a different surgical technique (3x two-level fusion, 1x additional anterior cage, 1x only local autograft). Another ten patients (five in each treatment group, all from one center) were later excluded due to poor quality of follow up CT-scans. Of the remaining 119 patients, neurological examination was missing for three patients, the preoperative questionnaire was missing for one patient, one patient was lost to follow-up, and one patient withdrew from the study due to a concurrent medical condition (brain tumor). In summary, the overall outcome was based on a total of 113 patients - 57 patients in the OP-1 and 56 patients in the autograft group respectively.

There were no major differences in baseline characteristics between the groups, except for the number of reported smokers, which was 48% in the OP-1 group and 31% in the autograft group (Table 2). There were no significant differences in blood loss, surgery time, and length of hospitalization between both groups (Table 3). The overall success was 40% for the OP-1 group and 54% for the iliac crest autograft group (risk difference -13.3%, 90% CI -28.6% to +2.10%). Non-inferiority was thus not achieved. The difference in the overall success was caused by the significantly lower radiological fusion rate in the OP-1 group: 54% *versus* 74% (95% CI -40.1% to -2.98%, p = 0.03).

Due to differences in smoking between the groups, a multiple logistic regression analysis was performed. Overall outcome was not affected by smoking (p = 0.52), or treatment group (p = 0.15). The radiological fusion was also not affected by smoking (p = 0.90), but was significantly influenced by the treatment group (p = 0.02), confirming the results of the primary analysis.

Figure 1 Flow Diagram for Overall Success

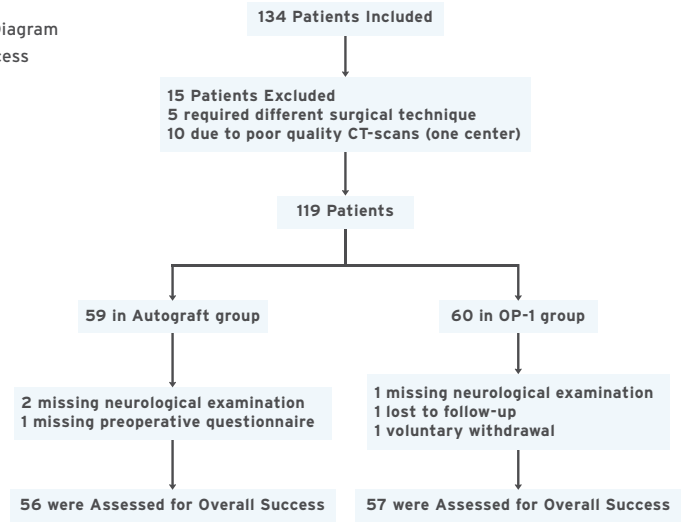

Table 2 Patients' demographic and clinical details. BMI = Body Mass Index.

	OP-1 Group (n=60)	Autograft Group (n=59)
Age (y)	54 ± 14	55 ± 13
BMI (kg/m^2)	26.6 ± 4	25.2 ± 5
Gender		
Male	27 (45%)	25 (42%)
Female	33 (55%)	34 (58%)
Smokers	29 (48%)	18 (31%)
Origin of Instability		
Degenerative Spondylolisthesis	31 (52%)	33 (56%)
Isthmic Spondylolisthesis	29 (48%)	26 (44%)
Level Fused		
L3-L4	9 (15%)	6 (10%)
L4-L5	24 (40%)	34 (58%)
L5-L6	0 (0%)	1 (2%)
L5-S1	27 (45%)	18 (31%)
Preoperative ODI	44 ± 16	44 ± 14

Table 3 Results. Plus-minus values are means ± SD; § An independent sample t-test were used to compare surgical data between the groups; ¶ Non-inferiority of OP-1 was tested with a 15% inferiority margin; ¥ The results of the individual components of overall success were summarized with the exception of the product related-adverse events, since this did not occur; * The ODI (Oswestry Disability Index) is a validated, and standardized instrument for outcomes in spinal pathology. The questionnaire is scored from 0% (no disability) to 100% (total disability). ∫ All components of overall success were compared using a two-sided Fisher exact test. † Repeated measurement analysis was used to assess any significant differences of ODI between both groups.

	OP-1 Group	Autograft Group	
Surgical Data			**p-value§**
Surgery Time (minutes)	156 ± 60	159 ± 61	0.78
Blood Loss (cc)	422 ± 280	428 ± 293	0.91
Hospitalization (days)	9.1 ± 5.4	8.1 ± 4.7	0.30
Overall Success	40%	54%	**risk difference¶ (CI 90%)** -13.3% (-28.6 to +2.10)
Components Overall Success¥			**p-value∫**
Fusion	54%	74%	0.03
ODI* Improvement	84%	86%	1.0
No Revision Surgery	93%	97%	0.44
No Neurological Deterioration	89%	89%	1.0
ODI* Scores			**p-value† 0.90**
Preoperative	44 ± 16	44 ± 14	
6 weeks	34 ± 19	35 ± 19	
3 months	21 ± 17	23 ± 17	
6 months	20 ± 17	21 ± 15	
1 year	18 ± 20	19 ± 18	

The classification of the fusion in the OP-1 group was: definitely fused 54%, doubtful 28%, and non-union in 18%; compared to respectively 74%, 10%, and 16%, in the autograft group. There were no significant differences (p = 0.90) in the mean Oswestry scores (Table 3).

Adverse events were reported in 40% of the patients in the OP-1 group, and in 52% in the autograft group (Table 4). In one patient in the OP-1 group, a primary brain tumor was diagnosed 11 months after the surgery, which was considered unrelated to the treatment. [15] In ten patients in the OP-1 group, and in two patients in the autograft group, re-operations were performed. The indications for reoperation in the OP-1 group were infection (4x), instrumentation failure (2x), neurological disturbances (3x), and instrumentation removal due to residual back pain (1x). The probable causes of the neurological disturbances were extra-pedicular screw placement and a hematoma, no cause was found in one patient. The indications for re-operation in the control group were infection, and instrumentation failure. None of the adverse events could be directly related to the use of OP-1. No complications occurred in conjunction with iliac crest autograft.

Table 4 Reported number of adverse events per treatment group. IVD = intervertebral disc. Miscellaneous consists of: tenosynovitis of the finger (OP-1); neck pain due to a degenerative condition (OP-1); knee pain for which an arthroscopy was performed (OP-1); anterior interosseous nerve syndrome (autograft); high tibial osteotomy for gonarthrosis (autograft); balance disturbances (autograft).

	Number of Subject with Complications	
	OP-1 Group (n=57)	Autograft Group (n=56)
Cardio/vascular	1	5
Respiratory	1	0
Gastro-intestinal	0	0
Urinary tract	0	2
Malignancy	1	0
Dural Tear	3	4
Surgical Infections Superficial Deep	 2 3	 2 2
Wound Dehiscence	0	1
Hematoma	1	1
Neurological Disturbances	3	3
Instrumentation Failure	2	3
Herniation of IVD	0	1
Excessive Back/Leg Pain	3	2
Miscellaneous	3	3

Discussion

In this first large multicenter, randomized, non-inferiority study comparing OP-1 to iliac crest autograft in single-level instrumented posterolateral spinal fusion, we found significantly lower fusion rates with OP-1 (54% *versus* 74%, p = 0.03). As a result of this lower radiologic fusion rate, non-inferiority was not achieved, despite similar clinical outcome.

Previous studies have also shown the lack of a clear relation between bony fusion, and clinical results. On the other hand clinical outcome may deteriorate within several years if intended bony fusion is not achieved. [23] Therefore, our follow-up period of one year may have been too short to show differences in clinical results. Since the purpose of the OP-1 was to create bony fusion, we believe the ability to do so, at least as good as autograft, is a minimal requirement.

Previously reported disadvantages of BMPs in spinal indications, such as ectopic bone formation, retrograde ejaculation, bone resorption, or soft tissue swelling [9] were not seen in the current study. However there were more re-operations in the OP-1 group (ten *versus* two patients). Although this could not be directly related to specific problems associated with BMP's this should be noted in the light of the recent discussions on the higher complication, and re-operation rates with the use of BMP-2. [11,12]

Clinical outcome was similar in both groups, despite avoidance of iliac crest bone harvesting in the OP-1 group. Since the reported incidence of donor site pain ranges from 8% to 41%, [3–5] some effect on clinical outcome, at least on the short-term, could have been expected. Previous trials on BMPs also failed to show a positive effect of avoidance taken iliac crest bone. [24] A possible explanation may be overestimation of donor site pain, due the inability of patients to differentiate between residual low back pain, and donor site morbidity. [3]

Three randomized prospective studies, besides the reported pilot phase of the current study, previously reported on OP-1 in posterolateral lumbar fusion. Johnsson *et al* [25] and by Kanayama *et al* [26] showed no significant differences, but lower fusion rates for OP-1. Although interesting on safety and feasibility, no conclusion can be made on the effectiveness of OP-1 due the limited number of patients (n ≤ 10). The only other large study, that evaluated OP-1 for noninstrumented fusion concluded that OP-1 Putty was a safe and effective alternative to autograft. [27] However, they also failed to show non-inferiority of OP-1 based on an overall success score that included plain radiographs, and as in our study, the low overall success was caused by a significantly lower fusion rate. When subsequently additional CT-scans were obtained at >3 years, these again showed a lower fusion rate in the OP-1 group (53% *versus* 83%, p = 0.001), but with a modified definition of the radiological success (presence of new bone on CT-scans instead of bridging bone, in combination with limited motion on dynamic radiograms), non-inferiority was reached, which lead to aforementioned conclusion.

Our study is not completely comparable with the previous studies. We used pedicle screw instrumentation to facilitate fusion. The use of instrumentation is generally believed to increase stability, and to enhance the bony fusion. [28,29] It is unlikely that instrumentation would disturb the working mechanism of BMPs. Another difference is the

composition of OP-1. Osigraft®, which we used, is available in Europe, and OP-1 Putty®, which is used in the United States. The active ingredient is the same amount of OP-1 in the same collagen carrier. The only difference is the carboxymethylcellulose additive in the OP-1 Putty® improving the handling characteristics. In the present study, we added fresh blood to the OP-1, which was allowed to clot to give it a more putty like structure. We also mixed the OP-1 with local decompression bone as this is a realistic scenario for BMP application where availability of some local autograft is inherent to the surgical technique. Although local autograft alone can achieve spinal fusion the efficacy compared to iliac crest autograft remains questionable in the absence of prospective studies.[30,31] Finally, patients with degenerative as well as isthmic spondylolisthesis were enrolled in the present study. This was done to make the conclusion relevant to a larger patient group with one-level low-grade spondylolisthesis requiring fusion surgery. Since instrumentation was used, no significant effect of biomechanical differences, influencing the fusion rates, are expected. For clinical outcome the etiology of the instability might be of relevance but not for fusion rates. In addition, there was no difference in the distribution between the OP-1 and control group.

There are several limitations to our study. The number of smokers was higher in the OP-1 group despite proper randomization (31% *versus* 48%). Smoking was not an exclusion criterion in order to represent the general patient population. Furthermore, OP-1 is specifically promoted for use in compromised patients including smoking, as specified in the Humanitarian Device Exception.[8] Moreover, several animal studies indicated that the inhibitory effect of smoking is overcome by using OP-1 instead of autograft.[32,33] In our study no effect was found of smoking on overall outcome or radiological fusion. Another limitation was that the patients were not blinded because of the occasional extra wound for iliac crest harvest. However, the surgeon was blinded until the graft type was disclosed as well as the observers that assessed the radiological data. CT-scans, as used in this study, present the lowest percentage of inaccuracy.[34] Finally, there were missing data, largely the result of inadequate CT quality in one center, resulting in a lower number of patients then required according the power calculation. Although this could have influenced the overall outcome assessment, this did not preclude the conclusion of a significantly lower fusion rate in the OP-1 group.

BMP use has multiplied several folds during the last decade to over a 100.000 cases annually in the USA.[35] Recent publications have expressed concerns regarding the structural underreporting of serious product related adverse events and significant design biases of industry-sponsored BMP-2 trials.[10–12,14,36] Whether there are differences in the effectiveness, and complication rates of different BMP's in clinical applications is not known. Due to the potential complications, and the lack of evidence of efficacy, caution is warranted. Adequate clinical trials need to clearly define the safety profiles and efficacy for each kind of BMP product, the dosage used, and specific carrier, before these compounds can be applied in spinal applications. Based on the results of the current study OP-1 cannot be recommended in instrumented posterolateral lumbar fusion procedures in place of autologous iliac crest bone.

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Chapter 9

Consequences of the Clinical Trials Directive form an academic perspective

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Introduction

In 2001 the European Union adopted the EU Clinical Trials Directive (2001/20/EC) as a framework for good management in trials of medicines.[1] Many concerns were expressed that this Directive would impede and inhibit publicly funded clinical trials.[2-4] The question is if the concerns regarding the Clinical Trials Directive were legitimate. In other words, has the Directive really made it practically impossible for a group of surgeons to initiate, organize, and conduct a European multi-center study? But also, has the new Directive achieved the goal of simplifying and harmonizing the administrative provisions governing clinical trials in EU countries?

As a University Medical Center, we have experienced the practical consequences of the Clinical Trials Directive at first hand during a European multi-center study on instrumented lumbar spinal fusions with the use of Osteogenic Protein-1 (OP-1). The objective of this paper is to provide insight in the difficulties involved while conducting such a European multi-center study under the new EU Clinical Trials Directive.

Background

The reason for implementation of the Clinical Trial Directive was that the rules and requirements concerning clinical trials diverged considerably in the Member States, resulting in delays and complications detrimental to the effective conduct of European trials in the Community. It was, therefore, necessary to simplify and harmonize the administrative provisions governing such trials by establishing a clear, transparent procedure, and creating conditions conducive to effective coordination of such trials.

Most objections to this directive were based on the conception that the Directive was conceived as a way of facilitating commercial drug development and that publicly funded trials were forced to fulfill the same requirements as their commercial counterparts.[3] The new requirements would impose a much greater administrative burden to independent and academic clinical research. Several articles addressed the essential role of independent research and expressed concerns about the increasing influence of industrial funding.[5-7] The research agenda can in this way be dictated by commercial profit only and thereby neglect research considered economically uninteresting.[7-9] These are real concerns as the objectivity of industry-driven trials have been frequently disputed.[6,10-12] Out of concern for the future of academic research after the implementation of the new Directive, the 'Save European Research' campaign was started, calling on the European Commission to repeal its Directive.[13] Despite the lobbying towards the policymakers to appreciate the role of non-commercial research, the European Union decided that the Directive must be incorporated into the national legislation in each Member State before May 2004.

Since our institution was responsible for the initiation and management of this aforementioned trial, we were, according to the definition of the Directive, considered 'the sponsor' of the trial. In this role we were one of the first academic centers to be faced with direct

consequences of the new Directive in the perspective of a European multi-center clinical trial. The participants of this study initially consisted of spinal surgeons of 12 hospitals located in Germany, The Netherlands, Spain, France, United Kingdom and Italy. During the course of this study we ran into a great deal of anticipated as well as unanticipated difficulties.

Encountered problems

Although the EU Member States were required to have implemented the Directive by May 2004, not all Member States were able to transpose the Directive in their national legislation by this date. As a consequence, we were obliged to fulfill the new guidelines of the Directive for some of participating centers, while for other centers their original national requirements were still applicable. Due to this dichotomy in requirements, the responsibilities and obligations of our hospital, as the initiator and coordinator of the study, were not completely clear, not even to the Ethic Committees or Competent Authorities involved.

The Directive and implementing guidelines imposed many administrative requirements that did not exist, or were not similarly developed in the Member States.[14] This included an obligatory new Database (EUDRACT), an authorization request to the competent authorities, as well as an Investigational Medicinal Product Dossier; annual reports for clinical trials; adverse reaction reports and final study reports. The Clinical Trials Directive also introduced Good Clinical Practice (GCP) principles to ensure that trials are conducted in accordance with high standards of ethics and science. Although the practice of GCP were standard in all Member States, only a minority of Member States had previously codified the obligations of the different parties and the involvement of the competent authorities as now imposed by the Directive. GCP is a standard for the design, conduct, monitoring, analyses, and reporting of clinical trials that provides assurance that the data and reported results are credible and accurate and that the rights of trial subjects are protected. The requirements of GCP are noted in a 53 pages containing manual describing practices, responsibilities and actions of all members involved. One of the requirements of GCP is that the sponsor establishes an independent data monitoring committee. The monitoring committee needs to assess at frequent intervals the progress of a clinical trial, the safety of data, and the critical efficacy endpoints. During these visits the reported trial data should be verifiable to the monitor from source documents, i.e. patient files, surgery reports, charting notes, nursing notes. Each participating site needs to be visited numerous times, making this a large, and time-consuming administrative activity, not only for the monitor, but also for the physicians involved. Additionally, monitoring is associated with high costs, especially when multiple international sites are included in the clinical trial.

A major difficulty of conducting a European multi-center study remains, despite the EU Clinical Trials Directive, that each country, and sometimes even different hospitals within the same country, requires separate approval of the study protocol by the local authorities. This implies that submission to the Ethics Committees and Competent Authorities needs to be repeated in each EU country for the same study. We have applied for approval to

conduct our study to the involved authorities 14 times. On top of that, the procedures to obtain this approval also differ greatly between different EU countries. The linguistic problems in the communication with the local authorities of some of the countries, demanding correspondence in their own local language, hampered communication considerably. For instance, we were repeatedly asked to sign official documents and declarations, sometimes even with an obvious legal status, in the local language. Surprisingly, in most cases an English translation was not accepted, since in certain countries official documents will only be issued in their local language. Obviously, official documents need to be in a mutually understandable language and the sponsor cannot sign a contract in a language he does not understand. Next to these linguistic problems, most official documents still refer to several national laws of which we did not know the exact content.

What practical consequences did the new Directive have for our study? Two hospitals were excluded due to the delays and difficulties caused by the new requirements. The completion of the study was delayed for at least one year. The delays were for a large extent due to the new administrative procedures of the Directive. Also, additional funding needed to be obtained to cover the extra costs of the monitoring requirements. Last, but certainly not least, the substantially increased administrative burden has undoubtedly reduced the enthusiasm and commitment of the physicians involved. In some cases even to the extent that they declared not to participate in these kind of studies again in the future.

The need for centralization

Were the concerns regarding the new EU Clinical Trials Directive legitimate? The answer is clearly: yes. The Directive has created many additional burdens for the conduction of academic trials independent of medical industry, while it did not meet the primary objective of harmonizing and simplifying the legislation in the Member States. It is now almost impossible for a group of physicians to conduct a European multi-center study independent of the industrial organizations and infrastructures. Clearly, this dependence is not desirable and it should be possible for surgeons and physicians to conduct studies independently.

The goal of the Directive to harmonize the legislation concerning clinical trials throughout the EU, was desirable, not only to strengthen Europe's economy by creating an internal common market, but also for European non-commercial research to be globally competitive. This legislation should guarantee the rights, safety and well-being of trial subjects, and insure that the results of the clinical trials are credible and reliable. It is obvious that the ethical and scientific quality should be verifiable to judging authorities and that this demands provision of sufficient information to these authorities. The information being requested, however, should bear relation with its purpose. Under the new Directive, application forms have grown to the size of books, carrying a message of general distrust of physicians, and it overlooks the fact that it is also in the best interests of the physicians to comply with demanded quality standards. The currently increased administrative requirements are inversely correlated to the physicians' enthusiasm to conduct independent clini-

cal trials. To avoid that the research agenda in the future will be dictated by the medical industry and to preserve the unique role of independent research, the load of the paperwork to physicians, while conducting such a study, should diminish significantly.

Despite the new Directive, separate approval from each national authority is still required before a clinical trial can start. This, in combination with a lack of uniformity in the procedures and communication in a mutually understandable langue, make conduction of clinical trials a needlessly laborious and frustrating experience. It is a missed opportunity of the European Commission that they did not promote centralization of approval procedures, meaning that one central European authority to give approval for all Member States involved. This should not only be a central Ethics Committee that approves the trial for all European countries, but a central organ that also provides the authorization for all Competent Authorities involved. This would have significantly contributed in simplifying and harmonizing the legislation regarding clinical trials.

We believe that the professional organizations of the European physicians and surgeons should make efforts to facilitate non-commercial studies and put pressure on political authorities to reduce the administrative requirements concerning such trials. One important aspect in achieving this is the implementation of central authority for application of trials that applies in all Member States.

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Chapter 10

Summary of the thesis

This thesis focused on alternatives for iliac crest autograft for spinal indications. One of the major disadvantages of iliac crest autograft is chronic donor site pain. The incidence ranges in literature from 6% to 39%.[1–8] We started with evaluating the incidence of donor site pain after posterior iliac crest bone harvesting. It should be realized that the incidence of donor site pain after bone graft harvesting is mainly reported from studies in patients who underwent low lumbar or lumbosacral surgery. We postulated that the close proximity of the primary surgery site to the iliac crest could interfere with the reported high incidence of donor site pain, thus leading to an overestimation. To evaluate this hypothesis, we compared in **Chapter 2** the donor site pain in spinal trauma patients, who underwent fusion of higher levels (> L3) with lower levels (≤ L3). We found significantly higher chronic donor site pain in the group with lower fusions compared to cephaled level of fusions (14.3% *versus* 40.9%), suggesting that a substantial percentage of the presumed donor site pain may be attributed to the lumbar surgery itself. Additionally, the majority (71%) of these patients graded the pain as mild. Although iliac crest pain does not seem to be a major problem, the quest for alternatives for iliac crest autograft remains, due to unacceptable high nonunion rates and the often insufficient amount that is available for grafting.

Next, we evaluated the most prominent feature of the autograft compared to the currently available alternatives, namely the presence of viable osteogenic cells. Little is known about the mechanism behind the clinical success of autograft. The presence of viable osteogenic cells is of dubious importance. Knowledge of the relevance of these cells has become crucial due to the emergence of cell-based bone tissue engineering. In **Chapter 3**, we investigated the effect of bone graft viability in a transverse process animal model that represents the initial bone formation in posterolateral fusion. Goats received viable and devitalized autologous bone grafts in chambers mounted on the decorticated lumbar transverse process. Histology and histomorphometry were performed after a 12-week implantation and sequential fluorochrome labeling monitored the dynamics of bone formation. An obvious qualitative effect of viability was demonstrated by the presence of early onset osteogenesis distant from the transverse process bone in the viable grafts only. Quantitative analysis indicated about 30% more bone in the viable grafts; however, this difference was not statistically significant. The potential advantage of bone graft viability appears to be predominately to the well-nourished peripheral implant areas, such as the submuscular region, making it likely to be relevant in posterolateral fusion, where nonunions often occur away from the transverse processes.

The aforementioned transverse process model was also used in **Chapter 4** to evaluate the osteopromotive effect of adding platelet gel to bone grafts. It is believed that the platelet-derived growth factors in platelet gel might lead to an improved and faster bone growth. To evaluate this effect, we implanted two 3-compartment cages onto the spinal transverse processes of goats containing iliac crest autograft, biphasic calcium phosphate, and trabecular metal. One cage was treated with platelet gel and the other cage was left untreated. After 9 weeks the amount of new bone of each graft was com-

pared between the platelet gel treated cages and untreated cages. Significant more bone growth was found in the platelet gel treated autograft and biphasic calcium phosphate samples. Fairly little bone growth was seen in treated or untreated trabecular metal scaffolds. The results of our study suggest a potential role for the application of platelet gel during surgeries in which autologous bone grafts or calcium phosphate scaffolds are used. Our model obviously serves only as a screening tool and the next step should be evaluating its effect in a more clinically relevant indication in a large animal model.

In **Chapter 5**, we determined whether a new putatively bioactive tricalcium phosphate (TCP) was a suitable bone graft substitute for spinal fusions in a large animal model. We compared this TCP to the currently most used grafts: iliac crest autograft and allograft. A total of nine goats underwent a two-level instrumented posterolateral lumbar fusion. Each side of the spine segment was randomized into one type of graft: iliac crest autograft; fresh-frozen allograft; TCP alone; or TCP combined with local autograft (1:1). The results demonstrate that TCP was capable of achieving fusion at a similar rate to autograft and allograft on computed tomography (CT) scans, while almost completely resorbing within 16 weeks. Calculation of the volume of the newly formed bone on CT images, however, showed significantly greater volume in the control groups. Despite the lower fusion mass volumes, the investigated new TCP is a promising alternative for autograft, even in challenging indication such as posterolateral fusion. Clinical trials need to determine its applicability as a stand-alone alternative.

In the second part of this thesis, we focused on bone morphogenetic proteins (BMPs) of which the usage in spinal fusions had rapidly emerged during the preceding decade.[9] The implementation of BMPs in the clinic could be achieved by combining the advances made by several distinct disciplines during the last century. First, in **Chapter 6**, we describe how the sequential converging of the technologies of biochemistry, biomaterial science, imaging and molecular biology has finally resulted in the development of a regenerative treatment in orthopedics.

Despite the extensive usage of BMPs, little scientific basis exists for most indications. We conducted a European multi-center trial, comparing OP-1 (BMP-7) to autologous bone in single-level instrumented posterolateral fusion, which is one of the most commonly used surgical techniques, in patients with symptomatic spinal stenosis and low-grade spondylolisthesis. The purpose of this study was to investigate for non-inferiority of OP-1 in terms of overall success, based on a combination of radiological fusion and clinical improvement. In **Chapter 7**, we reported, as analyzed for our interim analysis, on the results of the pilot phase consisting the safety and feasibility of the technique in the first 36 patients. Since we did not find large differences in clinical outcome or radiological fusion between the two groups, and could not identify product related adverse events, we continued enrolling the required total number of 134 patients. In **Chapter 8**, the result of the total study show that non-inferiority, based on overall success at one-year follow-up, was, however, not achieved for OP-1 compared

to iliac crest autograft (40% for OP-1 *versus* 54% for autograft). This was due to the significantly lower radiological fusion rate of OP-1 cases on CT scans (54% for OP-1 *versus* 74% for autograft). The clinical outcome, as measure by the Oswestry Disability Index, was similar between both groups. There were more re-operations in the OP-1 group in our study (ten *versus* two patients), but we could not relate these directly to OP-1 usage. Since the purpose of the OP-1 was to create a bony bridge, we believe the ability to do so, at least as good as autograft, is a minimal requirement. Therefore, OP-1 cannot be recommended in instrumented posterolateral lumbar fusion procedures as alternative to autologous iliac crest bone.

In **Chapter 9**, we discuss the drawbacks of the new legislation involving European multi-centers trials form an academic perspective. The requirements imposed many administrative requirements that did not exist for independent and academic clinical trials, making it needlessly difficult to perform investigator-driven, independent trials. To avoid that the research agenda in the future will be dictated by the medical industry and to preserve the unique role of independent research, it is necessary to simplify and harmonize the legislation regarding clinical trials in Europe. One important aspect in achieving this is the implementation of central authority for application of trials, which applies in all Member States.

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Chapter 11

General discussion and future perspectives

The aim of this thesis was to explore the potential of several promising bone graft substitutes using principles of the emerging regenerative medicine. Regenerative therapies have the potential to regenerate, and not only to repair or replace, specific tissues like bone. It was anticipated that by the beginning of this millennium we would have entered a new age in orthopedic treatment: The Age of Regeneration.[1] Despite the initial achievements and the great efforts of many scientists and clinicians, the clinical implementation of regenerative therapies is unfortunately still limited. Below, the current status and potential of the most promising bone grafts substitutes are discussed.

Cell-based bone graft substitutes

The therapy that was most appealing to the imagination was the application of progenitor cells for new tissue formation. A lot of research efforts focused on these cell-based therapies for bone regeneration. Many preclinical trials produced encouraging data using mesenchymal stem cells (MSCs) combined with different scaffolds including ceramics, allograft with or without additional BMPs.[2] Due to the emergence of cell-based tissue engineering, the knowledge of the exact role of the cells in bone grafts has become crucial. In this thesis, we demonstrated an obvious qualitative effect of cell viability in autologous bone grafts in terms of early onset osteogenesis distant from the host bone. This finding may be of importance in clinical settings, such as posterolateral fusions, since nonunions often occur at a certain distance from the transverse process. On the other hand, the total volume of bone in the viable bone grafts was not that much different and the qualitative difference may very well be clinically irrelevant. Several clinical studies investigated the use of cell-based bone graft alternatives and showed similar fusion rates when combining ceramic scaffolds seeded with cells to autologous bone.[3–5] Unfortunately though, none of these studies included a cell-free ceramic as a control group, making it unclear if the cells had any additive effect at all. In general, the basic and preclinical research literature clearly indicates that the use of MSCs for the reconstruction and repair of bone is feasible, but this has not been translated into any practical and convincing use in clinical trials yet.

The cell-based therapies are now hampered due to the cost and complexity of cell-based tissue engineering. The fundamental question is whether cellularity of bone grafts is an important feature for its clinical efficacy. It seems that most cells do not survive transplantation in clinically relevant sized grafts, [6,7] since they are deprived of vascularization for weeks.[8] Bone regeneration obviously depends entirely on the presence of sufficient numbers of osteoprogenitor cells. It remains, however, the question if these cells need to be directly provided by the graft, or that they can be better recruited from the surrounding tissues. The search for alternative therapies that recruits its osteogenic cells from the environment appears to be a more promising route, at least for the near future.

Ceramic scaffolds for posterolateral fusions

Ceramics are attractive as bone graft substitutes due to unlimited supply, low costs, and ease of sterilization and storage. Many varieties of ceramics are available with different composition, porosity, and surface structure, corresponding with distinctive mechanical properties, degradation times, and bioactivity. The most commonly used ceramic scaffolds for spinal fusion are calcium phosphates such as hydroxyapatite, tricalcium phosphate, and combinations of these. Of special interest are the tricalcium phosphate (TCP) ceramics due to their good bioresorbability that allows complete remodeling, in contrast to the non-resorbable hydroxyapatite. Ceramics in spinal fusion are currently used as bone graft extenders rather than bone graft substitutes as these types of materials are generally considered to be only a template for osteoconduction. Recently, however, the possibilities to improve the bone-forming capacities of ceramics have been recognized and are increasingly investigated.

A successful strategy appears the addition of trace elements, like silicon.[9,10] In a study that evaluated silicon-substituted calcium phosphates, the investigators found promising results in an instrumented sheep posterolateral fusion model.[11] However, it is not precisely clear whether the trace element itself or the resulting microstructure is responsible for the apparent improved performance.[12,13] Another promising approach was by changing the basic physicochemical parameters of the ceramic, such as surface textures, in a way that it could be endowed with biologically instructive properties.[14] This enhanced TCP was capable of possessing an intrinsic osteoinductive capacity comparable to the addition of BMPs.[14] In a comparative study, osteoinductivity of calcium phosphate ceramics appeared to be advantageous for bone defect healing when compared to non-osteoinductive phosphate ceramics.[15]

In this thesis, we evaluated a first generation of this microporous ceramic material in instrumented posterolateral fusions in a large animal model. The results showed that this TCP alone was capable of achieving fusion similar to iliac crest autograft while it was almost completely resorbed within 4 months. Despite lower volumes of newly formed bone, this TCP showed to be a promising alternative of autograft for posterolateral fusion. A huge advantage of this TCP and other ceramics is the synthetic production. This potentially allows many improvements of the material. Besides, little is known yet about optimal graft structure, volume and resorption characteristics. Together with ongoing research to improve the material, clinical trials will be needed to determine its applicability as a stand-alone alternative to iliac crest autograft.

The rise and fall of the current BMP products

The flagship of clinical regenerative therapy for bone healing has been without doubt the large-scale application of bone morphogenetic proteins (BMPs). They were discovered in the 1960s when proteins extracted from the bone matrix were shown to be capable of inducing bone formation when implanted at an ectopic site.[16] It then took several

decades before these BMPs could be identified, isolated and produced in sufficiently large quantities, eventually leading to the first clinical trial of purified human BMP in the early 1990s.[17] There are currently two recombinant human BMPs commercially available: BMP-2 and BMP-7, which is also known as osteogenic protein-1 (OP-1). For spinal indications BMP-2 on a collagen sponge has received FDA premarket approval for use in anterior lumbar interbody fusion, but only when used inside a specific cage.[18] A limited approval under the humanitarian device exemption (HDE) for BMP-2 combined with ceramic granules,[19] and OP-1,[20] was obtained for revisions of posterolateral fusions in cases where autologous bone graft harvesting was not feasible. Notably, this HDE is intended only for a small subset of patients (less than 4000) for which the manufacturer's research costs make FDA approval financially unattractive. Therefore, it does not require any clinical investigations demonstrating its effectiveness. Despite these limited approvals, the application of BMPs increased exponentially to more than 25% usage in primary spinal fusion surgeries in the United States of which the vast majority (85%) of cases represent off-label usage.[21,22]

Complications associated with BMP-2

The most commonly used BMP in the United States is BMP-2, accounting for approximately 90% of the cases. The enthusiasm in which supraphysiological dosages of this highly bioactive growth factor was applied around vulnerable anatomical structures, was somewhat hampered by increasing reports of complications, such as neck swelling, dysphagia and respiratory problems with its use in anterior cervical spinal fusion surgery.[23–26] These reports increased awareness on the potential dangers of using BMPs in the cervical spine, but remarkably did not significantly temper the usage in other off-label indications. One of the reasons for continuing its large-scale application was the favorable result on efficacy and safety of heavily industry-sponsored studies that were reported for off-label applications.[27] These publications were on posterior lumbar interbody fusions (PLIFs),[28] anterior cervical discectomy and fusions (ACDFs),[29] and posterolateral fusions.[30] All these reports concluded that BMP-2 was at least comparable, but most often superior, to iliac crest autograft in clinical as well as radiological outcomes. Surprisingly, there was not any product related adverse event reported. For posterolateral fusion, the manufacturer decided to increase the concentration of BMP-2 and to triple the dose based on preclinical studies.[31,32] This resulted in the product AMPLIFY™ which contained a whopping 2.0 mg/mL for a total dose of 40 mg compared to the already commercially available product InFuse®; which contained 'only' 1.5 mg/mL for a total dose of 12 mg. In addition, also a ceramic to resist the compressive forces was added to prevent the protein to be squeezed out of the collagen sponge due to soft tissue compression.[31,32] Interestingly, the same authors advocating the need for tripling the doses of BMP-2 for posterolateral fusions,[30] almost simultaneously, conducted clinical trials with the commercially available lower dose BMP-2 products.[33]

With the ever increasing application of BMP-2, the reports on potentially product-

related adverse events for indication also outside the cervical spine emerged during the second half of the 2000s.[25,34–46] The resulting anxieties culminated into a special issue on this subject of *The Spine Journal* in June 2011. One of the main criticisms expressed in various articles published in this issue was that adverse events were underreported in the industry-sponsored BMP-2 trials. This statement was based on a study that compared the complications reported in the publications of BMP-2 industry-sponsored trials with subsequently available Food and Drug Administration (FDA) data summaries, follow-up publications, and administrative and organizational databases.[47] This study revealed many originally unpublished adverse events with potential relationship with BMP-2 usage, including radiculitis, osteolysis, retrograde ejaculation, ectopic bone formation and cage subsidence. The authors stated that the complication rate of BMP fusion procedures was estimated at 5% to 15% for anterior procedures and 25% to 50% for posterior procedures, rather than the 0% rate reported in the original publications. Arguably, the most startling finding that was not addressed as potential complications were 9 cancer events in 9 patients (3.8%) receiving 40 mg of BMP-2 for posterolateral fusion, as opposed to 2 cancer events in 2 patients (0.9%) in the control group receiving autograft.[48] Although cancer could not be directly related to the BMP therapies yet, statistical analysis including additional FDA data with a longer follow-up period, revealed a 94% probability that this was product-related. [48]

After this intense discussion concerning the validity of the BMP-2 trials, the manufacturer announced that it would make all patient data, published and unpublished, available to Yale University for an independent review. This group was chosen based on their experience with the independent analysis of VIOXX (Rofecoxib), a nonsteroidal anti-inflammatory drug, after suspicions of cardiovascular risks associated with its use. The review process includes independent examinations of all relevant product data by two separate qualified research groups and making all patient-level clinical research data available for analysis by other external investigators. The results are expected to be released in June 2013. Not awaiting the outcome of this report, US lawyers already offer assistance to patients for preparing lawsuits against off label usage with resulting TV advertisements in all major TV channels in the US. Unsurprisingly, the use of BMP-2 has now decreased dramatically.

Concerns regarding the efficacy of OP-1

Less studies regarding OP-1 for spinal indication were performed as compared to BMP-2. Most randomized trials involved posterolateral fusions.[49–52] In this thesis, we performed a European investigator-initiated multicenter study of OP-1 in instrumented posterolateral fusions. The result of this study showed a lower fusion rate of OP-1 compared to iliac crest bone graft. The only other large clinical study that has been published is an industry-sponsored non-instrumented posterolateral fusion study concluding that OP-1 was an effective alternative to autograft.[52] This conclusion is remarkable as it could only be made after modifying the criteria for radiological success. Instead of bridging bone in combination with limited motion on dynamic radiograms representing spinal fusion, the pres-

ence of new bone on CT-scan was used as a new (post-hoc) endpoint. In accordance with our findings, the initial radiograms, but also the additionally obtained CT scans of this study, showed less bridging bone in the OP-1 group. Although one could argue whether bridging bone is a prerequisite criterion for success of any bone graft, the execution of a post-hoc analysis when the outcome of the study is not as expected, is not methodologically sound due to its potential biases. The many complications that were associated with the use of BMP-2 have not been reported for OP-1, although some case reports were published on ectopic bone formation. [53–55] In our study there were more re-operations in the OP-1 group (ten *versus* two patients). Although this could not be directly related to specific problems associated with BMPs, this should definitely be noted in the light of the discussions on the higher complication rates with the use of BMP-2.

Overall, based on the current literature the commercially available BMPs cannot be recommended for any off-label spinal indication when iliac crest bone is available, due to concerns with potential complications related to BMP-2 and lack of effectiveness of OP-1. This is at least the case until the review of the Yale University gives clarity about the potential product related adverse events of BMP-2. It is perhaps naive to assume that this report will provide definite answers to all our urging questions and uncertainties. Maybe we need to acknowledge that we currently do not have sufficient evidence that justifies the use of BMPs as now practiced in the overwhelming majority of patients.

Drawbacks of the available BMP products

The current commercially available BMP products contain extremely high amounts of growth factor, corresponding to thousands of kilograms of bone to be used in a single patient. [56] These growth factors are locally delivered in collagen-based 'carriers', which exhibit a large initial BMP burst release with a rapid decline of the released growth factors and retention of less than 5% after two weeks of implantation. [57,58] Although many investigators pointed at the disadvantage of this inadequate delivery system, a better delivery vehicle has so far not been applied clinically. It is reasonable to believe that most complications associated with the BMP usage are related to the extremely high dosages of these potent growth factors. The enormous amount that is released in a short time frame induces a massive inflammatory response in the proximity of vulnerable anatomical structures. Complications were indeed observed less frequently when lower growth factor amounts were implanted or improved growth factor containment had been applied.[59–61] The release of the bulk of the BMPs within the first days after implantation suggest an inefficient use, since it takes time before inducible cells are migrated to the target site. Several preclinical studies evaluating the effective dosage of BMPs, revealed that the length of time that BMPs were available at the graft site positively correlated with amount of bone formation.[18] Efficient use of BMPs, by improving the pharmacokinetic parameters, could increase fusion rates at lower dosages and subsequently decrease the risk of potential complications,

as well as the costs. Efforts are currently made to design an appropriate delivery vehicle for BMPs. This remains, however, complex due to the various biological, mechanical, pharmacological and economical aspects associated with biomaterials in bone regeneration.

The need of independent research

The controversies involving the industry-sponsored BMP-2 trials have instigated the discussion in the orthopedic community about the objectivity of industry-driven research. Over the last few decades, medical research and development has come to depend more and more on the financial support of the industry. Strong and consistent evidence shows that industry-sponsored research tends to draw pro-industry conclusions. [62] Irrespective of the answer whether the results of the BMP-2 trials were biased by conflict of interests, its intense discussion alone exposes the necessity of independent research.

However, two main elements prevent the execution of more independent trials. First, the current legislation in the European Union (EU) makes it almost impossible for a group of surgeons and physicians to conduct studies without any support from industrial organizations and their highly sophisticated infrastructures, due to the enormously increased administrative burden. [63] This is particularly the case in multi-center studies that exceed country borders, which is often essential to yield sufficient study power. In order to avoid that the research agenda will be dictated by professional researchers paid by the medical industry, it is essential to simplify the administrative provisions and to create uniform rules throughout the EU, including implementation of a central authority in the Union for application of clinical trials.[63] Secondly, substantial independent resources are still required, even if the administrative loads are reduced. It seems, on the other hand, unsuitable to use public resources to conduct trials for potential profits of the medical industry. One suggested possibility would be the development of an independent worldwide fund created by a small global tax on all medical products, which can provide the investigators with necessary grants to conduct their research independently. [64]

The importance of increasing the amount of independent research, however, does not imply that there is not any role for industry-sponsored trials. It is essential to fundamentally change the way sponsored studies are organized and published. One of the proposed aspects to improve transparency and prevent biased analysis is by sharing raw study data of clinical research for independent analysis. 65,66 To realize this, a central database should be publicly accessible, containing information on all available clinical research data of sponsored trials, including unpublished data, postmarketing studies and safety programs. On request, anonymised clinical research data on patient-level can be provided to independent researchers for analysis. To prevent that researchers with an agenda will selectively interpret and publish data, a comprehensive research proposal, including disclosure of potential conflict of interests, should be evaluated by an independent organization before the data will be provided. In line with the now generally

accepted clinical trial registration before conduction of RCTs, the study protocol should also to be openly assessable before any data is obtained. In this way we ensure prospective data analysis with sound results. Another step is to drastically sharpen the criteria for scientific publications in peer-reviewed journals. Although vigilance from the side of the editorial boards of the medical journals is absolutely necessary, not all biases can be appreciated by reviewing the manuscript with summarized (potentially incomplete) data by devoted clinicians. Thus, it seems reasonable that before publication of (industry-sponsored) trials on medicines or devices, an external independent organization should assess the accuracy of the data based on all available raw documents. Methodological aspects of the study should also be addressed by this organization, such as follow-up period, control group, primary outcome, and statistical analysis, since shortcomings in design will lead to incorrect conclusions. The additional costs associated with this comprehensive review process will have to be provided by the manufacturer of the investigated product.

Future perspective

This thesis focused on regenerative therapies for spinal indications. When the research project started in 2004, we believed that BMPs could soon be implemented in the routine clinical practice as one of the first regenerative therapies. By then, the available data indicated that these proteins gave consistent and abundant bone formation to be effective in numerous challenging indications. The only hurdles that needed to be overcome were establishing its effectiveness in a few more indications and of course its extreme high price. Today, the reported safety and efficacy issues involving the current available BMPs in spinal applications disillusioned us. This made its entrance as the long anticipated ideal alternative of the much disliked bone harvesting something of the past.

Despite the unsatisfactory outcome of the current BMP based therapies, they continue to be a unique product of which the exceptional features to induce bone when implanted in muscle should be equally appreciated. We may need to go back to the drawing table and redesign the product. The most logical step seems to be to improve the delivery vehicles, which need to provide a more efficient use of these growth factors by acknowledging the physiological processes of natural bone formation. The approach to simply increase the dose of these highly potent growth factors several folds to increase its efficacy, without correlating it to any physiological parameter, anatomic location or indication seems not longer justified. Obvious adjustments include sustained releases at a more physiological concentration and ultimately more complex sequential and adjustable release profiles of several growth factors including vasculogenesis and angiogenesis factors. Also, the osteoconductive properties of the carrier can be improved. Preferably this carrier or scaffold should resorb within reasonable time frame allowing for bone remodeling. Likely candidates are calcium phosphates that have the ability to function as drug-delivery vehicles as a result of the high binding affinities between ceramics and proteins, making the additional of growth factors such as BMPs possible.[67]

Although optimizing the pharmacokinetic parameters along with its carrier is the first logical step in improving growth factor therapies, this process is not easy and will take substantial time before it can be implemented in clinical practice. In the meantime, we still need effective, reliable and safe bone grafts. The risks of using alternatives of iliac crest autograft must carefully be weighed against its potential benefits. Since donor site morbidity does not seem to be a major concern in many cases, the potential benefits of the current alternatives do not support its routine use when sufficient iliac crest bone is available. The difficulty remains when iliac crest bone is not sufficiently available. Based on this thesis, the new generation of tricalcium phosphates with potential intrinsic osteoinductive capacity seems to be a promising alternative for spinal fusions in a large animal model. It is, however, unclear whether the proclaimed osteoinductive properties contributed to its success. Nonetheless, the results warrant further evaluation in clinical settings for which our department is currently initiating a multi-center prospective study.

Unfortunately, the Age of Regeneration is still further ahead than desired. Nevertheless, slow but consequent progresses are made and boundaries for regenerating bone are getting unraveled. We need, however, to recognize that there is an urgent need for structural reorganization between the medical industry and clinical research. Long persisting negative publicity may put a potentially promising innovation under too much pressure, leading to ignoring specific gains and eventually total abandonment of the innovation. We need to be careful that this will not happen to the BMP based therapies of the future.

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Addendum

Nederlandse samenvatting

In dit proefschrift zijn diverse alternatieven voor lichaamseigen (autoloog) bekkenbot voor wervelfusies geëvalueerd. Eén van de belangrijkste nadelen van lichaamseigen bekkenbot is chronische pijn ter plaatse van het donorgebied. De incidentie hiervan varieert in de literatuur tussen de 6% en 39%. Wij zijn in dit proefschrift begonnen met het evalueren van de pijn in het donorgebied na het verkrijgen van bekkenbot via een posterieure benadering. Het is belangrijk te realiseren dat de incidentie hiervan met name is gerapporteerd in studies waarbij patiënten een laag lumbale operatie hebben ondergaan. Wij stelden dat de nabijheid van de primaire operatie tot de bekkenkam de gerapporteerde incidentie zou kunnen beïnvloeden, resulterend in een overschatting van de incidentie van de bekkenpijn. Om deze hypothese te evalueren hebben wij in **Hoofdstuk 2** de pijn in het donorgebied vergeleken tussen patiënten met wervelfracturen die een hoge fusie (> L3) ondergingen met fusies van lagere niveaus (≤ L3). De resultaten toonde significant minder bekkenpijn in het donorgebied in de groep van de hoge fusies (40.9% *versus* 14.3%). Dit zou veroorzaakt zou kunnen worden doordat een aanzienlijk percentage van de pijn dat verondersteld werd afkomstig te zijn uit het donorgebied, uiteindelijk afkomstig is van de primaire (lumbale) operatie. Deze pijn werd bovendien door het meerderdeel van de patiënten (71%) geclassificeerd als milde pijn. Hoewel pijn in het donorgebied een minder groot probleem lijkt te zijn dan eerder werd verondersteld, blijft de noodzaak voor alternatieven bestaan gezien de hoge pseudoartrose percentages en het vaak onvoldoende beschikbare hoeveelheid van het bottransplantaat.

In het vervolg van het proefschrift hebben wij de meest prominente eigenschap van lichaamseigen bekkenbot vergeleken met de huidige beschikbare alternatieven; namelijk de beschikbaarheid van levende, botvormende (osteogene), cellen. Hoewel bekkenbot zeer frequent wordt gebruikt, is er nog weinig bekend over het werkingsmechanisme. Een fundamenteel aspect is de onduidelijke rol van levende cellen in het klinisch succes van lichaamseigen bekkenbot. Door het opkomen van cel-gebaseerde bot *tissue engineering* is kennis hiervan van cruciaal belang geworden. In **Hoofdstuk 3** hebben wij de invloed van levende (vitale) cellen in lichaamseigen bekkenbot onderzocht in een processus transversus model. Dit model bootst de initiële botformatie bij posterolaterale wervelfusies na. Er werden hiervoor cassettes op de gedecortiseerde processi transversi van geiten bevestigd. Elke cassette bevatte afgescheiden kamers, waarin vitaal en gedevitaliseerd lichaamseigen bekkenbot in werd geplaatst. Twaalf weken na de implantatie werden histologische en histomorphometrische analyses verricht. Daarnaast werd de botdynamiek geëvalueerd middels fluorochromen labeling. De resultaten toonde een toegevoegde waarde van levende (vitale) cellen in de vroege botvorming gelokaliseerd op afstand van de processus transversus. Kwantitatieve analyses toonde circa 30% meer bot in de groep met levende cellen; dit verschil was echter niet statistisch significant. De meerwaarde van levende cellen lijkt met name te zijn bij de periferie zone van de implantaten, zoals de submusculaire regio. Dit zou van klinische belang kunnen zijn gezien pseudoartroses vaak in deze regio plaatsvind.

Het genoemde processus transversus model werd ook gebruikt in Hoofdstuk 4 om de botvormende capaciteit van trombocyten concentraat (platelet gel) op diverse bottransplantaten te evalueren. Er zijn aanwijzingen dat groeifactoren afkomstig uit trombocyten kunnen leiden tot meer botformatie. Om dit effect te onderzoeken hebben wij twee cassettes geïmplanteerd op de processi transversi van geiten. Elke cassette bevatte lichaamseigen bekkenbot, bifasisch calcium fosfaat (BCP) en tantalium (trabecular metal). Eén cassette werd behandeld met trombocyten concentraat en andere cassette werd onbehandeld gelaten. Na 9 weken werd de hoeveelheid nieuw gevormd bot vergeleken. In het lichaamseigen bekkenbot en het BCP was er significant meer bot gevormd in de met trombocyten concentraat behandelde groep. Er werd weinig bot gevormd in de tantalium groep, ongeacht de behandeling met het concentraat. Deze studie toonde aan dat trombocyten concentraat de botvorming bij lichaamseigen bekkenbot of calcium fosfaat transplantaten stimuleert. Het gebruikte model is echter bedoeld als screening. Alvorens dit bij patiënten gebruikt kan worden dient dit nog wel verder onderzocht te worden in een klinisch relevant proefdier model.

In **Hoofdstuk 5** hebben wij in een diermodel onderzocht of een nieuw bioactief tricalcium fosfaat (TCP) geschikt is als alternatief voor lichaamseigen bot bij wervelfusies. Wij hebben dit TCP vergeleken met donorbot en lichaamseigen bekkenbot. In totaal hebben 9 geiten een geïnstrumenteerde posterolaterale fusie op twee niveaus ondergaan. Elke zijde van beide niveaus kreeg een andere behandeling toegewezen. Hierdoor konden 4 groepen worden getest: lichaamseigen bekkenbot; donorbot; TCP; en TCP gecombineerd met lokaal lichaamseigen bot (1:1). Na 16 weken werd de mate van fusie tussen de wervels en de volume van nieuw gevormd bot vergeleken. Hierbij werd er een vergelijkbaar fusie percentage van TCP, donorbot en lichaamseigen bekkenbot gezien. Het TCP zelf was nagenoeg volledig geresorbeerd en vervangen door bot. Kwantificatie van het botvolume op de CT-scans toonde echter minder bot in de TCP groep. Ondanks het mindere botvolume, is het nieuwe TCP een veelbelovend alternatief voor lichaamseigen bekkenbot bij posterolaterale fusies. Echter, klinische studies moeten dit nog verder uitwijzen.

In het tweede deel van het proefschrift hebben wij ons gericht op bone morphogenetic proteins (BMPs). Dit is een groeifactor die in staat is botvorming te induceren en in de kliniek op grote schaal reeds wordt gebuikt. In **Hoofdstuk 6** beschrijven wij hoe de combinatie van de vooruitgang binnen verschillende disciplines heeft geleid tot de ontwikkeling van klinische BMP producten. Er is ondanks het veelvuldig gebruik van BMPs weinig wetenschappelijke onderbouwing aanwezig over de effectiviteit en veiligheid. Derhalve hebben wij een Europese multi-center studie gedaan, waarbij wij OP-1 (BMP-7) hebben vergeleken met lichaamseigen bekkenbot in geïnstrumenteerde posterolaterale wervelfusies. Deze operaties zijn verricht bij patiënten met afgegleden wervel (spondylolisthesis), welke een zenuwbeklemming veroorzaakte. Het doel van de studie was om aan te tonen dat er met OP-1 een vergelijkbaar resultaat kon worden verkregen als met lichaamseigen bekkenbot in termen van radiologische fusie en klinische verbetering. In **Hoofdstuk 7** worden de resultaten van de eerste 36 patiënten beschreven. Hierbij werden geen

complicaties gezien die geassocieerd konden worden met OP-1. Tevens was OP-1 in staat om wervelfusies te induceren bij patiënten, hoewel de exacte effectiviteit nog bepaald moest worden. Op basis van deze interim analyse konden wij de studie voortgezet. De volledige studie beschrijven wij in **Hoofdstuk 8**, waarbij in totaal 134 patiënten zijn geïncludeerd. De resultaten van deze studie toonde geen vergelijkbare uitkomst tussen OP-1 en lichaamseigen bekkenbot. Bij OP-1 was er significant minder radiologische fusie in vergelijking met lichaamseigen bekkenbot (54% *versus* 74%). Het klinische resultaat vanuit patiëntenperspectief, gemeten met gevalideerde vragenlijsten, was vergelijkbaar tussen de groepen. Er waren meer re-operaties in de OP-1 groep (tien *versus* twee), die echter niet direct gerelateerd konden worden aan het OP-1 gebruik. Het doel van de operatie is om een benige verbinding tussen twee wervels te creëren. Aangezien OP-1 hierin minder effectief is dan lichaamseigen bekkenbot adviseren wij dit niet te gebruiken als alternatief bij geïnstrumenteerde posterolaterale fusies.

Tijdens het verrichten van deze multi-center studie hebben wij veel hinder ondervonden van de nieuwe Europese wetgeving omtrent klinische trials. In **Hoofdstuk 9** beschrijven wij de nadelen van deze nieuwe wetgeving, welke heeft geresulteerd in een forse toename van de administratieve eisen voor onafhankelijke en academische studies. Deze eisen maken het bijna onmogelijk om dit soort studies in Europees verband onafhankelijk van de industrie te doen. Onafhankelijk onderzoek blijft echter essentieel om klinische vooruitgang te boeken. Derhalve zou deze regelgeving vereenvoudig en geharmoniseerd moeten worden. Een belangrijk aspect hiervan is centralisatie van de procedures binnen Europese Unie.

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Curriculum Vitae

The author of this thesis was born on August 22, 1978 in Amersfoort, The Netherlands. In 1997 he graduated from high school (VWO, Vallei College, Amersfoort) and started to study medicine at the University of Utrecht. In 1999, he joined research projects as a student on familial combined hyperlipidaemia at the Department of Vascular Medicine (supervisor: dr. M. Castro Cabezas) of the University Medical Center Utrecht. In 2003 he did his last internship at the Department of Orthopaedic Surgery in the University Medical Center Utrecht. After receiving his Medical Degree in 2004, he started working as a fulltime researcher at the University Medical Center Utrecht on a PhD project on bone regeneration therapies for spinal surgery (head: prof. dr. W.J.A. Dhert and prof. dr. F.C. Oner; co-supervisor: dr. M.C. Kruyt). This work has lead to several publications, a number of presentations at international conferences, and this thesis.

In September 2007 he started his surgical training at the Diakonessenhuis in Utrecht and Zeist (head: dr. G.J. Clevers) as part of his orthopaedic training. He continued his orthopaedic residency at the University Medical Center Utrecht in 2009 (head: prof. dr. D.B.F. Saris) and the Antonius Hospital in 2010 until 2012 (head: dr. M.R. Veen). He is currently working as a resident at the University Medical Center Utrecht (head: prof. dr. D.B.F. Saris). He will complete his orthopaedic residency in December 2013. During his last year of residency he performed a 3-months fellowship in arthroplasties in the Diakonessenhuis (head: dr. A. de Gast). After his residency he will further specialise in spine surgery.