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BIOACTIVE GLASS IN LUMBAR SPONDYLODESIS

A Pre-Clinical and Clinical Study

by

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To my family

TIIVISTELMÄ

Janek Frantzén

BIOAKTIIVINEN LASI LANNERANGAN LUUDUTUSLEIKKAUKSISSA: PRE-KLIININEN JA KLIININEN TUTKIMUS

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Bioaktiivinen lasi (BL) kuuluu synteettisiin silikaattipohjaisiin koostumuksestaan riippuvaisiin pinta-aktiivisiin biomateriaaleihin joilla on osteokonduktiivisia, osteopromotiivisia, angiogeneettisiä ja antibakteerisia ominaisuuksia.

Kansallinen tutkimusryhmä joka toimi TEKESin Combio Teknologia ohjelmassa (2003–2007), kehitti BL 1–98- ja polymeerikuiduista huokoisen, kuormaa kantavan komposiitin kirurgisiin sovelluksiin. Tämän väitöskirjan pre-kliininen osuus keskittyi komposiitin in vitro- ja in vivo- tutkimuksiin kanin reisiluun ja selän posterolateraalisella luudutusmallilla. Reisiluumallissa ei voitu osoittaa BL 1–98:n aiemmin todettua osteogeneesiä stimuloivaa vaikutusta. Tämä johtuu todennäköisesti BL:n kuitumuodon aiheuttaman resorption muutoksesta. Selän luudutustutkimus oli keskeytettävä odottamattoman haittavaikutuksen vuoksi. In vitro- soluviljemissä havaittiin kasvun estymistä ihmisperäisissä mesenkymaalisissa kantasoluissa BL- kuitujen läheisyydessä, sekä radikaaleja pH-muutoksia.

Kliinisessä osuudessa suoritettiin vuosina 1996–1998 leikattujen potilaiden prospektiivinen pitkäaikaisseurantatutkimus. Tutkittiin BL S53P4:n ja autogeenisen luunsiirteen käyttöä lannerangan degeneratiivisen spondylolisteesin (n=17) ja instabiilien burst-nikamamurtumien (n=10) instrumentoidussa posterolateraalisessa luudutuksessa. Leikkaustulos arvioitiin röntgenkuvin ja tietokonetomografialla (TT), sekä kliinisellä tutkimuksella. Spondylolisteesiryhmässä todettiin vahva luutuminen TT-tutkimuksen perusteella BL-puolella 12 potilaalla ja osittainen luutuminen viidellä potilaalla. Luutumisaste oli yhteensä 88% sekä L4/5- että L5/S1-tasolla, luudutetuista nikamaväleistä (n=41). Nikamamurtumatutkimuksessa vahva luutuminen todettiin viidellä potilaalla ja osittainen luutuminen niin ikään viidellä potilaalla. Luutumisaste oli 71% luudutetuista nikamaväleistä (n=21).

Prekliiniset tuloksemme viittaavaat siihen että määrätyissä olosuhteissa BL:n fyysinen muoto on kemiallista koostumusta merkittävämpi suunnitellessa kliinistä sovellusta. Ensimmäiset pitkäaikaisseurantatulokset BL S53P4:n käytöstä luunkorvikkeena lannerangan instrumentoidussa posterolateraalisessa luudutuksessa osoittivat sen käytön olevan turvallista ja komplikaatiot harvinaisia. BL S53P4 ei yksinään käytettynä edistänyt kiinteän luusillan muodostumista yhtä tehokkaasti kuin autogeeninen luunsiirre.

Avainsanat: bioaktiivinen lasi, biohajoava, luunkorvike, lannerangan luudutus

ABSTRACT

Janek Frantzén

BIOACTIVE GLASS IN LUMBAR SPONDYLODESIS, A PRE-CLINICAL AND CLINICAL STUDY

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Bioactive glasses (BGs) form a group of synthetic, surface-active, composition-dependent, silica-based biomaterials with osteoconductive, osteopromotive, and even angiogenic, as well as antibacterial, properties.

A national interdisciplinary research group, within the Combio Technology Program (2003–2007), developed a porous load-bearing composite for surgical applications made of BG 1–98 and polymer fibers. The pre-clinical part of this thesis focused on the in vitro and in vivo testing of the composite materials in a rabbit femur and spinal posterolateral fusion model. The femur model failed to demonstrate the previously seen positive effect of BG 1–98 on osteogenesis, probably due to the changed resorption properties of BG in the form of fibers. The spine study was terminated early due to adverse events. In vitro cultures showed the growth inhibition of human mesenchymal stems next to BG 1–98 fibers and radical pH changes.

A prospective, long-term, follow-up study was conducted on BG–S53P4 and autogenous bone used as bone graft substitutes for instrumented posterolateral spondylodesis in the treatment of degenerative spondylolisthesis (n=17) and unstable burst fractures (n=10) during 1996–1998. The operative outcome was evaluated from X-rays and CT scans, and a clinical examination was also performed. On the BG side, a solid fusion was observed in the CT scans of 12 patients, and a partial fusion was found in 5 patients, the result being a total fusion rate in all fusion sites (n=41) 88% for levels L4/5 and L5/S1 in the spondylolisthesis group. In the spine fracture group, solid fusion was observed in five patients, and partial fusion was found in five resulting in a total fusion rate of 71% of all fusion sites (n=21).

The pre-clinical results suggest that under certain conditions the physical form of BG can be more critical than its chemical composition when a clinical application is designed. The first long-term clinical results concerning the use of BG S53P4 as bone graft material in instrumented posterolateral spondylodesis seems to be a safe procedure, associated with a very low complication rate. BG S53P4 used as a stand-alone bone substitute cannot be regarded as being as efficient as AB in promoting solid fusion.

Keywords: bioactive glass, biodegradation, bone substitute, spinal fusion

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LIST OF ORIGINAL PUBLICATIONS

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I **Alm JJ, Frantzén JP, Moritz N, Lankinen P, Tukiainen M, Kellomäki M, Aro HT**. In vivo testing of a biodegradable woven fabric made of bioactive glass fibers and PLGA80 – a pilot study in the rabbit. J. Biomed. Mater. Res. B. Appl. Biomater. 2010 May;93(2):573–80.

II **Frantzén JP, Alm JJ, Lankinen P, Moritz N, Röyttä M, Aro HT**. Serious adverse event of woven fabric made of bioactive glass fibers of the Na_2O-K_2O-MgO-CaO-B_2O_3-P_2O_5-SiO_2 system in the rabbit spinal fusion model. (Manuscript)

III **Frantzén JP, Rantakokko J, Aro HT, Heinänen J, Kajander S, Gullichsen E, Kotilainen E, Lindfors NC**. Instrumented spondylodesis in degenerative spondylolisthesis with bioactive glass and autologous bone: a prospective 11-year follow-up. J. Spinal Disord. Tech. 2011 Oct;24(7):455–461.

IV **Rantakokko J,* Frantzén JP,* Heinänen J, Kajander S, Kotilainen E, Gullichsen E, Lindfors NC**. Posterolateral spondylodesis using bioactive glass S53P4 and autogenous bone in instrumented unstable lumbar spine burst fractures. Scand. J. Surg. 2012;101(1):66–71.
(*equal contribution)

ABBREVIATIONS AND TERMINOLOGY

ALIF	Anterior lumbar interbody fusion
BG	Bioactive glass
BMA	Bone marrow aspirate
β-TCP	β-tricalcium phosphate
HA	Hydroxyapatite
µCT	High-resolution microcomputed tomography
ODI	Oswestry Disability Index
PLIF	Posterior lumbar interbody fusion
pQCT	Peripheral quantitative computed tomography
SBF	Simulated body fluid
TLIF	Transforaminal lumbar interbody fusion
VAS	Visual analogue scale

Allograft	Graft obtained from another person.
Angiogenic	The physiological process promoting the growth of new blood vessels from pre-existing vessels.
Autograft	Graft obtained from another anatomic site in the same person
Bioactivity	The characteristics of implant material that allows it to form a bond with living tissue.
Bioactive glass	Non-crystalline silica-based solid material with the ability to form a calcium phosphate layer on its surface in vivo.
Bioactive material	Material designed to elicit or modulate biological activity.
Biocompatibility	Characteristic of material used in a medical device to perform an appropriate host response in a specific location.
Biomaterial	Material intended to interface with biological systems to evaluate, treat, augment, or replace any tissue, organ or function of the body.
Bone bonding	Establishment, by physico-chemical processes, of continuity between an implant and bone matrix.
Bone remodeling	Process during which bone gradually alters its morphology in an attempt to adapt to any new external load.
Ceramic	Inorganic, non-metallic solid prepared by thermal treatment and subsequent cooling. Ceramic material may have a crystalline or partially crystalline structure, or it may be amorphous.
Foreign body reaction	Variation in normal tissue behavior caused by the presence of a foreign material.

Implant	Medical device made from one or more biomaterials intentionally placed within the body, either totally or partially buried beneath an epithelial surface.
Osteocondutive	Ability of a graft to function as a scaffold for the ingrowth of new bone and sprouting capillaries.
Osteogenic	Composed of or originating from any tissue involved in the development, growth, or repair of bone.
Osteoinductive	Ability for bone formation de novo or in non-osseous tissue by the stimulation of the phenotypic conversion of undifferentiated cells (mesenchymal stem cells) from the surrounding tissues and their differentiation into bone-forming osteoblasts.
Osteopromotive	Ability of a material to promote the de novo formation of bone.
Resorption	Reduction of biomaterial because of cellular activity or simple dissolution.

1. INTRODUCTION

Lumbar spondylodesis is a generally accepted surgical procedure. Degenerative spondylolisthesis is a common cause of low-back pain and radiculopathy in adults older than 40 years (Booth et al. 1999). Secondary changes such as facet hypertrophy and thickening of the ligamentum flavum lead to spinal stenosis due to compression of the neural elements that can cause neurogenic claudication. If conservative treatment fails, surgery with decompression, restoration of the intervertebral disc space, and fusion is justified. Acquired diseases such as infection, tumors, or scoliosis often require fusion of affected segments of the spine as part of their treatment. Most spine fractures can be treated conservatively with braces and physiotherapy. Unstable vertebral burst fractures with neurological deficits require prompt surgery to decompress the neural elements, to realign the vertebrae, and to stabilize the spine by fusion (Reinhold et al. 2010).

Autograft is considered the gold standard of bone grafts to be used in spine surgery because it contains the patient's natural combination of osteogenic, osteoconductive, and osteoinductive factors that help stimulate bone formation and fusion. This additional bone harvesting procedure is associated with a prolonged operation time and increased blood loss, and it causes additional postoperative pain beyond the time that the spine heals after surgery (Banwart et al. 1995, Robertson and Wray 2001). Allografts and various synthetic bone graft substitutes are in clinical use but have certain limitations.

Bioactive glasses (BGs) are a group of synthetic, surface-active, composition-dependent, silica-based biomaterials with osteoconductive, osteopromotive, and even angiogenic, as well as antibacterial, properties. The bone bonding properties of the two original BGs (Bioglass® 45S5 and S53P4) were first delineated by Larry Hench in the early 1970's (Hench et al. 1971, Hench 2006). The reactivity of BGs depends on the dissolution of surface ions, and the rate of dissolution can be adjusted by the choice of glass composition. However, the relative proportion of the four main BG components (SiO_2, Na_2O, CaO, P_2O_5) must be within a narrow range to get the desired biological response. For optimal function in vivo, medium-rate bioactivity has been suggested, with controlled and moderate ion release that does not create ion excess. In order for a large working range of glass melts to be achieved for the manufacture of BG fibers and microspheres for microporous scaffolds, a system of Na_2O-K_2O-MgO-CaO-B_2O_3-P_2O_5-SiO_2 was introduced to match the degradation rate of BGs to clinical conditions (Brink et al. 1997).

This doctoral thesis was initiated as part of an interdisciplinary project called the Combio Technology Program (2003–2007), which was coordinated by the National Agency for Technology and Innovation in Finland (TEKES). The aim was to develop a porous load-bearing composite for surgical applications made of bioactive/biodegradable glass and polymer fibers. The pre-clinical part of this study focused on in vitro and in vivo testing of the composite materials. The prospective clinical studies targeted the long-term outcome of the use of BG S53P4 in lumbar instrumented posterolateral fusions for spondylolisthesis and instable burst fractures.

2. REVIEW OF THE LITERATURE

2.1. Clinical lumbar spinal fusion

2.1.1. History

Spinal disorders and injuries have been recognized as a serious asperity of humans since ancient times. The oldest known prehistoric spine fracture dates back to 32100 B.C. It was found in a skeleton named Statten1 in excavations made by Riek in 1931 in the Vogelherd Cave in Germany (Weber et al. 2004). Traditionally, such injuries were treated conservatively with the addition of a traction table introduced by the Greek physician Hippocrates of Cos (460–370 B.C). This table was used for the closed reduction of spine injuries and for spinal deformity. Improvements were introduced to the traction table by a Greek physician named Oribasius (325–400 A.D.), who added a cross bar that could be used as a lever for achieving a reduction in the fracture dislocation. This treatment modality was still being recommended at the end of the Middle Ages (Gruber and Boeni 2008).

The first successful surgical spinal decompression in the form of a laminectomy was performed by Alban G. Smith (1788–1862) in 1829. Many surgeons failed, mainly due to pain and infections. Aseptic surgery was introduced in 1866 by a famous English surgeon named Joseph Lister (1827–1912), who achieved this goal with the use of a weak solution of carbolic acid (Lister 1867). Aseptic surgery was further improved during the last part of the 1900th century by the introduction of steam sterilizers and surgical rubber gloves. After the discovery of penicillin by Alexander Fleming (1881–1955) in 1929, surgical infections could be treated.

The development of a comprehensive treatment regime for spinal disorders made a giant leap forward after the discovery of X-rays by William C. Roentgen (1845–1923) (Roentgen 1959). He received the Nobel prize for his discovery in 1901. Air ventriculography and myelography were introduced in 1918 by the American neurosurgeon Walter E. Dandy (1886–1946) (Dandy 1918). This method allowed the spinal cord and nerve roots to be visualized, and pathologic compression to be delineated, for the first time. It was later refined by the injection of contrast agent that was lipid-based requiring removal after imaging and was therefore later replaced by water-soluble agents. The step-by-step development of computed tomography (CT) in the early 1970s revolutionized the diagnostic assessment of the central nervous system and spine (Ambrose 1973, Hounsfield 1973). The diagnostic imaging modality of choice for most clinical conditions involving the spine and spinal cord today is magnetic resonance imaging (MRI), which was patented by Raymond Damadian (1936–) in 1974 and which is based on the principle discovered by Swiss physicist Felix Bloch (1905–1983), who received a Nobel prize for this work (Damadian 1971).

The aforementioned improvements in antiseptic surgical methods and the introduction of anesthesia, first in the form of nitrous oxide (N_2O) in the middle of the 1900th century and followed by the more potent anesthetics chloroform and cocaine, led to more invasive and effective spinal surgery. In 1887, the first successful internal fixation of the spine was performed by the American surgeon William F. Wilkins (1848–1935). The patient was a 6-day-old child with a fracture and dislocation of Th12 and L1 caused by the mother sustaining a severe injury the day before giving birth. This condition was treated by the placement of a carbolized silk ligatures around the pedicles; the procedure thus stabilized the fracture. Four years later in Austin, Texas Berthold E. Hadra (1842–1903) operated on a 30-year-old waiter who had been treated conservatively for a C6/C7 fracture after a fall injury. Ten months after the initial injury the patient's condition deteriorated after a sudden movement of the head. He was then successfully surgically treated by Dr. Hadra who performed a wiring of the spinous processes together in a figure of 8, using a silver wire (Hadra 1891).

In the early 20th century, the first attempts to stabilize a tuberculous spine with the use of steel rods, first with silk and later with silver wires, were made

by the German surgeon Fritz Lange (1864–1952) (Lange 1910). Fred H. Albee (1876–1945) was an American orthopedic surgeon who is cited as being the first surgeon to perform a successful spinal fusion. He also treated a patient suffering from spinal tuberculosis with a strip of autologous tibia between split spinous processes (Albee 1911). Techniques involving posterior lumbar interbody fusion were described by the neurosurgeon Ralph B. Cloward, who became more known for his work with anterior decompression and fusion of the cervical spine (Cloward 1953). Improvements were made in the instrumentation, and noncorrosive metallic implants were developed. The incidence of pseudoarthrosis was still too high until Paul Harrington (1911–1980) introduced his hook and rod fixation system. It was originally developed for scoliosis surgery but was later also applied to fractures and degenerative conditions; it dominated the field of surgery for a quarter of a decade (Harrington 1962).

The next breakthrough in the development of instrumentation was made by the French surgeon Raymond Roy-Camille (1927–1994) in 1963; he used a combination of pedicle screws and a posterior osteosynthesis plate (Roy-Camille et al. 1970). In 1977, an external fixation system using pedicle screws and rods was introduced by the Austrian surgeon Friedrich Magerl. This system formed the basis for angle–stable fixation (Magerl 1984) and was later converted to an internal fixator that led to the further development of similar devices worldwide, making it a routine surgical procedure for stabilizing the spine (Dick et al. 1985) (Figure 1).

2.1.2. Clinical indications

The indications for surgical stabilization of the spine are numerous, starting with the basic four first cited by Panjabi and White (i.e., restoration of stability due to trauma or degenerative changes, maintenance of alignment after the correction of deformities, prevention of further alignment deformities, and the alleviation of pain related to instability or pathologic movement (Panjabi and White 1980, Schlenzka et al. 1993, Kotilainen et al. 1997). Other indications are stabilization as part of tumor resection or debridement after infections (Murrey et al. 2002, Lindfors et al. 2010a). Rheumatoid arthritis has a predilection for the cervical spine, causing instability in the occipito-cervical junction, for which stabilization does not seem to decrease mortality but instead results in increased quality of life (Ronkainen et al. 2006). In cases in which the inflammatory process of ancylosing spondylitis causes severe kyphotic deformity and myelopathy correction involving osteotomies, stabilization is required (Smith-Petersen et al. 1969, Hehne et al. 1990). For the purpose of this thesis the emphasis is on spinal injuries and degenerative disorders in the lumbar region.

2.1.2.1. Spine trauma

Thoracolumbar spine injuries are the most frequent among men (2/3) with a peak age between 20 and 40 years, and they are generally caused by a fall from a height (Reinhold et al. 2010). The most important classification is between stable and unstable fractures. Frank Denis made the first classification of a three-column spine in 1983. He considered the middle column, consisting of the posterior longitudinal ligament (PLL), the dorsal annulus fibrosus, and the dorsal part of the vertebral bodies, to be the key structures in evaluations of the stability of injuries. The Denis classification is still widely used because of its simplicity, and it still covers the main injury patterns (Denis 1984). Nowadays, the type of injury is generally classified according to the AO-Magerl classification, which is increasingly being accepted as the gold standard (Magerl et al. 1994). This comprehensive classification for the documentation and treatment of spine injuries is based on 1400 cases and is divided into three main categories of fractures (A B C). These categories are further classified into groups (1 2 3) and detailed subgroups (.1 .2 .3) according to morphological findings. The severity of injury increases for each type and group.

Type A is characterized by a shortening of the anterior column with the focus on injuries of the vertebral body. There are three subtypes for each type. Impaction, split, and burst fractures comprise type A. Type B injuries describe distraction injuries with disruption of the posterior or anterior column. Type C injuries result from an axial torque superimposed on type A, B or shearing injuries.

It is important to document a neurological examination in order to identify patients with a

progressive course of injury. The documentation is carried out according to the guidelines of the American Spinal Injury Association (ASIA). The main classification is paraplegia referring to a loss of motor and/or sensory function at level T2-S5. Impairment at level C0-T1 results in tetraplegia. Further rating on the Frankel/ASIA impairment scale is graded as A through E as follows:

Grade A Complete. The lesion is found to be complete at both the motor and sensory level below the marked segment.
Grade B Sensory only. There is some sensation present below the level of the lesion, but the motor paralysis is complete below that level. This column does not apply when there is a slight discrepancy between the motor and sensory level, but it does apply to sacral sparing.
Grade C Motor useless. There is some motor power present below the lesion, but it is of no practical use to the patient.
Grade D Motor useful. There is useful motor power below the level of the lesion. Patients in this group can move their lower limbs, and many can walk, with or without aids.
Grade E Recovery. The patient is free of neurological symptoms (i.e., no weakness, no sensory loss, no sphincter disturbance). Abnormal reflexes may be present. (Frankel et al. 1969)

Vaccaro and coworkers introduced a new classification according to which the neurological status is integrated into the grading system of the injury (Vaccaro et al. 2005). According to the Thoracolumbar Injury Classification and Severity Score (TLICSS), a score of 5 or higher would warrant surgical treatment, each group having a maximum score as follows: morphology 4 points, integrity of the posterior ligament 3 points, and neurological status 3 points.

2.1.2.2. Degenerative disease

Degenerative lumbar spondylosis is a degeneration of the lumbar motion segment starting at the microscopic level with a decrease in functional proteoglycans in the cartilage affecting shock absorption (Bayliss et al. 2001).The age-related decline in the transport of nutrients to the avascular matrix of intervertebral discs leads to a loss of functional ability and an increase in the vulnerability to injury (Horner and Urban 2001). When these degenerative changes coincide with facet joint osteoarthritis and segmental instability, the biomechanics of the lumbar segment is altered. Age-related postural changes in the sagittal profile of the spine leads to altered stress distribution in each segment. Progression of the degenerative changes can result in spinal stenosis with or without spondylolisthesis, and, in some cases, it can lead to degenerative scoliosis.

Presenting symptoms due to structural changes in the intervertebral discs are deep-aching low-back pain with aggravated flexion and pain radiation in the anterior thigh without a radicular pattern. Pain originating from the facet joints is often improved during motion and aggravated by extension and rotation due to increased pressure on the joints. Low-back pain induced by segmental instability is worsened during motion and vibration (e.g., driving a car). The clinical signs of instability described as "instability catch", "painful catch", and "apprehension" have been shown to be successful when used as selection criteria for the selection of patients with chronic back pain for treatment with spondylodesis (Kotilainen et al. 1997). Compromise of the neural element can lead to neurogenic claudication if the spinal canal is stenosed. If the neural foramina are narrowed, this can result in radicular nerve pain according to the affected dermatome.

2.1.3. Surgical procedure of posterolateral fusion

A normal spine is balanced in a sagittal profile. The primary thoracic kyphosis is counterbalanced by secondary cervical and lumbar lordosis. Facet joints, intervertebral discs, and ligaments stabilize each spinal motion segment. Identifying the level of instability exceeding the passive stability of these structures can be challenging. The goal of internal fixations is to reconstruct and create a bony fusion between the affected motion segments. The fixation is successful when the implanted hardware can withstand mechanical stress until the segment is fused.

There are three main approaches to achieving lumbar arthrodesis. Posterolateral fusion,

first described by Watkins in 1953, still remains the gold standard for fusion (Watkins 1953). This technique was originally based on the use of large cortico-cancellous iliac bone blocks placed over decorticated transverse processes, pars interarticularis, and facet joints. Later, the technique was modified to use thinner strips of iliac bone in order to avoid dislocation of the graft. The second approach is the use of lumbar interbody fusion performed anteriorly (ALIF), posteriorly (PLIF), or transforaminally (TLIF). This process consists of disc removal, endplate decortication, and bone grafting. The third approach is a combination of both techniques, yielding a 360-degree fusion.

The first attempts to use posterior spinal instrumentation as described by Harrington suffered from overdistraction, which caused the flat back syndrome, while the laminar hooks that could cause neural compression, together with a lack of segmental corrective force, drove the development towards the use of pedicle screws (Harrington 1962) (Figure 1A). This technique requires the exact assessment of the pedicle size and its orientation. Its use is based on preoperative plain X-rays, but also on MRI or CT scans. Anatomical landmarks are used as the proper entry points on the spine. These vary depending on the level of the spine. For the lumbar spine, it is the intersection of a horizontal line bisecting the transverse process with a vertical line tangential to the lateral part of the facet joint. The screw should converge15–20 degrees at the L5 level and decrease in the upper part of the lumbar spine to 5–10 degrees. In the thoracic spine, the entry point is just below the rim of the upper facet joint. The convergence should be between 7 to 10 degrees, the caudal angulation being 0–20 degrees, especially if polyaxial screws are used. In order to achieve a solid purchase of bone in the sacrum, bicortical screw placement with a convergence of 15–20 degrees towards the anterior corner of the promontorium is recommended. The entry point can vary due to the variability of the anatomy, usually found inferior and lateral to the S1 facet (Figure 2). The accuracy of pedicle screw insertion can be increased with image-guided computer-navigated surgery from a pedicle perforation rate of 13.4% to 4.6%, compared with conventional methods (Laine et al. 2000, Schlenzka et al. 2000). In trauma surgery, when reduction is attempted, a slight ascending direction of the screws towards the cranial endplate and a downward course at the caudal end are used in order to achieve biomechanical leverage.

A variety of materials are used for pedicle screw and rod constructs. Along with the increasing use of CT and MRI scans in the 1990s, the stainless steel constructs needed to be modified to titanium alloy because titanium causes fewer artifacts on the images (Ebraheim et al. 1994). The change of material itself enhanced the pedicle screw bone on-growth when compared with the use of stainless steel implants (Christensen et al. 2000). The material has been developed further by adding a hydroxyapatite coating to the screw for improved integration, especially for dynamic stabilization surgery and for patients suffering from osteoporosis (Sanden et al. 2002). The fatigue of titanium implants, particularly at notches, that results from rod contouring is more prominent than with implants manufactured of stainless steel (Dick and Bourgeault 2001). Cobalt-chromium has been shown to be superior with respect to wear and fatigue properties, as well as having improved imaging characteristics, in comparison with its precursors (Nguyen et al. 2011).

Polyetheretherketone (PEEK) polymer has a modulus of elasticity between cortical and cancellous bone (3400MPa), and it allows limited motion when used as rods for stabilization, resulting in reduced stress on the adjacent segment (Highsmith et al. 2007).

2.1.4. Surgical procedures of intercorporal fusion

Interbody fusion can be used in combination with translaminar or transpedicular fusion. Spacers or cages can be implanted through an anterior, posterior, or lateral approach, providing restoration of disc height and, most importantly, the opening of the neural foramina.

With the posterior technique the disc space is approached by a laminotomy of the upper lamina and resection of the medial half of the facet joints. A bilateral discectomy is performed, and the endplates are prepared. After distraction, a

spacer packed with autologous bone or bone substitute is implanted under fluoroscopic guidance (Freeman et al. 2000).

The transforaminal approach differs by being unilateral, and the distraction can be facilitated by inserting pedicle screws that are connected to a distractor. The foraminal window is further lateral and requires removal of the facet joint. Special care needs to be taken to avoid injury to the exiting root. Decompression of the contralateral side is indirect. Since there is no compression on the dural sac, this procedure is suitable for all levels of the lumbar region (Hackenberg et al. 2005).

ALIF, using interbody cages, has the advantage of recreating lumbar lordosis and, due to the larger exposition, decompression of the neural elements is easier. The larger footprint of the implant reduces the risk of subsidence and increases the initial stability (Pavlov et al. 2004). The anterior approach often requires an access surgeon familiar with abdominal and vascular surgery. The interbody spacers are manufactured out of PEEK, titanium, or machined femoral rings. All of these methods require complementary fixation (e.g., translaminar or pedicular fixation) (Figure 1A). There are a few stand alone interbody cages on

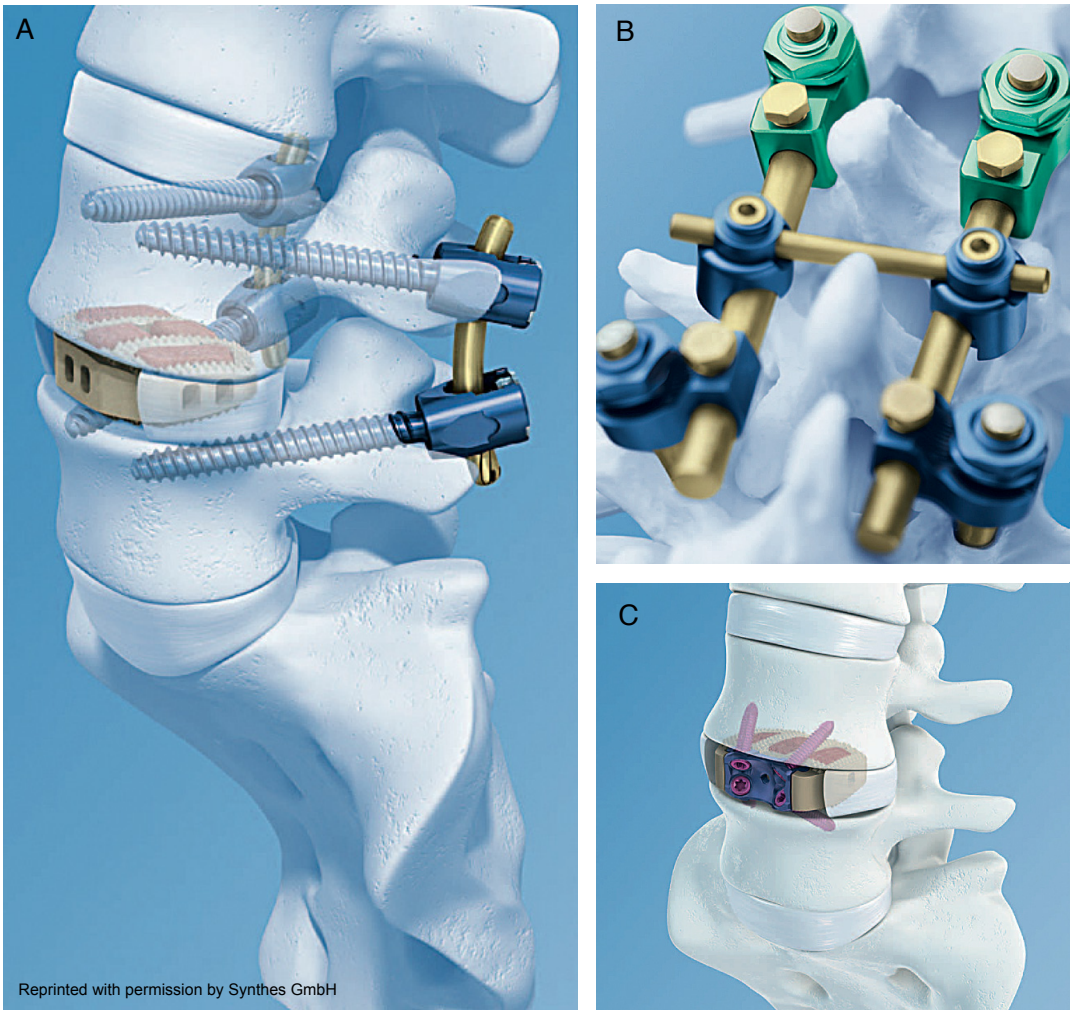
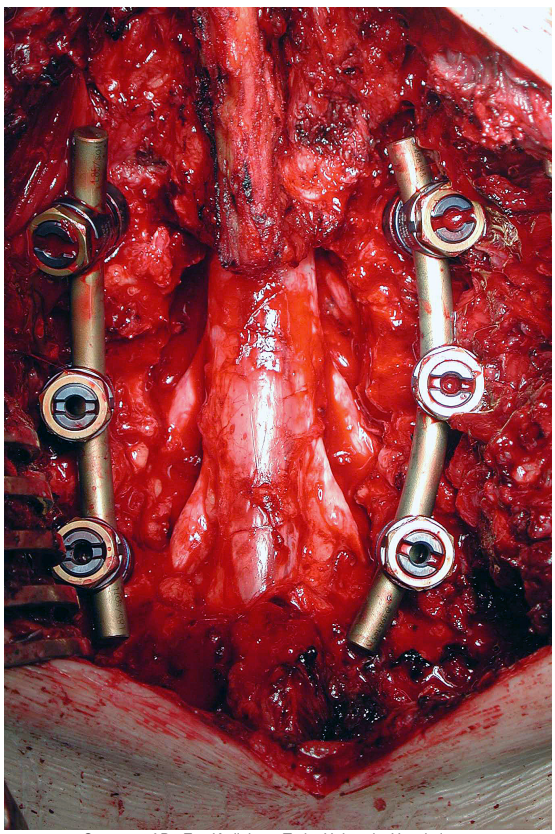


FIGURE 1. *Instrumented spinal fusion. A. Transpedicular fusion. B. USS trauma fixation. C. Intercorporal fusion.*

the market with a built-in anterior locking plate and screws providing an anterior tension band with results comparable to those of a combined approach (Strube et al. 2011) (Figure 1C).

2.1.5. Clinical outcome measures

In spine surgery, as in many fields of medicine, the outcome is multivariable, requiring standardized and validated, well-designed outcome instruments to cover these characteristics. Pain is the most common cause of spine surgery, and therefore pain relief serves as a means to evaluate the effectiveness of treatment. The duration of 4 weeks to 1 year of pain is regarded as chronic, but no consensus exists (Raspe et al. 2003). The experience of pain is a very subjective interpretation, and it is therefore difficult to assess objectively. The degree of emotional arousal and action readiness is used as a measure of intensity (Von Korff et al. 2000). In another study, the authors advocated the use of a multimodal and cognitive-behavioral approach for the assessment of chronic pain because of the weak correlation between pain intensity and pain behavior (McCahon et al. 2005).



Courtesy of Dr. Esa Kotilainen, Turku University Hospital

FIGURE 2. *Transpedicular fusion L4-S1 using Variable Angle Screw (VAS) (Synthes GmbH, Zuchwil, Switzerland) and decompression of the neural elements.*

The visual analogue scale (VAS)/graphic rating scale (GRS) is a line with the defined end points of "no pain" and "pain as bad as it could be" (Huskisson 1974). The GRS gives additional descriptive terms such as "mild", "moderate", and "severe" on a line that is preferably 10 or 15 cm long or with numerical scaling. These measures have correlated well with other self-reporting measures and were found to be sensitive to treatment effects (Jensen et al. 1986).

For the purpose of telephone interviews, when pain needs to be evaluated verbally, the numerical rating scale (NRS) or verbal rating scale (VRS) can be useful.

The most commonly used tools for assessing disability that is caused by back pain is the Roland-Morris Disability Questionnaire (RMDQ) (Roland and Morris 1983) and the Oswestry Disability Index (ODI) (Fairbank et al. 1980). Both of these tools have been validated in nine languages. The RMDQ has been shown to detect changes over time with a higher sensitivity than the ODI (Beurskens et al. 1996). With regard to functional status, the ODI is useful in specialty care settings or in situations in which the disability level is likely to remain relatively high throughout a trial.

According to the World Health Organization (WHO), its Quality of Life (WHOQOL) questionnaire should assess people's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards, and concerns. The questionnaire consists of 100 questions in six main fields, covering such aspects as physical, psychological, level of independence, social relationships, environment, and spirituality/religion (Group 1996).

WHO has developed a tool for a new, unified, holistic approach with which to describe the im-

pact of a disease on a patient's functioning, covering different areas of health and disability, the International Classification of Functioning, Disability and Health (ICF). These domains are classified from a list of body functions and structure, as well as a list of domains of activity and participation, including a list of environmental factors. The ICF was officially endorsed by all 191 WHO Member States in the Fifty-fourth World Health Assembly on 22 May 2001. The ICF is a highly standardized, comprehensive, qualitative and quantitative framework for measuring functional limitations at both the individual and population levels. While the conventional approach determines a patient's level of functioning as a sum of social, psychological, and physical functional limitations (emphasizing the level of disability), the ICF describes altered functioning using a logical chain which starts with the impairment of body structure and function and results in the limitation of a person's participation in personal or social life. The ICF also takes into account the possible positive and negative influence of environmental factors. The practical implementation of the ICF has been delayed due to its complexity. Simplified versions and checklists have been introduced for the ICF, and a core set for evaluating low-back pain was introduced in 2004 (Cieza et al. 2004). Other frequently used standardized tools of health outcome are the EQ-5D by the EuroQol Group (Brooks 1996), the Psychological General Well-Being Index (PGWBI) (Dupuy 1984) and, specifically designed for scoliosis patients, the Scoliosis Research Society questionnaires SRS-22/-30 (Haher et al. 1999, Baldus et al. 2011).

2.1.6. Radiological evaluation of fusion

The gold standard for the assessment of spinal fusion is even today direct surgical exploration of the fusion site (Cleveland et al. 1948, Kant et al. 1995). In clinical practice, plain radiographs are commonly used to assess fusion, yielding a precision of 68% (Kant et al. 1995). Especially cumbersome is the interpretation of radiographs if anterior titanium cages are used, warranting a thin-slice (2 mm) CT scan with sagittal and coronal reconstructions with which to examine for the possible presence of bridging bone and the ruling out of a locked pseudoarthrosis (Carreon et al. 2008). CT scans and functional radiographs taken in flexion and extension increase the sensitivity in posterolateral fusions, but fusion evaluation based on radiographs agrees with CT scans only in 50% of the cases (Carreon et al. 2007).

2.1.7. Randomized clinical trials

Until recent years, prospective randomized controlled trials comparing non-operative and operative treatment, as well as different forms of operative management for thoracic spine fractures, have not been available. As in most areas of neurological trauma, significant controversy still remains regarding the best treatment for a given injury. In many cases, we have to rely on the class III and class II evidence that is available. In a recent study, the anterior approach was compared with posterior fusion for thoracolumbar burst fractures. The authors found significantly less intraoperative blood loss and complications, a shorter operative time, and better pulmonary function after operations with the posterior approach (Lin et al. 2011). In a study in which AO Type A spine fractures without neurological deficit were randomized into conservative treatment or short-segment posterior stabilization, the result for operative treatment was superior for all of the functional outcome measures (Siebenga et al. 2006). When conservative treatment options were randomized for thoracic and lumbar compression fractures, brace treatment with supplementary physical therapy scored significantly better on a VAS and ODI than did plaster of Paris cast treatment (Stadhouder et al. 2009). The latest Cochrane database review identified 31 randomized controlled trials on surgery for degenerative lumbar spondylodesis published by 31 March 2005. Most of the studies still compared different surgical techniques, and few answered the question of whether surgery provides effective relief for presenting symptoms. There was conflicting evidence on the clinical effectiveness of fusion. Instrumentation seemed to produce a higher fusion rate, but any improvement in clinical outcomes was probably marginal (Gibson and Waddell 2005). In a meta-analysis of randomized clinical trials on techniques of spine

fusion in lumbar spondylosis (PLIF or ALIF), the authors found a greater fusion rate and less bleeding when fused by the interbody technique than when posterolateral fusion was used. On the other hand, posterolateral fusion outperformed interbody fusion in operative length and perioperative complications. No difference was found for the outcome measures (ODI) return to work, residual lumbar pain, and lower-limb pain (Umeta and Avanzi 2011).

In a recent study, posterior interbody fusion or posterolateral fusion was used with decompression and transpedicular instrumentation for low-grade isthmic spondylolisthesis with an average follow-up of 3.3 years. In the clinical evaluations, good or excellent results were obtained for 88% of the patients in the PLIF group and 76% of those in the PLF group. The fusion ratios were 100% for the PLIF group and 84% for the PLF group. Both lumbar lordosis and the segmental angle showed greater improvement in the PLIF group. The authors found no difference in the complication rates of each group (Musluman et al. 2011).

There is still insufficient evidence on the effectiveness of surgery according to clinical outcome, and no firm conclusions can be drawn. High-quality randomized clinical trials are required that compare surgical treatment with placebo or conservative treatment while bearing in mind the natural history of degenerative disease of the spine.

2.2. Bone grafting and bone graft substitutes

The challenge in spinal fusion is to fuse an anatomic region with bone that is not normally supported by viable bone. With respect to the treatment of injuries, disease, and deformities of the spine, it is essential to understand its constituent parts, bone being the main one, along with muscles, tendons, neural structures and blood supply, together with an understanding of altered states of bone metabolism.

The structure of bone is constantly being reorganized and remodeled. According to the law of Wolff, bone can adapt to changes in external load with an increase in bone matrix due to an increased load, but also resorption of bone can occur if sufficient load is not present (Wolff 1986). The essential cell types important to bone growth and formation are osteogenic precursor cells, osteoblasts, osteoclasts, osteocytes, and the hematopoietic elements of bone. Resorption of bone is caused by osteoclasts, and new bone is produced by unmineralized organic matrix (osteoid) that is secreted by osteoblasts. The mineralization gives bone its strength and rigidity (Buckwalter et al. 1996a, Buckwalter et al. 1996b).

Bone is often misconceived as being only a rigid structural element, but it is now known that it is in constant change that is orchestrated by hormonal impulses. Calcified bone consists of two main components, the organic extracellular matrix and the mineralized substance. The inorganic mineral substance of bone consists of biological apatite which accounts for 70% of the mass and 50% of the volume of bone (Aerssens et al. 1994). Since almost all of the body calcium resides in the skeleton, homeostasis is maintained by the uptake or release of calcium from bone, together with the interplay of intestinal absorption and renal excretion. Serum calcium levels are important for the maintenance of normal cellular functions, and they are regulated by the parathyroid hormone 1,25-dihydroxy vitamin D and calcitonin.

2.2.1. Autogenous and allogeneic bone grafting

Autograft is considered the gold standard of bone grafts because it contains the patient's natural combination of osteogenic, osteoconductive, and osteoinductive factors that help stimulate bone formation and fusion. Cancellous autografts are rich in cells and growth factors but have a low weight-bearing capacity, making it especially suitable for the filling of structural grafts. Cortical autograft is more suitable when structural support is needed, although with slow incorporation due to the limited number of bone marrow cells. Autogenous bone, such as strut graft, tricortical graft, bicortical graft, unicortical graft, and cancellous pieces, can be harvested from the anterior and posterior iliac crest, depending on patient positioning for surgery. The bone harvesting procedure is associated with a prolonged

operation time and increased blood loss, and it causes additional postoperative pain longer than the time needed for the spine to heal after surgery (Banwart et al. 1995, Robertson and Wray 2001). Since the supply of the optimal bone graft is limited and there are complications associated with the harvesting of autogenous bone, allograft bone is an alternative. By definition, it is bone harvested within the same species, tested, preserved, and sterilized. The osteoinductive properties are reduced, and the osteogenic properties are eliminated by the required processing. An allograft is remodeled to new bone by creeping substitution, which is a slow process resembling that of fracture healing or bone infarction (Ehrler and Vaccaro 2000). Recent changes in the legislation concerning bone banking within the European Union (EU Tissues and Cells Directive 2004/23/EC) has made the use of allograft more demanding in terms of cost and administration.

2.2.2. Calcium phosphate ceramics

Synthetic bone-graft extenders have gained popularity due to the unlimited supply and lack of risk for disease transmission associated with allografts. Among the synthetic bioactive components, β-tricalcium phosphate (β-TCP) has been used for decades in various orthopedic applications due to its osteoconductive and biodegradable nature (Bohner 2000). Although β-TCP has several favorable properties, it has poor mechanical properties and hence an elevated risk of fracture. Hydroxyapatite (HA)-based ceramics have been used clinically as bone graft expanders in posterior spinal surgery and as a bone graft substitute in cervical fusions. It has proven to be useful as a carrier for bone growth factors and as an augmenting coating on pedicle screws (Sandhu and Boden 1998, Spivak and Hasharoni 2001). Artificial bone graft substitutes (HA and β-TCP) seem to effectively promote posterolateral lumbar non-instrumented and instrumented fusions when added to autografts (Epstein 2008).

2.2.3. Composite grafts and bone marrow aspiration

Osteoconductive composites (e.g., Type 1 collagen/HA matrix) turn osteoinductive when soaked in bone marrow aspirate (BMA). In a clinical setting, the composite with BMA showed no significant difference from autografts in posterolateral fusion but was inferior in interbody fusion (Neen et al. 2006). A later study using the transforaminal approach for interbody fusion achieved anterior bridging in 20 of 22 patients (Carter et al. 2009). In a recent prospective randomized study, BMA was combined with calcium sulfate pellets to yield a fusion rate significantly inferior to iliac crest bone graft in one level lumbar posterolateral fusion (Niu et al. 2009).

2.2.4. Recombinant growth factors

Marshall R. Urist published his discovery of the bone inducing capability of bone morphogenetic proteins (BMP) in 1965, when implanting demineralized bone matrix (DBM) in the muscle of rabbits (Urist 1965). The low extraction rate of BMP from allograft led to the development of recombinant technology that provided unlimited and quality-controlled BMP. Two rhBMPs have been approved by the US Food and Drug Administration (FDA). rhBMP-2 has been approved for anterior interbody spinal fusions, and rhBMP-7 (rhOP-1) has been approved as an alternative to autograft for compromised patients undergoing revision surgery for posterolateral lumbar fusion.

The use of rhBMP has increased rapidly, from 5% of lumbar fusion cases in 2003 to 28% of fusion cases in 2008 (Deyo et al. 2012). The off-label use of rhBMP-2 in anterior cervical fusions has been found to be associated with inflammatory soft-tissue reactions, making it contraindicated for use with respect to the cervical spine (Smucker et al. 2006). Strong criticism has recently been directed towards some of the authors that have published articles on the use of rhBMP in industry-sponsored studies, suggesting possible study design bias in the original trials, as well as a clear increased risk of complications and adverse events with respect to patients receiving rhBMP-2 in spinal fusion (Carragee et al. 2011).

2.2.5. Bioactive glasses

Bioactive glasses (BGs) are a group of surface-active, composition-dependent, silica-based

biomaterials. The bone bonding properties of the two original BGs (Bioglass® 45S5 and S53P4) were first discovered by Larry Hench in the early 1970's (Hench et al. 1971). The relative proportion of the four main BG components (SiO_2, Na_2O, CaO, and P_2O_5) must be within a narrow range to get the desired biological response in bone and soft tissues (Hench and Wilson 1984, Hench 2006). Among other modifications, a system of Na_2O-K_2O-MgO-CaO-B_2O_3-P_2O_5-SiO_2 was introduced to achieve a larger working range to ensure better processing properties for BG fibers and microspheres (Pitkänen et al. 1995, Brink et al. 1997, Itälä et al. 2001). Since then, basic research has largely expanded, including the genetic design of BGs (Hench 2009), the manipulation of basic BG compositions (Rahaman et al. 2011), the introduction of new therapeutic ions and drugs into BG compositions (Hoppe et al. 2011), the combination of viral gene therapy with BG microspheres (Välimäki et al. 2005), improvement in the manufacturing techniques used for melt and sol-gel processes (Arcos and Vallet-Regi 2010, Wu et al. 2011), the production of composite scaffolds of polymers and bioactive ceramics (Rezwan et al. 2006) , the creation of porous BG scaffolds with an optimized nanostructure (Jones et al. 2006, Jones 2009), the creation of micro-roughness on BG surfaces (Itälä et al. 2003), and improvement in the mechanical properties of BG composites (Fu et al. 2011).

There is a vast variety of clinical applications for BGs, ranging from the middle-ear replacement of ossicles (Merwin 1986, Rust et al. 1996) and the filling of frontal sinuses and mastoid cells (Peltola et al. 2006, Stoor et al. 2010) to larger orbital and cranial reconstructive surgery utilizing casted plates and composites (Suominen and Kinnunen 1996, Kinnunen et al. 2000, Aitasalo et al. 2001, Peltola et al. 2008, Peltola et al. 2012). In a recent meta-analysis of the treatment of periodontal intrabony defects, BG outperformed active controls and open flap debridement (Sohrabi et al. 2012). BG has been used in several applications as bone filler in orthopedics (Lindfors et al. 2009, Lindfors et al. 2010b, Pernaa et al. 2011).

The first clinical publication on the use of BG S53P4 in the treatment of osteomyelitis in the lower extremities and spine showed that it was effective as a one-stage procedure with a favorable outcome for 10 of 11 patients, lasting for a mean of 24 months (Lindfors et al. 2010a).

There are only a few publications to date on the use of BG in instrumented spine surgery. In a study on lumbar spondylodesis using a HA-BG composite (Chitra-HABg) as graft material, a high resorption rate and poor consolidation was reported for 95% of the BG composite cases. The Chitra-HABG used in this study, which had to be terminated early, contained 80% HA and 20% of a BG (composition unknown). The outcome of this study was excellent with respect to autografts (Acharya et al. 2008).

An apatite-wollastonite-containing BG-ceramic (A/W glass-ceramic), developed at the Kyoto University in 1982, was used in 30 patients undergoing spine surgery. An implant manufactured as a load bearing implant was used for trauma, tumor surgery, and degenerative disease. In a 14.9-month follow-up, good bone formation was noted around the prosthesis (Yamamuro and Shimizu 1994).

A combination of BG 45S5 (Novabone®) and autograft bone was compared with autograft bone alone in the treatment of 88 patients with adolescent idiopathic scoliosis, the result being similar results to those obtained using autograft alone. The loss of correction of the main thoracic curve was slightly less for the BG group. Moreover, the blood loss and the complication rate were also significantly lower for the BG group (Ilharreborde et al. 2008).

In a recent prospective, randomized FDA-IDE trial, investigators compared the treatment results of 162 patients receiving Cortoss™ with that of 94 patients receiving poly(methy)methacrylate (PMMA) for vertebroplasty injection as treatment for vertebral compression fractures. Cortoss™ consists of 33% di-functional methacrylates (bis-GMA, TEGDMA, bis-EMA) that form a highly cross-linked, three-dimensional polymer, reinforced with 67% radiopaque and BG ceramic particles. Its mechanical properties closely match those of bone in compression.

A non-inferiority of Cortoss™, relative to that of PMMA was observed, and 87% of the Cortoss™-treated patients experienced significant pain relief at 3 months compared with 75% of the patients treated with PMMA.The improvement in function was greater in the Cortoss™ patients at 24 months, resulting in a difference of 8% (p=0,0299) (Bae et al. 2012).

2.2.5.1. Chemical compositions

The main component of melt-derived BGs is silica (SiO_2), which forms the basic network, and alkali metals or alkali earth metals act as modifiers of the biological properties (Table 1). BGs containing 45–52 wt% silica bond the fastest to bone and soft tissue, and higher levels of silica 55–60 wt% result in low bioactivity and a loss of bonding to soft tissue (Hench and West 1996). Sol-gel-derived glasses in the Na_2O-CaO-SiO_2 system show bioactivity in a broader composition range, up to 90 wt% SiO_2 (Li et al. 1991).

2.2.5.2. In vitro testing of bioactivity

The engineering of different properties of bone bonding materials requires that the material must be bioactive. There are two ways to test bioactivity in vitro: chemical testing in physiological solutions for analyses of reaction layers, ion dissolution, and ion exchange and biological testing in cell cultures.

One essential requirement for an artificial material is the formation of bonelike apatite. The formation of apatite seen in vivo can be reproduced in vitro. The traditional way of testing bioactivity in vitro is to soak the biomaterial in a "physiological solution". There are several different protocols and solutions in use, but the most used are simulated body fluid (SBF) (Kokubo 1991), tris(hydroxymethyl)aminomethane buffer (Tris-buffer), and phosphate-buffered saline (PBS). After the biomaterial has been soaked in physiological solution for different time periods, the apatite formation can be analyzed in surface and cross-sections of the implants with, for example, a scanning electron microscope with energy dispersive X-Ray analysis (SEM-EDXA). Ion concentrations in the immersion solutions can be measured spectroscopically. An examination of apatite formation on a material in SBF is a useful method of predicting the in vivo bone bioactivity of a material, and the number of animal experiments can be reduced (Kokubo and Takadama 2006). Still, these tests are limited in predicting the whole biological response, since they only provide information at the chemical level.

In the further investigation of the biocompatibility and bioactive properties of bone-bonding material, the growth and differentiation of osteogenic cells can be of value. Traditionally, different osteogenic cell lines have been used, but today primary progenitors are more widely used, since they provide a representative response to biomaterials. Mesenchymal stem cells (MSCs) derived from human and animal bone marrow are widely used, as also human adipose stem cells (hASCs) are (Haimi et al. 2009). Immortalized cell lines are still in use to some extent, even though there is some uncertainty of the clinical value that they

Table 1. *Compositions of various bioactive glasses (expressed as weight-%)*

Glass	Na_2O	SrO	TiO	K_2O	MgO	CaO	B_2O_3	Al_2O_3	P_2O_5	SiO_2
45S5/Bioglass®	24,5	0,0	0,0	0,0	0,0	24,5	0,0	0,0	6,0	45,0
S53P4/Bonalive®	23,0	0,0	0,0	0,0	0,0	20,0	0,0	0,0	4,0	53,0
StronBone®	26,2	3,8	0,0	0,0	0,0	18,7	0,0	0,0	9,7	41,5
Biorestore®	11,1	0,0	0,4	16,2	3,1	13,8	1,3	0,0	3,1	51,0
1-98	6,0	0,0	0,0	11,0	5,0	22,0	1,0	0,0	2,0	53,0
13-93	6,0	0,0	0,0	12,0	5,0	20,0	0,0	0,0	4,0	53,0

represent (Modglin et al. 2012). The parameters analyzed from the cell culture testing of biomaterials include cell attachment, viability and apoptosis, and the osteogenic differentiation of MSCs and ASCs (evaluated by alkaline phosphatase (ALP) expression and mineralization by von Kossa staining for example). Testing biocompatibility in cultures with MSCs or primary human osteoblasts provides important additional information on the interaction between cells and biomaterial. This phenomenon is important since in vivo it is the surrounding cells that dictate the outcome.

2.2.5.3. Bone bonding and bone turnover

The reactivity of the BG surface, resulting in a controlled release of biologically active soluble silica and calcium ions, is the key trigger mechanism of the recruitment and activation of osteoprogenitor cells and the rapid formation of a silica-rich hydroxycarbonate apatite surface layer for chemical bonding with on-growing new bone (Hench 2009). Hench and coworkers suggested the following surface-reaction stages in bone generation and repair (Hench et al. 2004):

1. Formation of Si-OH and the release of soluble silica in the form of $Si(OH)_4$
2. Polycondensation of Si-OH +OH-Si = Si-O-Si +H_2O (hydrated silica gel)
3. Adsorption of amorphous Ca^{2+}, PO_4^{3-} and CO_3^{2-} groups
4. Crystallization of hydroxyl-carbonate-apatite (HCA).

The original notion of Hench was that a relative proportion of the four main BG components of the SiO_2-Na_2O-CaO-P_2O_5 system must be within a narrow range in order to get the desired osteopromotive response without toxicity (Wilson et al. 1981). In addition, preclinical studies have shown that the BG surface is not only conductive, but also osteopromotive in facilitating migration, replication, and the differentiation of osteogenic cells and their matrix production (Välimäki and Aro 2006). The cellular response in defects filled with BG granules was characterized by a continuous overexpression of type III collagen and osteogenic mesenchymal cells prior to their differentiation to osteoblasts, organized as a dense periosteum-like layer on the surface the BG granules (Virolainen et al. 1997). Within hours in human primary osteoblasts BG 45S5 has shown the activation of several genes encoding nuclear transcription and growth factors (Xynos et al. 2000, Xynos et al. 2001).

2.2.5.4. Antibacterial effects

From the clinical point of view, BGs seem to have several unmatched properties as a bone graft substitute. The antibacterial properties are attributed to an increased osmotic pressure caused by the ions leaching from the surface of the glass. The surface reaction leading to the production of sodium hydroxide leads to an increase in the pH and initially contributes to the antibacterial effect of BG (Gubler et al. 2008). The early results of BG S53P4 showed a bacteriostatic effect in experimental and clinical otorhinological use (Stoor et al. 1998) Furthermore, BG has been shown in vitro to have effective bacterial growth inhibiting properties towards 17 anaerobic bacteria (Leppäranta et al. 2008), as well as bactericidal effects on 29 clinically important aerobic bacteria (Munukka et al. 2008).

The first clinical publication on the use of BG S53P4 in the treatment of osteomyelitis in the lower extremities and spine showed that it is effective as a one-stage procedure with a favorable outcome in 10 out of 11 patients, the effect lasting a mean of 24 months (range 10–38 months) (Lindfors et al. 2010a). BG can be tailored for a more specific antibacterial effect by adding gallium ions (Ga^{3+}), which have been shown to decrease bacterial iron (Fe^{2+}) uptake and interfere with Fe signaling in *Pseudomonas aeruginosa* (Kaneko et al. 2007, Valappil et al. 2009). The continuous release of silver ions (Ag^{+}) is bactericidal, and it has been successfully added to BG without the bioactivity being destroyed (Bellantone et al. 2000, Clupper and Hench 2001, Bellantone et al. 2002).

2.2.5.5. Angiogenetic effects

Angiogenesis is a complex process that forms new blood vessels. In addition, it is a necessity for wound healing and plays a pivotal role in tissue engineering.

BG 45S5 has been shown to significantly increase the endothelial cell proliferation of vascular endothelial growth factor (VEGF) in conditioned medium from human fibroblasts (Day 2005, Keshaw et al. 2005). When BG was compared with poly (D,L lactide) (PDLLA), a higher vascularization occurred in BG-containing scaffolds, and the difference was fivefold when the VEGF levels were compared with PDLLA films (Gerhardt et al. 2011). Leu and coworkers made the interesting finding that BG promotes bone healing in irradiated calvarial defects among rats through an increase in angiogenic response (Leu et al. 2009).

2.2.6. Clinical complications of bone grafts or graft substitutes

Allografts are associated with the risk of disease transmission; cases of human immunodeficiency virus (HIV) and hepatitis C (HCV) infections have been reported in the literature (Simonds et al. 1992, Tomford 1995). The processing of an allograft by freeze-drying and sterilizing with ethylene oxide or gamma irradiation may further diminish the osteoinductive and mechanical properties (Zimmermann and Moghaddam 2011). Synthetic bone grafts like rhBMP have been found to be associated with life-threatening inflammatory soft-tissue reactions in cervical anterior fusion, retrograde ejaculation, radiculitis, and subsidence of an implant in lumbar fusion surgery (Carragee et al. 2011). The biodegradation of β-TCP can be too fast and therefore result in non-unions, since the bone mass could still be immature at 6 weeks. HA is relatively inert, and its slow resorption could hinder bone remodeling (Sandhu and Boden 1998). In general, it can be stated that, in cases of an infection in a surgical field all bone graft substitutes will be affected as if they were foreign objects, except for BG, which has antibacterial properties and can potentially withstand bacteria to some extent.

2.3. Pre-clinical studies of spinal fusion

2.3.1. Animal models

The use of quadrupeds in spine research is often criticized due to the horizontal position of the spine when compared with the position in humans. A biomechanical analysis of the spine showed that the main load is, in fact, axial and that trabeculae in goat spine are oriented between the endplates and the vertebrae themselves, having a higher density than that of humans (Smit 2002).

The use of non-human primates for spine research is considered ideal in establishing the burden of proof; this practice is difficult however to carry out in practice (Drespe et al. 2005). The most commonly used model for the posterior approach is the New Zealand white rabbit (Boden et al. 1995). The range of motion (ROM) of sheep spines for the different load directions is qualitatively similar to those of humans (Wilke et al. 1997). The differences in disc height in the cervical spine and the significant difference in the stiffness of the spine have been shown in radiographic and biomechanical studies (Kandziora et al. 2001). Canine models are suitable for different spinal techniques, lumbar fusion being the most common (Cook et al. 1995).

Table 2 summarizes the results of experimental studies on bone graft substitutes in the rabbit lumbar fusion model.

2.3.2. Evaluation of fusion

The evaluation of fusion in animal models is multimodal. In addition to regular imaging modalities available for human use (X-ray, CT, MRI), peripheral quantitative computed tomography (pQCT) and high-resolution micro-computed tomography (μCT) are available. Since the specimens are available after the termination, biomechanical analysis is performed as plain manual palpation, or for more precise and quantitative information pull-apart testing or multidirectional flexibility testing is used (Erulkar et al. 2001).

Table 2. *Bone graft substitutes used for experimental spinal fusion in rabbit.*

Author Year	Investigated material	Timepoint (weeks)	Radiology/ MP	Fusion rate	Foreign-body reaction
(Walsh et al. 2011)	AB	12	CT	92%(n=6)	No overt inflammation or presence of giant cells observed.
	AB	24	CT	86%(n=6)	
	Poly(Lactide-co-Glycolide) with	12	CT	67% (n=7)	
	Hyaluronic Acid	24	CT	71% (n=7)	
(Tanaka et al. 2011)	AB	12	CT	67%(n=12)	No inflammation or FBR around the surgical site
			MP	58%	
	Single strip	12	CT	17% (n=12)	
	u-HA/ PdlLA		MP	17%	
	Morselized	12	CT	67% (n=12)	
	u-HA/ PdlLA (BMA)		MP	75%	
	Single strip	12	CT	92% (n=12)	
	u-HA/ PdlLA (BMA)		MP	92%	
(Matsumoto et al. 2011)	AB	8	MP	20% (n=10)	No robust inflammation
	ß-TCP PLA-PEG+	8	MP	0% (n=6)	
	rhBMP-2(0µg)	8	MP	0% (n=6)	
	rhBMP-2(30µg)	8	MP	40% (n=10)	
	rhBMP-2(60µg)	8	MP	100% (n=10)	
	rhBMP-2(120µg)	8	MP	100% (n=10)	
	sham	8	MP	0% (n=6)	
(Huang et al. 2011)	AB	6	MP	60% (n=5)	Histology randomly on 40%. No FBR was reported.
		12	MP	100% (n=5)	
	Poly(Lactide-co-Glycolide) /HA/	6	MP	60% (n=5)	
	Collagen+MSC	12	MP	100% (n=5)	
(Dodds et al. 2010)	AB	18	X-ray	75% (n=8)	Not reported
	Bioset			69% (n=7)	
	Pro Oston			30% (n=9)	
	Bioset/AB 50%			56% (n=9)	
(Urrutia et al. 2010)	AB	8	X-ray	53%(n=15)	Not reported
	BMSC		MP	0% (n=12)	
(Chen et al. 2009)	AB	6	MP	60% (n=5)	Not reported
		12		100%(n=5)	
	MSC/Pluronic F127/	6		60% (n=5)	
	coralline HA hybrid graft	12		100%(n=5)	
(Dohzono et al. 2009)	AG	8	CT	23% (n=8)	Not reported
	BMP 5µg/β-TCP	8		32% (n=8)	
	BMP15 µg/β-TCP	8		55%(n=8)	
	BMP50 µg/β-TCP	8		85% (n=8)	
	BMP150 µg/β-TCP	8		87% (n=8)	
(Walsh et al. 2009)	AB	6	CT	50% (n=3)	A mild inflammatory/ FBR was present adjacent to the mineral and collagenous phases at 6 weeks, although this subsided by 12 weeks
		12		75% (n=6)	
	collagen/HA/β -TCP	6		N/A	
		12		N/A	
	collagen/HA/β –TCP+BMA	6		92% (n=12)	
		12		N/A	
	collagen/HA/β -TCP +BMA+AB	6		92% (n=12)	
	sham	12		N/A	
		6		N/A	
		12		N/A	
(Smucker et al. 2008)	AB	6	MP	63% (n=8)	No detection of adverse inflammatory reaction
			X-ray	55%	
	HA/β -TCP		MP	33% (n=10)	
			X-ray	66%	
	HA/β –TCP+ B2A 50 µg		MP	78% (n=9)	
			X-ray	88%	
	HA/β –TCP+ B2A 100 µg		MP	89% (n=9)	
			X-ray	89%	
	HA/β –TCP+ B2A 300 µg		MP	80% (n=10)	
			X-ray	80%	
	Sham		MP	0% (n=10)	
			X-ray	0%	
(Motomiya et al. 2007)	AB	5	MP	100% (n=6)	Not reported
	HA 15% porosity +AB		MP	62% (n=11)	
	HA 50% porosity +AB		MP	75% (n=9)	
	HA 85% porosity +AB		MP	62% (n=10	

Table 2. *Bone graft substitutes used for experimental spinal fusion in rabbit.*

Author Year	Investigated material	Timepoint (weeks)	Radiology/ MP	Fusion rate	Foreign-body reaction
(Lawrence et al. 2007)	AB+nicotine 4,5µg/kg/min Re-spondylodesis: No graft AB +nicotine 4,5µg/kg/min rhBMP-2 (2.25mg)/ACS +nicotine 4,5µg/kg/min rhBMP-2 (3.0mg)/CRM +nicotine 4,5µg/kg/min	5	MP MP MP MP MP	3% (n=72) 6%(n=16) 29%(n=17) 100%(n=15) 100%(n=16)	Not reported (3 samples were lost)
(Minamide et al. 2007)	AB MSC MSC+5µg BMP MSC+12.5µg FGF MSC+12.5µg FGF	6	MP X-ray MP X-ray MP X-ray MP X-ray MP X-ray	57%(n=7) 57% 0%(n=7) 0% 29%(n=7) 29% 43%(n=7) 43% 86%(n=7) 86%	Not reported
(Choi et al. 2007)	AB DBX strips DBX strips+AB	9	MP X-ray MP X-ray MP X-ray	38% (n=16) 69% 94% (n=16) 100% 100% (n=16) 100%	No evidence of any chronic or acute inflammation in any of the groups.
(Magit et al. 2006)	AB collagen/HA coating collagen/HA coating+ rhGDF-5 0,5mg/ml collagen/HA coating+ rhGDF-5 1.0 ,mg/ml collagen/HA coating+ rhGDF-5 1.5mg/ml	8	MP X-ray MP X-ray MP X-ray MP X-ray MP X-ray	38%(n=13) 54% 0% (n=13) 0% 100% (n=13) 77% 100% (n=13) 85% 100% (n=13) 92%	Islands of residual carrier, mixed into a fibrous stroma and neutrophils.
(Hile et al. 2006)	AB PPF scaffold PPF scaffold +AB	6	MP X-ray MP X-ray MP X-ray	40% (n=5) 60% 50% (n=6) 50% 67% (n=6) 67%	No significant inflammatory changes where noted.
(Namikawa et al. 2005)	AB ß-TCP/PLA-DX-PEG+ rhBMP-2(0µg) ß-TCP/PLA-DX-PEG+ rhBMP-2(7.5µg) ß-TCP/PLA-DX-PEG+ rhBMP-2(15µg) ß-TCP/PLA-DX-PEG+ rhBMP-2(30µg)	6	MP X-ray/CT MP X-ray/CT MP X-ray/CT MP X-ray/CT MP X-ray/CT	40%(n=5) 100% 0% (n=5) 0% 29%(n=5) 80% 100% (n=5) 100% 100% (n=5) 100%	Not reported
(Minamide et al. 2005)	AB collagen/HA+ rhBMP-2(100µg) collagen/HA+ low number BMC(cult.) collagen/HA/+ high number BMC(cult.)	6	MP X-ray MP X-ray MP X-ray MP X-ray	57% (n=7) 57% 100% (n=7) 100% 0% (n=7) 0% 71% (n=7) 71%	No signs of foreign bodies, such as intervening soft tissue.
(Kraiwattanapong et al. 2005)	collagen/HA/+BMA collagen/HA/β –TCP+ rhBMP- 2(1.29mg)	8	MP X-rayCT MP X-ray/CT	0% (n=12) 0% 100% (n=12) 100%	There was not a substantial inflammatory response present in either group. Some necrotic muscle and soft tissue was seen.

Table 2. *Bone graft substitutes used for experimental spinal fusion in rabbit.*

Author Year	Investigated material	Timepoint (weeks)	Radiology/ MP	Fusion rate	Foreign-body reaction
(Cinotti et al. 2004)	AB	8	MP X-ray	25% (n=8) 25%	Not reported
	HA/β –TCP+MSC		MP X-ray	57% (n=7) 86%	
	HA/β –TCP+BMA		MP X-ray	38% (n=8) 50%	
	HA/β –TCP		MP X-ray	10% (n=10) 30%	
(Yee et al. 2003)	AB 0.7g	9	MP X-ray	0% (n=22) 9%	Not reported
	AB 1.4g		MP X-ray	13% (n=23) 42%	
	DBX–Hyaluronan 0.7g+ AB 0.7g		MP X-ray	13% (n=23) 57%	
	DBX–Hyaluronan 1.4g + AB 0.7g		MP X-ray	38% (n=21) 86%	
(Louis-Ugbo et al. 2002)	BCP+ rhBMP-2	5	MP X-ray	100% (n=9) 100%	Not reported
	3BCP/collagen+ rhBMP-2		MP X-ray	100% (n=9) 100%	
(Cheng et al. 2002)	Allograft HA/β –TCP	7	MP Microrad. MP	100% (n=6) 0% 100% (n=6)	Not reported
	HA/β –TCP+ rhBMP-4(1.25µg)		Microrad. MP	0% 100% (n=6)	
	HA/β –TCP+ rhBMP-4(5µg)		Microrad. MP Microrad.	50% 100% (n=6) 100%	
(Lindfors et al. 2002)	AB	4 12	CT	25% (n=4)* 50% (n=4)	Not reported
	S53P4 30%+AB 70%	4 12		25%(n=4) 75% (n=4)	
	S53P4 100%	4 12		100% (n=4) 75% (n=4)	
(Minamide et al. 1999)	AB	6	MP X-ray	40% (n=5) 60%	Not reported
	TBC		MP X-ray	0% (n=5) 20%	
	TBC/collagen		MP X-ray	20% (n=5) 40%	
	TBC/collagen+ rhBMP-2 100µg		MP X-ray	100% (n=5) 80%	
(Boden et al. 1999)	coralline HA + AB coralline HA + BMA coralline HA + rhBMP-2	5	MP	50% (n=14) 0% (n=14) 100% (n=11)	There was no inflammatory reaction to the carrier
(Tay et al. 1998)	AB collagen/HA collagen/HA+BMA collagen/HA+heparinized BMA	8	X-ray	75% (n=12) 18% (n=11) 100% (n=10) 100% (n=9)	Not reported
(Silcox et al. 1998)	AB rhBMP-2+DMX rhBMP-2 + AB	5	MP	0% (n=16) 64% (n=14) 100% (n=14)	No histology
(Morone, Boden 1998)	AB DBX+ AB (50:100) DBX+ AB (50:50) DBX+ AB (75:25)	6	MP	73% (n=11) 73% (n=11) 70% (n=7) 67% (n=9)	Not reported
(Schimandle et al. 1995)	AB rhBMP-2 rhBMP-2 rhBMP-2	5	MP	42% (n=14) 100%(n=14) 100%(n=14) 100%(n=14)	Not reported
(Boden et al. 1995)	AB at different timepoints	4 5 6 10	X-ray	20% (n=5) 40% (n=5) 50% (n=6) 38% (n=8)	Not reported

FBR=foreign body reaction, MP=manual palpation, u-HA/PdlLA=hydroxyapatite/ polydllactide, PEO-PPO-PEO=poly(ethyleneoxide)-poly(propylene oxide)-poly (ethylene oxide), MSC=mesenchymal stem cell, B2A=synthetic peptide B2A2-K-NS, ACS=absorbable collagen sponge carrier, CRM=compression resistant matrix, BCS=bovine collagen hydroxyapatitetricalcium, PCC=phosphate composite carrier, FGF=fibroblast growth factor, DBX=demineralized bone matrix, rhGDF-5= recombinant human growth and differentiation factor-5, PPF poly(propylene glycol-co-fumaric acid), PLA-DX-PEG polymer=poly-D,L-lactic acid-p-dioxanone/ polyethylene glycol block copolymer, BCP biphasic calcium phosphate.

Histology with appropriate stainings on decalcified or non-decalcified samples gives precise information on bridging bone and characteristics. Histomorphometric methods quantify the fusion mass. Reverse transcriptase polymerase chain reaction is one of the molecular techniques used to analyze the different factors related to the fusion process (Morone et al. 1998).

2.3.3. Comparison of bone grafting procedures

Bone grafting is limited for smaller species. Especially if BMA is needed, a larger animal is required. In addition to the anterior and posterior part of the iliac crest, the caudal part of the sternum can be used for bone grafting in anterior procedures in goat and sheep due to their sternal protecting fat pad (Drespe et al. 2005).

3. AIMS OF THE STUDY

This study started as the pre-clinical phase of a TEKES (National Agency for Technology and Innovation) sponsored, multi-institutional research project on load-bearing biodegradable implants made of composite fibers (contract 40172/06). The pre-clinical studies were intended as tests for the surgical performance and osseointegration of the composites and the evaluation of fusion in the spinal model.

The prospective clinical trials involved investigating the use of BG granules as bone graft substitutes in lumbar spondylodesis in both degenerative and trauma spine surgery.

The following issues were addressed:

1. An intra-animal comparison of biodegradable woven fabrics made of bioactive glass (BG 1–98) fibers and poly(L-lactide-co-glycolide) 80/20 copolymer ($PLGA_{80}$) fibers, or $PLGA_{80}$ fibers alone, in the surgical stabilization of bone graft.

2. The in vitro and in vivo performance of three different biodegradable woven fabrics. The fabrics had a bioactive BG 1–98 component and were designed to act as osteoconductive and osteopromotive surfaces for autogenous bone grafting in an established model for posterolateral intertransverse lumbar arthrodesis in the rabbit.

3. The long-term clinical and radiological findings in patients with degenerative spondylolisthesis treated with instrumented spondylodesis using BG S53P4 as bone graft substitute.

4. The long-term clinical and radiological findings for patients with unstable lumbar spine fractures treated with instrumented spondylodesis using BG S53P4 as bone graft substitute.

4. MATERIALS AND METHODS

4.1. Biomaterials

4.1.1. Bioactive glass compositions

In the pre-clinical study, BG 1–98 fibers were used: SiO_2 53%, NaO_2 6%, CaO 22%, K_2O11%, MgO 5%, P_2O_5 2%, B_2O_3 1% by weight. In the clinical trials commercially available BG S53P4 53% SiO_2, 23% Na_2O, 20% CaO, 4% P_2O_5 granules were used.

4.1.2. Preparation of bioactive glass 1–98 fiber composites

Biodegradable fabrics measuring 15 mm x 45 mm were manufactured by weaving (Department of Biomedical Engineering, Tampere University of Technology, Finland). For the weaving, $PLGA_{80}$ fibers were drawn by extruding granules to rods. The rods were chopped to pellets and drawn to monofilament fibers (200 µm in diameter). The control fabric ($PLGA_{80}$ fabric) was manufactured as a plain weave with a canvas-type structure. For the BG 1–98/$PLGA_{80}$ fabric, BG fibers with a diameter ranging from 20 to 30 µm were prepared from BG 1–98 by heating BG blocks in a platinum crucible, and fibers were drawn from the glass melt at 950˚C and coated with $PLGA_{50}$ to protect the BG fibers throughout the manufacturing and sterilization process. For the woven structures, $PLGA_{80}$ monofilaments were used as warp threads. BG fibers were multiplied to threads containing 70–140 filaments and used as weft threads.

The weaving started and ended with $PLGA_{80}$ monofilament weft threads that were fused using ultrasonic welding to seal the ends of the fabric and stabilize the structure. It was done manually using a small-scale loom. To further stabilize the BG fabrics, a $PLGA_{50}$ membrane was attached to the fabrics (Figure 3A). The fabrics were gas-sterilized at 55˚C, or gamma sterilized using 25 kGy.

To form a 3D composite plate, nine hybrid knitwear and ten PLA_{96} knitwear were stretched biaxially and piled in alternating layers in a metal frame. A 3-mm thick porous plate was obtained by putting the frame in a planar mold. It was then initially compressed, heated to 140º C, and further subjected to 10 MPa pressure against limiter sleeves, and finally cooled to room temperature (Figure 3B).

4.1.3. Preparation of S53P4 bioactive glass granules

S53P4 BG granules were prepared from a crushed fraction of S53P4 plate. The batch consisted of European Pharmacopoeia (Ph.Eur) analytical grade reagents, Na_2CO_3, $CaCO_3$, ($CaHPO_4$) ($2(H_2O)$) and commercial crushed quartz (Norwegian Quartz 99.99% pure) as silica raw material. The glass was melted, cast, annealed, crushed at 520°C for 1 hour, allowed to cool to room temperature, and re-melted using the same procedure to ensure homogeneity. Finally, the glasses were crushed and sieved to the desired fractions.

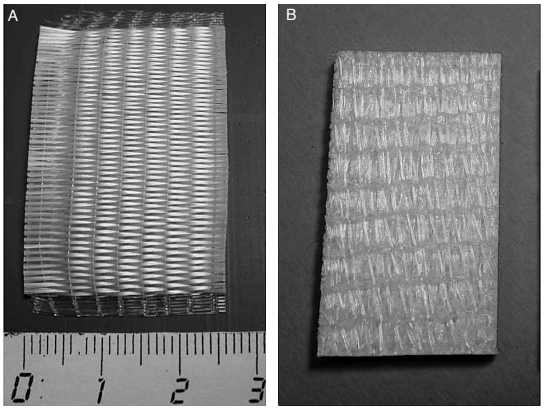


FIGURE 3. *Biodegradable BG1–98/$PLGA_{80}$ fabric was manufactured by weaving.* BG 1–98 fibers (diameter 20–30 µm, coated with $PLGA_{50}$) were multiplied to threads containing 70–140 filaments and used as weft threads. The $PLGA_{80}$ monofilament fibers (diameter 200 µm) were used as warp threads (3A). 3D composite plates were formed of multiple layers of fabric that was heated to 140º C and compression molded (3B).

4.2. Pre-clinical studies

4.2.1. In vitro testing using mesenchymal stem cell cultures

4.2.1.1. Cell culture testing

The tested materials included BG 1–98 fibers, BG 45S5 fibers, E-glass fibers, $PLGA_{80}$ fibers, PLA_{96} fibers, BG 1–98 discs, and biodegradable fabrics made of BG 1–98/$PLGA_{80}$ or $PLGA_{80}$ fibers.

Characterized passage 2 human-bone-marrow-derived MSCs from young donors (<25 years of age) were used (Alm et al. 2010). The cell-culture testing of the materials was performed in osteoblastic induction medium consisting of basal medium (αMEM with 10% fetal calf serum and 100 U/ml penicillin-streptomycin) supplemented with 10 mM sodium β-glycerophosphate and 0.05 mM ascorbic acid-2-phosphate and 100 nM dexamethasone during the first 7 days, as described previously (Alm et al. 2012).

One disc or 3–5 fibers were placed in a well of 24-well cell culture plates. 10 000 hMSCs in 50-µl culture medium were carefully added onto the materials. As controls, three parallel wells with only materials and culture medium (without cells) were used, in addition to cell cultures without material. Cell attachment and growth was studied by light microscopy and photographed. After 14 days, the cells were fixed and stained for alkaline phosphatase (ALP).

The pH of the cultures was measured at 3 days and after 1, 2, 3, 4, and 5 weeks, as described earlier. For the BG 1–98/$PLGA_{80}$ fabrics and titanium discs, adhered hMSCs were visualized at 3 days and again at 5 weeks by fixing the cells and staining for Hoechst and actin according to standard protocols.

4.2.1.2. Twelve-hour pH measurements

To carefully follow the changes in pH during the first 12 hours in the osteoblastic culturing medium, BG 1–98/$PLGA_{80}$ fabrics were incubated in 5-ml and 50-ml cell culture medium, and 3D composite plates were incubated in 5-ml cell culture medium at 37°C, 5% CO_2. The pH was measured every hour for 12 hours and then at 24 hours, 4 days, and 7 days. There were three parallel samples for each material, and the pH was measured twice from each sample.

4.2.2. In vivo testing of woven fabric made of bioactive glass fibers

4.2.2.1. Ethical approval of the animal experiments

The animal experiments were a part of a multi-institutional research project on load-bearing biodegradable implants made of composite fibers sponsored by National Agency for Technology and Innovation in Finland (TEKES) (contract #40172/06). Ethical consent for the study protocol was given by the Animal Ethical Committee of The Provincial State Office of Western Finland (permits #1342/03 and #1539/05). All of the procedures were carried out in accordance with the guidelines of the local Animal Welfare Committee.

4.2.2.2. Animal anesthesia and pain medication

All of the animal experiments were performed under general anesthesia. A standard intra-institutional perioperative protocol was followed. Preoperatively, a single prophylactic dose of 500 000 IU benzylpenicillin was given intramuscularly 30 minutes prior to the incision. Anesthesia was induced by using a subcutaneous injection of fentanyl citrate-fluanisone followed by another injection before the surgery. Functional activity was not limited postoperatively. The animals received standard postoperative pain medication for 3 days.

4.2.2.3. Experimental design

An intra-animal comparison of biodegradable woven fabrics made of BG 1–98/$PLGA_{80}$ fibers or a control fabric made of $PLGA_{80}$ fibers alone were alternately placed distally or proximally in a randomized order during the surgical stabilization of the bone graft (Figure 4). Altogether 7 adult male New Zealand white rabbits (HB Lidköpings Kaninfarm, Sweden), weighing 2.4–3.4 kg, were used. The bone graft attachment, the bone formation, and the integration of the fabrics were com-

pared at 12 weeks. In addition to qualitative X-ray and histological analyses, quantitative pQCT was performed using the contralateral intact femur as reference.

4.2.2.4. Surgical procedure

The left hind leg and lower back was shaved, disinfected, and draped. A dorsal skin incision was made over the iliac crest for the harvesting of the bone graft. The bone chips were divided into four equal aliquots, on averaging 3x4x4 mm each, for each fabric (Figure 4A). The exposed surface of the iliac bone was sealed with bone wax, and the wound was closed in layers. The lateral intermuscular approach was used to expose the femoral shaft. The periosteum was preserved. The bone grafts were placed against the dorsolateral femoral surface and the fabrics were wrapped around the bone and fixed with three resorbable sutures (Figure 4B). The surgical incisions were closed in layers.

4.2.2.5. Harvesting of specimen

After 12 weeks of follow-up, the animals were euthanized with sodium pentobarbital, and both femurs were harvested for analysis.

4.2.2.6. Peripheral quantitative computed tomography

For the quantitative evaluation of bone-graft healing and the integration of the fabrics, pQCT imaging was performed. The operated and intact femurs of each animal were imaged simultaneously with a Stratec XCT Research M device (Norland Statec Medizintechnik GmbH, Birkenfeld, Germany). For scanning, the femurs were fixed in a custom-made holder, and continuous cross-sectional images were obtained for the entire length. The slice distance was set at 1 mm, and a voxel size of 0.07x0.07x0.050 mm was used. The 3D images of the femurs were re-constructed from the pQCT data set.

For the quantitative pQCT analysis, pQCT images and histological sections were used together to select a representative volume of interest, consisting of seven images for each fabric. From the seven most representative images for each fabric, the cross-sectional total area (mm^2), the bone volume (mm^3) of the femur, and the area (mm^2) and volume (mm^3) of the intramedullary canal, as well as cortical thickness, were calculated. The strength strain index (SSI, expressed mm^3) was calculated to estimate the mechanical strength of the bone (Ferretti 2000). The intact contralateral femurs were used as paired controls.

4.2.2.7. High-resolution micro-computed tomography

For visualization of the time-related changes, one animal was scanned with pQCT 3 days after the surgery. After the harvesting at 12 weeks, the operated femur of the same animal was scanned with both high-resolution μCT (SkyScan 1072, SkyScan n.v., Kontich, Belgium) and pQCT imaging.

4.2.2.8. Histology

After the imaging, the operated femurs with deep soft tissue layers were fixed in 70% ethanol. The specimens were dehydrated and embedded in isobornyl methacrylate. The pQCT scans were

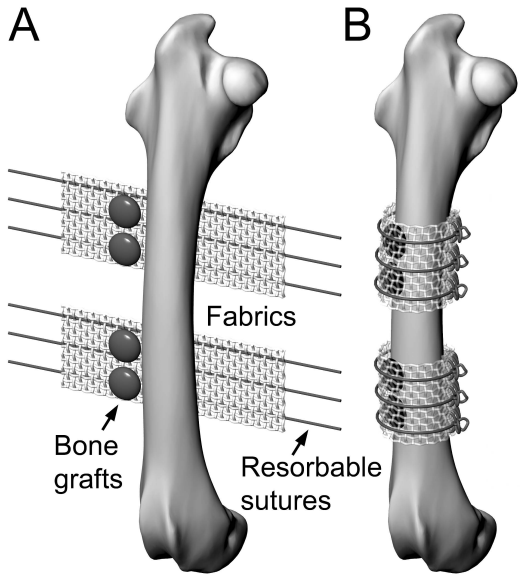


FIGURE 4. *Schematic drawing of the experimental model.* The bone grafts were placed on two woven fabrics (A), which were wrapped around the bone and fixed with resorbable sutures (B). The two fabrics (BG 1–98/$PLGA_{80}$ and $PLGA_{80}$) were placed around each bone were placed distally or proximally in random order (figure adapted from Study I).

used to determine the exact anatomical locations for the histological sectioning. Five hard-tissue sections of 20-µm were prepared and stained by a modified Van Gieson's method. The histological sections were imaged and analyzed qualitatively using an Olympus B51 virtual microscope with a U-CMAD3 camera attached (Olympus Optical, Tokyo, Japan).

4.2.3. Experimental posterolateral spinal fusion

4.2.3.1. Experimental groups

Thirty-seven 1-to-2-year-old adult New Zealand white rabbits (HB Lidköpings Kaninfarm, Sweden), weighing 3.1–4.3 kg, were used for the posterolateral spinal fusion. The animals were divided into the following four groups: BG 1–98/$PLGA_{80}$ (Group 1), control $PLGA_{80}$ fabric (Group 2), or 3D BG 1–98/$PLGA_{80}$ composite (Group 3), and a group treated with the autograft alone without biomaterial (Group 4).

4.2.3.2. Surgical procedure

The applied surgical protocol followed the published reports on surgical anatomy (Palumbo et al. 2004) and the improved operative technique of Boden (Boden et al. 1995, Valdes et al. 2004). Under strict sterile surgical conditions, each animal underwent surgery for a single-level posterolateral intertransverse fusion at the level of the 5^{th} and 6^{th} lumbar spine without instrumentation. The skin preparation involved careful clipping and scrubbing and disinfection. A dorsal midline skin incision was made, followed by two paramedian fascial incisions. The transverse processes of the 5^{th} and 6^{th} lumbar spine were exposed. The posterior parts of the transverse processes were decorticated with a high-speed drill. The posterior iliac crest was exposed for the harvesting of the bone graft. The tested biomaterial (one of the two fabrics or the 3D composite) was placed over the decorticated transverse processes with the autograft. In Group 4, the autograft was placed over the fascia between the decorticated transverse processes. The wound was closed in layers. Functional activity was not limited after the animals recovered from the anesthesia.

4.2.3.3. Harvesting of specimens

After a follow-up of 6 weeks, the animals were euthanized with an intravenous administration of sodium pentobarbital, and the lumbar spine segment was harvested for further analyses.

4.2.3.4. Radiographic analysis

Standard anteroposterior radiographs were taken of the retrieved spine segments on digital image plates (Fuji IP cassette, Fuji Photo Film Co Ltd, Tokyo, Japan).

4.2.3.5. Computed tomography

The success of the spinal fusion was evaluated by means of computed tomography (CT) performed using a 16-row detector CT system (LightSpeed16, GE Medical Systems, Milwaukee, Wisconsin, USA). Coronal and sagittal reconstructions and 3D volume rendering were made using an open source image analysis software package (OsiriX v.3.8 and 2.8-Ghz Intel Core i7 iMac, Apple Inc, Cupertino, CA, USA). The radiographs and CT images were examined by two independent observers for the success of the spinal fusion and for the quantity of new bone formation in the bone-grafted regions.

4.2.3.6. Biomechanical testing

The stability of the fused level was tested manually and compared with the two adjacent levels by two independent observers, and it was graded as fused or non-solid fusion. A three-point flexion-bending test was performed to assess the rigidity of the fusion. A universal testing device (Avalon Technologies, Rochester, MI, USA) was used for the three-point bending test with a 30-mm inter-support distance and a 1mm per minute loading rate. The bending moment was determined at 1.5-mm middle-span deflection (Figure 5).

4.2.3.7. Histopathology

The fused level of the retrieved spinal segment was fixed in 70% ethanol, dehydrated, and embedded in isobornyl methacrylate. Specimens were cut in the sagittal plane, and 20-µm sections were stained with hematoxylin-eosin and modified Van Gieson's stain. For the three animals that died or had to be euthanatized due to a postoperative complication, explorative biopsies of mus-

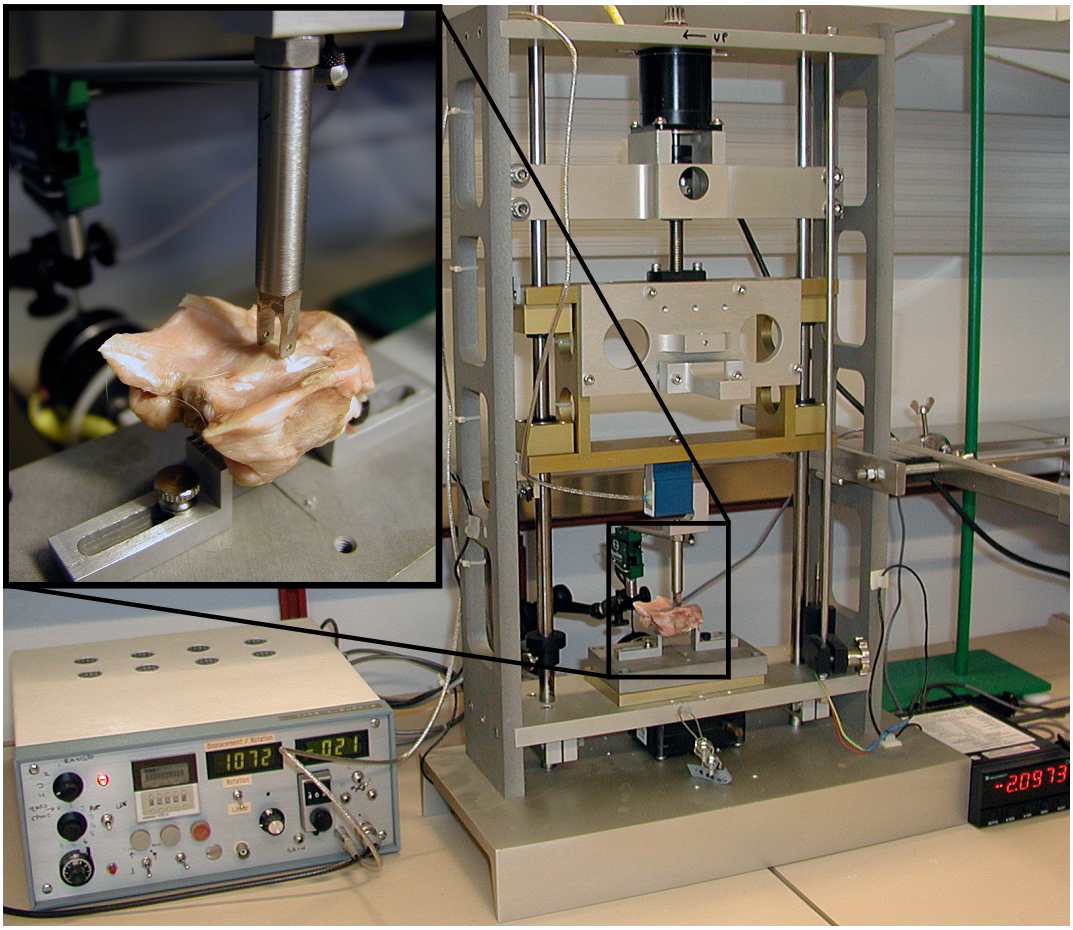


FIGURE 5. *Universal testing device (Avalon Technologies, Rochester, MI, USA) used for the three-point bending test of the rabbit spine.*

cles, the spinal cord, and peripheral nerve roots were taken from the surgical site at the level of fusion. As internal control samples, corresponding soft-tissue biopsies were taken one level above the fusion. The samples were fixed in formalin and embedded in paraffin, and 4-µm transverse sections were cut and stained. Histological section imaging and analyses were performed using an Olympus B51 virtual microscope with a U-CMAD3 camera attached (Olympus Optical Co. Ltd, Tokyo, Japan). A neuropathologist was consulted concerning the findings.

4.3. Clinical studies

4.3.1. Design of clinical trials

Prospective long-term follow-up trials were conducted on BG S53P4 used as bone graft substitute for posterolateral spondylodesis in the treatment of degenerative spondylolisthesis and unstable lumbar spine burst fractures during 1996–1998. The surgical procedure was a standardized instrumented posterolateral fusion. BG S53P4 was implanted on the left side of the fusion bed, and autograft bone (AB) was inserted on the right side. The granules used in this clinical trial were based on a four-component BG not containing potassium or magnesium, as opposed to the BG 1–98 fibers used in the pre-clinical study. The op-

erative outcome was evaluated from X-rays, CT, and MRI scans, and a clinical examination was also performed.

4.3.2. Ethical approval

The trials were conducted in accordance with the ethical principles detailed in the latest version of the Declaration of Helsinki, which stipulates applicable regulatory requirements, including the standards of the International Organization for Standardization, to Finnish law and regulations. The study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland. The trials were registered at www.clinicaltrials.gov.

4.3.3. Patient populations

All of the patients were treated in the Department of Surgery, Turku University Hospital. Twenty patients (12 women, 8 men), aged 39–61 (mean 49, SD 6) years, with low-back pain due to lumbar spondylolisthesis participated in the degenerative prospective trial. All of the patients had undergone conservative physiotherapeutic treatment pre-operatively. Nine patients had undergone previous spine surgery, discectomies, or foraminotomies before the fusion procedure.

Sixteen patients, aged 31–58 (mean 49, SD 10) years, with an unstable lumbar burst fracture (one patient had sustained two fractures), classified according to the Denis classification (Denis 1984), participated in the prospective trauma trial. Two patients had incomplete spinal cord injuries classified as Frankel C (Frankel et al. 1969); the others were neurologically intact. None of the patients had undergone previous spine operations.

4.3.4. Surgical procedures

4.3.4.1. Degenerative spine surgery

The patients were operated on in the Department of Surgery of the Turku University Hospital from March 1996 to August 1997. The surgical procedure was a standardized instrumented posterolateral fusion using USS/VAS® instrumentation with steel or titanium screws (Synthes GmbH, Zuchwil, Switzerland). After decortication of the transverse processes and the facet joints, BG S53P4 with a granule size of 1000 to 2000 µm (Abmin Technologies Ltd., Turku, Finland) was implanted onto the left side of the posterolateral fusion bed. The mean amount of implanted BG S53P4 was 25 g (20 to 40 g) depending on the length of the attempted fusion. Autograft bone harvested from the left posterior iliac crest, and also bone from the laminas obtained during the decortication procedure, was implanted onto the contralateral fusion bed.

4.3.4.2. Lumbar spine fractures

All of the patients were operated on in the Department of Surgery, Turku University Hospital, during September 1996 through December 1998 by three senior surgeons. Within 72 hours of the injury the lumbar fractures were reduced and fixed using posterior USS® instrumentation with titanium Schanz screws (Synthes GmbH, Zuchwil, Switzerland). Posterolateral bone grafts were harvested from the posterior laminae and the left posterior iliac crest and implanted on the right posterolateral fusion bed. A mean amount of 23 g (10–35 g) of BG S53P4 (Abmin Technologies Ltd., Turku, Finland) was implanted on the left side.

4.3.5. Postoperative follow-up

For both groups the primary follow-up included visits to the outpatient department at 3, 6, and 12 months postoperatively. All of the patients were contacted for the 10- and 11-year long-term follow-up visits.

4.3.5.1. Clinical evaluation

The clinical examination included documentation of the patients' medical history, ongoing medication, smoking and alcohol consumption, possible adverse effects, self-assessment for pain on a visual analogue scale (VAS) (0–10), and a subjective satisfaction score graded as excellent, good, fair, or poor. The Oswestry Disability Questionnaire was also filled out. The result was graded as excellent (0–20), good (21–40), poor (41–60), or very poor (>61).

4.3.5.2. Radiographic evaluation

The imaging protocol included X-rays in flexion and extension (lateral views), as well as lateral and anteroposterior images with the patient in a neutral standing position (Philips Bucky Diagnostics C, Philips Medical Systems, Eindhoven, The Netherlands).

4.3.5.3. Computed tomography

For the CT General Electric HiSpeed Qxi (GE Medical Systems, Milwaukee, Wisconsin, USA) of the lumbar spine, both the transaxial and the reconstructed coronal and sagittal images were assessed. The images were viewed for the bridging of bone between the transverse processes, in addition to the incorporation of bone between the transverse processes as a solid fusion. Radiological fusion, as seen in CT scans, was evaluated and assessed for the AB and BG side. The radiological outcome was graded as 0 = no fusion, 1 = partial or incomplete fusion, and 2 = complete fusion. Possible resorption of the BG granules and AB was separately evaluated.

4.3.5.4. Magnetic resonance imaging

From the MRI (Philips 1.5T Intera Power (Philips Medical Systems, Best, The Netherlands), sagittal T1- and T2-weighted images, in addition to transaxial T2-weighted and coronal short-tau-inversion-recovery (STIR) images, were acquired. Stenosis of the spinal canal or foramina, together with the level of disc degeneration, was assessed from the MRI. The adjacent segment degeneration was graded as mild, moderate, or severe.

4.4. Statistical analyses

The results were expressed as the mean and (+/-) and standard deviation. Continuous data from the biomechanical testing, cell culture experiments, and the 12-hour pH measurements were tested for normal distribution and for equal variances with the Shapiro-Wilk test and Levene's test, respectively.

The paired t-test was used for comparisons of the pQCT parameters of the operated femurs against those of the intact contralateral femurs and between the two fabrics. The comparisons were presented as the mean differences with 95% confidence intervals (CI). A significance level of 0.05 was applied.

For the investigation of whether the frequency of adverse events was statistically significant between the spine implant groups, the chi-square test was used, and the differences between the biomechanical properties of the spine implant groups were tested using the one-way ANOVA with Tukey's post hoc test.

Time-related changes in pH and the effect of different materials were analyzed using repeated-measures ANOVA with the material as a co-factor. The analyses were performed with the use of SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

The evaluations of the clinical trials were based on summary tables, and no formal statistical analyses were performed. As there were no formal power calculations or hypotheses set, therefore the statistical tests were deemed not to give additional information. The results were expressed as the mean and (+/-) and the standard deviation, as well as the median and range. Data analysis, tables and subject data listings were performed by StatFinn Oy, Turku, Finland with SAS® 9.1 for Windows (SAS Institute Inc., Cary, NC, USA).

5. RESULTS

5.1. Pre-clinical studies

5.1.1. In vitro testing using mesenchymal stem cell cultures

5.1.1.1. Cell attachment, growth and differentiation

In the hMSC cultures with BG 1–98 fibers, empty areas with rather sharp borders were observed between the fibers and the confluent cell layers at 1 and 2 weeks. With all of the other fibers (BG 45S5, E-glass, $PLGA_{80}$, PLA_{96}), the cells grew close to the fibers, and even onto the E-glass and PLA fibers. The same empty area was visible between the BG 1–98 discs and the confluent hMSC layer. As demonstrated by Hoechst and actin staining, hMSCs were detected only sparsely on the biodegradable fabrics (BG 1–98/$PLGA_{80}$, $PLGA_{80}$), whereas a confluent layer of cells could be seen on the controls (titanium discs, cultures of hMSCs alone).

The hMSCs cultured in osteoblastic medium with BG 1–98 fibers and discs showed a low degree of ALP expression, there being only a few positively stained cells. In the control hMSC cultures, ALP positive cells were evenly spread over the confluent culture.

5.1.1.2. Effects of fibers and fabrics on the pH of the cell cultures

For the BG 1–98 fibers, the pH was elevated to 7.7±0.01 already at 3 days; this level was significantly higher when compared with that of all the other fibers (BG 45S5, E-glass, $PLGA_{80}$ and PLA_{96}) ($p<0.001$ for all). At 7 days, the pH of the other fiber cultures started to approach the level of the BG 1–98 cultures.

The cultures with BG 1–98/$PLGA_{80}$ fabric showed a very distinct pattern for the pH changes, reaching almost 8.2 after 3 days, a level significantly higher than the pH of the other cultures ($p\leq0.001$). The cultures with the BG 1–98/$PLGA_{80}$ 3D composite showed a more stable pH that the discs and fabrics did.

In the control cultures (titanium discs), the pH followed the pH trends of the control cultures of hMSCs without materials. At 14 days, an increase in pH appeared, indicating optimal alkaline conditions for bone cell differentiation.

5.1.2. In vivo testing of woven fabric made of bioactive glass fibers

5.1.2.1. Surgery (femur model)

The surgeries were uneventful, with no postoperative complications. Intraoperatively, the BG 1–98/$PLGA_{80}$ fabric was flexible, but a partial opening occurred in the woven structure as a result of the surgical handling; this occurrence was not seen with the control $PLGA_{80}$ fabric. All of the animals were fully weight-bearing within 12 hours and maintained full ambulatory status throughout the study. Macroscopically, no signs of inflammation or necrosis were detected in the bone or in the surrounding soft tissues at the time of the harvesting.

5.1.2.2. Digital radiography

At 12 weeks postoperatively, the radiographs showed signs of periosteal ossification at the implantation sites and also areas of cortical thickening. No apparent differences were observed at the sites of the two fabrics. There were no signs of adverse local bone reactions.

5.1.2.3. Qualitative and quantitative pQCT analysis

The integration of the two fabrics and bone grafts with bone was qualitatively evaluated from the pQCT images. Both fabrics were still detectable, and the cross-sectional 3D images showed endosteal and intracortical resorption cavities, suggesting a process of natural adaptive remodeling. Overall, both fabrics seemed to be well integrated with the cortical bone surfaces. With the BG 1–98/$PLGA_{80}$ fabric, there was a 25% increase in the bone volume (95% CI 16–35%) when compared with that of the intact contralateral site ($p < 0.001$). With the control

$PLGA_{80}$ fabric, the increase was 28% (95% CI 12–47%, p = 0.006).

The pQCT measurements confirmed a significant increase in the cortical thickness, by 23% (95% CI 10–36%), for both the BG 1–98/$PLGA_{80}$ and the $PLGA_{80}$ fabrics in comparison with the intact contralateral bone (p=0.001 and p=0.005, respectively).

The increased bone volume of the operated femurs, reflected as a decreased volumetric bone mineral density when compared with that of the intact contralateral bones. With BG 1–98/$PLGA_{80}$ fabric, the local BMD was 94% of the corresponding anatomical site at the contralateral bone (95% CI 91–96%, p=0.001). With $PLGA_{80}$ fabric, the local BMD was 95% of that of the contralateral site (95% CI 92–97%, p=0.002).

5.1.2.4. Histological evaluation

In the qualitative histological evaluation, both the BG 1–98/$PLGA_{80}$ and the $PLGA_{80}$ fabrics were still visible at 12 weeks. Only a few BG 1–98 fibers were visible, suggesting an almost complete resorption by 12 weeks. Both fabrics had integrated well with the surrounding periosteal new bone tissue, which tended to form a neocortex. Extensive remodeling of the original cortical bone was observed at the sites of the bone grafting; it involved circular intracortical and endosteal resorption cavities. There were no signs of foreign body reactions in the bone or surrounding connective tissues.

5.1.3. Experimental posterolateral spinal fusion

5.1.3.1. Surgery and postoperative complications

Altogether 37 rabbits were utilized for the study. Of this number sixteen were lost (three preoperatively, four peroperatively and nine postoperatively). Of the remaining 30 animals surviving surgery, 21 completed the follow-up. The total rate of postoperative complications was 9 of 30 (30%). Seven of these complications occurred in the BG groups, and two were in the autograft group. No complications were found in the $PLGA_{80}$ group. The chi-square test showed a statistically significant higher frequency of complications for the BG 1–98/$PLGA_{80}$ groups when they were compared with the autograft and $PLGA_{80}$ groups (p=0.020). In the autograft group, one postoperative death occurred after surgery, probably caused by anesthetic complications. In the BG 1–98/$PLGA_{80}$ group, two animals had to be euthanized due to intractable pain, and one ambulatory animal developed paraparesis at day 1 postoperatively. One animal in the 3D composite group had to be euthanized at a late stage, 5 weeks after surgery, due to intractable pain and autophagia of the hind leg (Table 3). Wound healing was uneventful for all of the animals.

5.1.3.2. Radiographic evaluation of fusion

Posterioanterior (PA) radiographs revealed bone formation in all eight animals in the autograft group. In the $PLGA_{80}$ group, only four of the six animals (67%) and, in the BG 1–98/$PLGA_{80}$ group, five of the seven (67%) animals showed bone mass at both sides at the index level. A continuous fusion, according to the CT images, was seen on both operated sides of five of the eight animals (62%) in the autograft group, in none of the animals in the $PLGA_{80}$ group, and in one of the seven animals (14%) in the BG 1–98/$PLGA_{80}$ group.

5.1.3.3. Manual palpation

Manual palpation was done blind, and it showed that all except one was fused in the autograft group, whereas only one-third in the $PLGA_{80}$ group and one out of six in the BG 1–98/$PLGA_{80}$ group were fused.

5.1.3.4. Biomechanical testing

The three-point flexion-bending test showed a consistently higher bending moment for the autograft group (n=7) (mean 0.42 Nm, 95% CI 0.18–0.66) than for the $PLGA_{80}$ group (n=6) (mean 0.23 Nm, 95% CI 0.10–0.36) and for the BG 1–98/$PLGA_{80}$ group (n=4) (mean 0.23 Nm, 95% CI 0.05–0.40) (p=0.046). There was no difference in the bending moment between the BG 1–98/$PLGA_{80}$ and $PLGA_{80}$ groups.

TABLE 3. *Qualitative outcome of spinal fusion in the different implant groups.*

Fusion implant	Animal#	Completed Followup (6 weeks)	Serious adverse event	Histology Foreign body reaction
Autograft	5D2188*	Yes	None	none
	5D479	Yes	None	none
	5D3848	No	Unexplained death 4 days postop.	N/A
	5D2174	Yes	None	none
	5D3832	Yes	None	mild
	5D850	No	Unexplained death 1 day postop.	N/A
	5D506	Yes	None	none
	5D365	Yes	None	none
	5D512	Yes	None	none
	5D3652	Yes	None	mild
$PLGA_{80}$ fabric + autograft	5D4008	Yes	None	none
	5D872	Yes	None	none
	5D4143	Yes	None	mild
	5D4380	Yes	None	moderate
	5D809	Yes	None	moderate
	5D3662	Yes	None	none
BG1-98/ $PLGA_{80}$ fabric +autograft	5D2182*	Yes	None	severe
	5D848	No	Unexplained death 1 day postop.	N/A
	5D822	No	Paraparesis, euthanized 1 day postop.	N/A
	5D811	Yes	None	severe
	5D3666	No	Unexplained death 1 day postop.	N/A
	5D823	No	Unexplained death 3 days postop.	N/A
	5D4302	Yes	None	moderate
	5D4322	Yes	None	severe
	5D4599	No	Unexplained death 1 day postop.	mild (artefact)
	5D4080	Yes	None	severe
	5D857	No	Euthanized due to pain 1 day postop.	severe
BG1-98/ $PLGA_{80}$ 3D Composite + autograft	6D126	No	Euthanized due to intractable pain, autophagia (5 weeks)	severe
	3FQD	Yes	None	severe
	3FID	Yes	None	severe
Non- Randomized	3FPU	No	Preoperative death	N/A
	5D808	No	Preoperative death	N/A
	6D140	No	Preoperative death	N/A
	5D3751	No	Peroperative death	N/A
	6D1677	No	Peroperative death	N/A
	3GHE	No	Peroperative death	N/A
	5D3662	No	Peroperative death	N/A

* pilot study animals
(Table adapted from Study II)

5.1.3.5. Histological examination

In the autograft group, a complete fusion was seen in five of the six animals (83%). A normal postoperative situation was found, with a slight increase of fibrosis beside the autograft and a few inflammatory cells, but no significant necrosis was found.

In the $PLGA_{80}$ group, one unilateral and one bilateral complete fusion were identified. Only a few inflammatory cells and postoperative fatty degeneration of the muscle tissue were found in all except one animal, in which multiple accumulations of inflammatory cells were encountered, accompanied with dense fibrosis towards the investigated material.

In the BG 1–98/$PLGA_{80}$ group, none of the animals that completed the follow-up were histologically fused. Marked resorption of the autograft was noted, and areas were identified in which the decorticated transverse process had an indentation under the BG 1–98/$PLGA_{80}$. Altogether 10 of the 14 animals in this group were investigated histologically, and severe inflammation and necrosis were found in all of the samples. Muscle samples at the level of fusion adjacent to the investigated materials showed pale muscle fibers, and no nuclei were identified, similar to the findings for coagulative necrosis. Beside the necrotic area, macrophages with multinuclear macrophages were found. The sensory ganglia at the level of fusion showed signs of edema, and the control samples were intact above the fusion site (Figure 6).

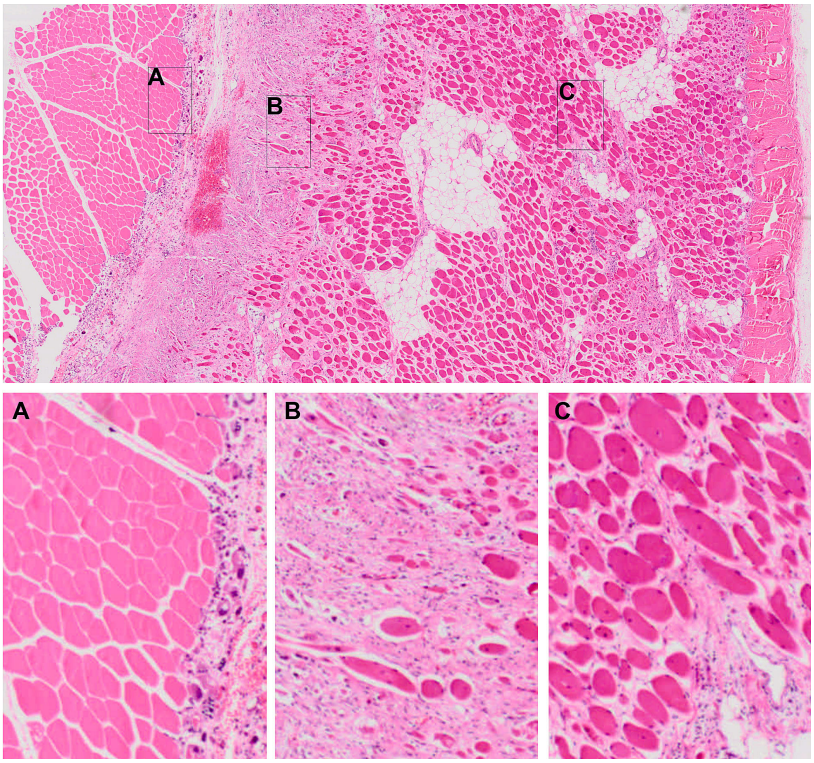


FIGURE 6. *H-E stain, axial cut of the muscle tissue overlying the implant at the index level (animal #6D126, 3D composite).*
Overview showing the 3D composite on the left side (removed).
A. Pale muscle fibers and no nuclei were identified similar to that noted in a coagulative necrosis. Beside the necrotic area, macrophages with multinuclear macrophages were found.
B. Increased fibrosis was observed with numerous atrophic but otherwise well preserved muscle fibers were observed.
C. Increased endomysial fibrosis with multiple accumulations of inflammatory cells that consisted of mononuclear inflammatory cells and macrophages. In this area focal necrosis of muscle fibers was noted with subsequent phagosytosis of necrotic muscle fiber.

5.2. Clinical studies

5.2.1. Degenerative spine surgery

Altogether 17 of 20 patients (12 women, 5 men, follow-up rate 85%) participated in the 11-year follow-up. Two patients died during the follow-up due to unrelated causes, and one patient did not accept our invitation. Six patients had returned to their occupation at the 12-month follow-up, and six were concurrently working at the completion of the long-term study.

Compared with the preoperative situation, the general subjective long-term outcome improved in 15 of the 17 patients. At the 6-month follow-up, the ODI score had decreased by 61%, and the VAS score by 50%. At 12 months a further decrease was found, as the ODI score had decreased by 74% and VAS score by 68% when compared with the preoperative scores. The mean ODI score at the 11-year follow-up was 21 (range 0–52), compared with the corresponding preoperative score of 49 (range 32–64). The preoperative median VAS (0–10) scores for back pain were 5.6 (range 4–9). The median VAS score for back pain at the 11-year follow-up was 3.5 (range 0–8), and, for radicular pain, it was 2.9 (range 0–8), compared with the pre-operative situation, which was 7.3 (range 4–9) and 7.3 (range 4–9), respectively.

Eleven years after the surgery, five patients reported discomfort in the bone harvesting area, and one patient complained of disabling pain in this area.

A solid bony fusion was observed on the AB side in all of the patients. On the BG S53P4 side, a solid bony fusion was detected in the CT scans of 12 patients. The fusion rate of all the fusion sites (n=41) for BG S53P4 as bone substitute was 88% at the L4/5 level and 88% at the L5/S1 level. The fusion was not solid at eight levels in five patients. In two of these patients, hardware breakage was seen in their X-rays, and, for one patient, radiolucency was observed around the hardware. However, the subjective outcome of four of these five patients was excellent or good. Screw breakage was found in five patients, and radiolucent lines around implanted screws were detected in three patients. Malposition of the pedicle screws was detected in the CT scans of two patients.

The bending lateral X-rays were examined for evidence of functional spondylolisthesis adjacent to the fused level. Spondylolisthesis was detected in three patients (Figure 7).

5.2.2. Lumbar spine fractures

Altogether 10 of 16 patients (1 woman, 9 men, follow-up rate 63%) participated in the 10-year follow-up. Three patients had died from unrelated causes, and three did not want to participate for personal reasons. No additional operations or hardware removals had been performed after the primary operation.

Seven patients rated their back as good or excellent, and three as fair. The mean ODI score was excellent, 12 (range 0–46). The mean pain score (VAS 0–10) for radicular and back pain was 1 (range 0–4). All of the patients had returned to their occupations. At the time of the 10-year follow-up, 5 of the 10 patients were retired on the basis of their age, none because of their medical condition.

No persistent problems associated with the bone harvesting area were detected in the 10-year follow-up.

Mild limping but walking without an aid was observed for two patients. Both patients showed preoperative neurological compromise (i.e., partial spinal cord injury or spinal nerve root injury).

All of the fractures had healed. The mean compression rate of the injured vertebral body was 25% (range 5%–36%). The hardware appeared to be undamaged in the X-rays at 12 months. At the long-term follow-up, hardware breakage was observed in one patient for one of the four screws.

A solid bony fusion was seen in the CT scans on the side of the AB implantation in all ten patients. The resorption of the implanted graft was mild in seven patients. On the BG S53P4 implantation side, a solid fusion was seen in the CT scans of five patients, and a partial fusion or a discontinuation in the fusion mass was found in five patients. The fusion mass was more solid on the AB side. On the BG S53P4 side, a fusion-rate of

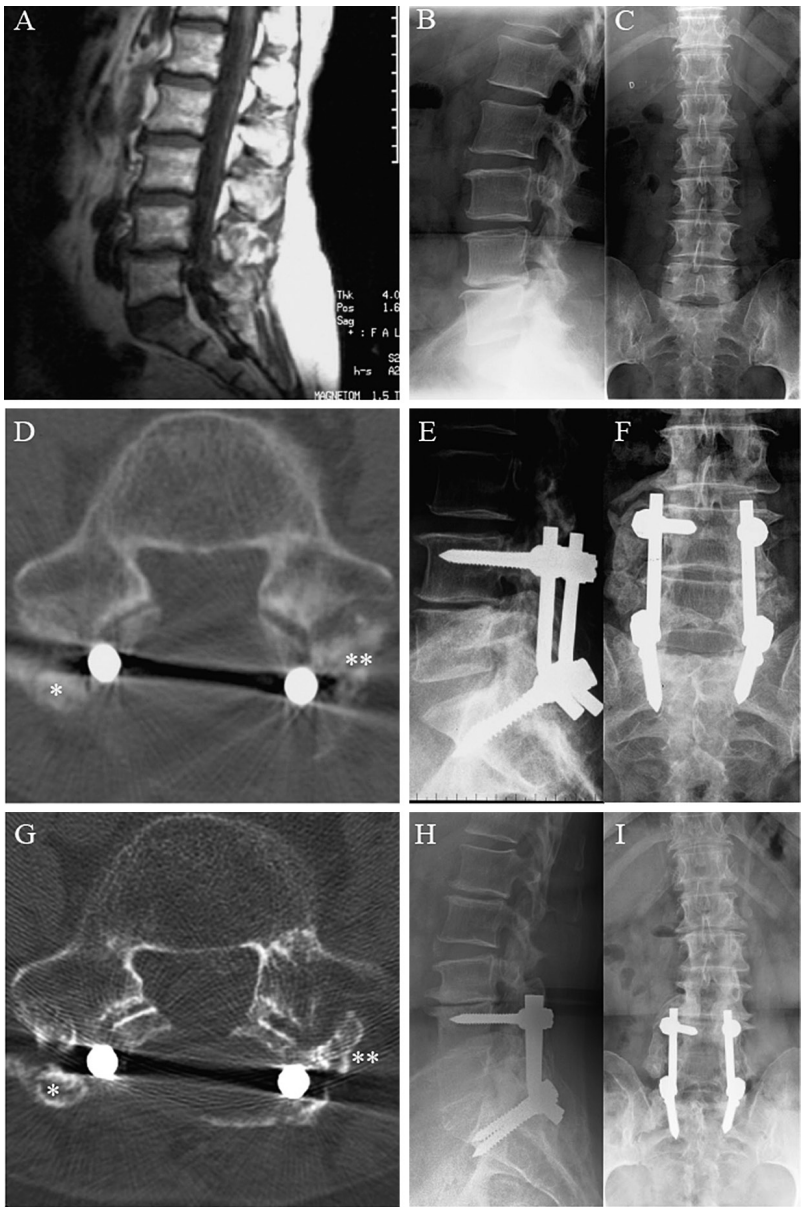


FIGURE 7. *(rtg/CT/MRI). The patient is a 76-year-old female treated for L4/5 degenerative spondyloslisthesis with instability symptoms and radicular pain in the lower extremities.*
A. Preoperative, T1-weighted, sagittal MRI shows disc degeneration at L4/5 and segmental stenosis.
B-C. Lateral and AP X-ray images show loss of disc height and traction spurs at L4/5 and a straight posture with no signs of scoliosis preoperatively.
D. The 1-year postoperative axial CT image at the L4 level shows solid fusion on both the (*)autograft and the (**)BG side.
E–F. Plain X-ray images show strong fusion on the autograft side and bridging ostephytes from L3.
G. The 11.5-year postoperative axial CT image at L4 shows solid fusion on both the (*)autograft and the (**)BG side.
H-I. Plain X-ray images show severe loss of disc height at the adjacent L3/4 level with prominent anterior osteophytes and slight degenerative retrolisthesis of L3. Shown in the AP view is L3 fused to L4 on the right side and slight degenerative scoliosis above the fusion. (Figure adapted from study III)

71% was achieved (15 of 21 fusion sites). However, in two patients in the BG S53P4 group, an ectopic fusion was observed above the fusion site. The resorption of BG S53P4 was mild in three patients, moderate in two, and severe in five. BG S53P4 granules were still partially visible in the CT scans of six of the ten patients.

No malpositioned Schanz screws were observed. Spinal stenosis was not observed; only limited disc degeneration was present in the adjacent segments. No significant deformities or signs of instability were clinically or radiologically observed. The flexion-extension radiographs showed degenerative spondylolisthesis of 6–10 mm in two patients. The spondylolisthesis was not at an adjacent level and was not associated with clinical or radiological signs of spinal stenosis.

5.2.3. Bioactive glass granules as a bone graft substitute

At the time of the long-term follow-up, BG S53P4 granules were partially visible in the CT scans for 19 out of a total of 27 patients. Severe resorption of BG was observed in 10 patients, but it was not associated with non-fusion in the treatment area. No re-operations were attributed to the use of BG S53P4 granules.

6. DISCUSSION

6.1. In vivo testing of BG 1–98 composite woven fabric

This is the first in vivo test of a composite woven fabric made of BG fibers. However, several groups have described mechanical properties and in vitro behavior of BG fibers (Pazzaglia et al. 1989, Domingues et al. 2001, Clupper et al. 2003, Clupper et al. 2004, Pirhonen et al. 2006a, Pirhonen et al. 2006b, Brown et al. 2008, Modglin et al. 2012) and in vivo properties of compressed composites with BG fibers as a component (Marcolongo et al. 1998). Previously, Moimas and coworkers made a pilot rabbit study on the use of short (3 mm) BG fibers in a sintered porous scaffold (Moimas et al. 2006). In another study, Asikainen and coworkers investigated PDTE [poly(desamino tyrosyl-tyrosine ethyl ester)] carbonate membranes alone or in combination with a nonwoven implant of BG 13-93 fibers fixed with chitosan in a rabbit, mandible, critical-size defect model (Asikainen et al. 2006). They found more new bone formation with PDTE carbonate membrane alone than in combination with BG 13-93.

Engineered composite scaffolds made by degradable polymer matrices with bioactive components play a key role in tissue engineering and in simple clinical applications as bone graft substitutes (Rezwan et al. 2006, Walsh et al. 2011). As anticipated, new solutions always have potential complications and side effects, and these effects increase as the number of components in a composite increase. In the case of BGs, the anticipated complication is that the local biological microenvironment can be influenced negatively by their degradation (Rahaman et al. 2011).

The kinetics of BG surface reactions, leading to the chemical bonding to bone, have mainly been established on the basis of in vitro studies using BG in the forms of solid plates, rods, and granules in simulated body fluid (SBF) or Tris buffer (Hench and LaTorre 1992, Greenspan et al. 1994). BG fibers offer extended possibilities for manufacturing scaffolds of different dimensions, porosities, and surgical handling properties. In addition, the use of BG fibers would enlarge the surface-to-volume ratio, and this enlargement has been expected to result in enhanced osteoconductive capabilities (Pirhonen et al. 2006b, Moimas et al. 2006). It should be noted that the surface area of BG fibers, rather than their volume, affects the degradation rate, and therefore the resorption rate of BG fibers may be much faster than that of BG with the composition normally exhibited as solid forms. This situation could possibly explain the resorption of BG fibers in study I in 12 weeks. In addition, the possibility of an impact from by-products of the degrading $PLGA_{80}$ component on BG fiber resorption cannot be completely ruled out. Still, the fraction of PLGA in BG 1–98/$PLGA_{80}$ fabric was only 4.5 wt%, and the impact on BG fiber degradation was probably minimal.

Study I failed to demonstrate the previously found positive effect of BG 1–98 and 13-93 on osteogenesis in the form of solid discs in vitro (Gao et al. 2001, Itälä et al. 2002) or implants of sintered microspheres in the rabbit femur (Itälä et al. 2003, Välimäki and Aro 2006), and inlays made of BG 1–98 or 13-93 microspheres failed to promote osteogenesis in slots of titanium alloy implants in sheep (Keränen et al. 2010, Keränen et al. 2011). The resorption rate of the BG 1–98 fibers exceeded the balanced rate of dissolution necessary for an optimal biological response; although no side effects or toxic tissue reactions were observed in these animals.

Study II not only showed a failure in the promotion of osteogenesis, but also even in the toxic tissue response to the BG 1–98/$PLGA_{80}$ composite in a rabbit spinal fusion model. The mechanisms behind the adverse events remain unclear.

In previous studies using the same animal model, the rate of per- and postoperative complications was 3% and 5%, respectively, according to 26 publications (a total of 1138 rabbits) (Table 4). Therefore, in the present study, termination was necessary at the point when 34 rabbits were operated on and 9 rabbits died or had to be eu-

thanized due to complications. The complication rate was 50% for the groups containing BG 1-98 fibers, which was extremely high in comparison with that of those not containing BG fibers. This occurrence proves that the high complication rate in the BG group could not have been the result of poor surgical technique being used in the experiments.

In the autograft group, seven out of eight animals showed complete fusion. This figure is well above the average, as the fusion rates described in the literature average 66% (range 53%–82%) at 8 weeks (Bozic et al. 1999, Long et al. 2002, Lehman et al. 2004, Lehman et al. 2010, Urrutia et al. 2010a, Urrutia et al. 2010b, Smucker et al. 2011), confirming successful surgery and setup.

Table 4. *Complications in posterolateral fusion in rabbit.*

Author(s)	Animals Total	Animals lost peroperatively	Animals lost postoperatively
Ritsilä et al. 1975	36	0 (0%)	0 (0%)
Bouchard et al. 1994	27	0 (0%)	0 (0%)
Schimandle et al. 1995	48	4 (8%)	8 (17%)
Boden et al. 1995	60	4 (7%)	8 (13%)
Silcox et al. 1995	28	0 (0%)	11 (39%)*
Morone et al. 1998	48	1 (2%)	3 (6%)
Silcox et al. 1998	48	2 (4%)	1 (2%)
Boden et al. 1999	40	0 (0%)	1 (2%)
Minamide et al. 1999	20	0 (0%)	1 (5%)
Erulkar et al. 2001	10	1 (10%)	1 (10%)
Cheng et al. 2002	24	0 (0%)	0 (0%)
Louis-Ugbo et al. 2002	18	0 (0%)	0 (0%)
Long et al. 2002	72	2 (3%)	5 (7%)
Liao et al. 2003	64	2 (3%)	2 (3%)
Yee et al. 2003	100	2 (2%)	0 (0%)
Lehman et al. 2004	50	2 (4%)	5 (10%)
Cinotti et al. 2004	40	4 (10%)	1 (2%)
Minamide et al. 2005	28	0 (0%)	2 (7%)
Magit et al. 2006	67	2 (3%)	0 (0%)
Choi et al. 2007	48	1 (2%)	1 (2%)
Lawrence et al. 2007	72	4 (6%)	0 (0%)
Motomiya et al. 2007	36	0 (0%)	0 (0%)
Yao et al. 2008	32	1 (3%)	2 (6%)
Dodds et al. 2009	37	0 (0%)	0 (0%)
Wang et al. 2009	27	0 (0%)	0 (0%)
Matsumoto et al. 2011	58	5 (9%)	0 (0%)
Total	1138	37 (3%)	52 (5%)

* 16 animals was were operated with a different technique (a.m. Wiltse)

The rates of non-union were similar in the BG 1–98/$PLGA_{80}$ and $PLGA_{80}$ control groups; the major difference was adverse effects found only in the BG 1–98/$PLGA_{80}$ group.

The onset of pain occurred within 72 hours after surgery in the affected animals. One animal suffered from intractable pain and bit its leg, as a possible sign of neuropathic pain. This occurrence was interpreted as a radicular symptom of the L5 root. Five animals died, most probably from pain; one was euthanized because of intractable pain. In cases of severe ischemia in the spinal musculature, pain could be mediated by the sensory ramus of the spinal roots. One animal suffered from hindleg paralysis and was euthanized.

For paralysis to develop, either the spinal cord or the sacral plexus must be injured (Craigie 1948). In contrast to that of humans, the rabbit intervertebral foramen is situated dorsal to the plane of the transverse processes and transmits the associated spinal nerve that pierces the intertransverse ligament just cranial to the base of the caudal transverse process (Palumbo et al. 2004). The foramen is an opening to the spinal canal and the spinal cord, and it offers a potential route for the distribution of various changes in pH and/or ion concentrations caused by the investigated materials. This occurrence could trigger tissue responses and lead to edema and inflammation and therefore lead to dysfunction of the neural elements. In addition, the sciatic nerve can be injured during bone graft harvesting if dissection is close to the posterior third of the iliac wing (Valdes et al. 2004). Other causes of paralysis in the early postoperative phase would be fracture of the pelvis, hemorrhage, or infection as complications to the harvesting of bone. Unfortunately, the first animals that died unexpectedly were not subjected to a full autopsy in order to rule out other causes for the adverse reactions.

A histopathological examination of the implant area revealed areas with numerous multinuclear macrophages, inflammatory cells, and ghost cells at the site of the fusion with the BG 1–98/$PLGA_{80}$ composites. The inflammation was probably the cause of extensive coagulative necrosis. Resorption of the grafted bone tissue and also the

transverse spinoses showed clear signs of resorption and degeneration when in contact with BG 1–98/$PLGA_{80}$. None of these adverse reactions were detected in such a large scale in the autograft group or in the $PLGA_{80}$ control group.

Critical concentrations of dissolution ions from BG have beneficial biological effects (Xynos et al. 2001), and silicon is known to increase bone formation, but, at high concentrations, it causes apoptosis and necrosis (Gough et al. 2004a, Gough et al. 2004b). A local increase in intracellular silicon or calcium exceeding critical concentrations activates inflammatory responses (Christensen et al. 2002, Peters et al. 2004).

In a report for government use, Hench (1978) had already reported necrosis and mineralization of surrounding tissue caused by an increased BG surface area for reaction with the surrounding tissues and fluids (Hench 1978). Brink and coworkers found that a few glass cones out of 26 different compositions implanted in rabbit tibia caused moderate inflammation with mononuclear inflammatory reaction in the bone marrow (Brink et al. 1997). Another early study by Pazzaglia and coworkers showed an intense inflammatory response to BG fibers implanted in muscle, with a milder reaction when co-implanted with MSCs (Pazzaglia et al. 1989). When a BG 13-93 fiber mesh with 3% chitosan was subcutaneously implanted in rabbit, foreign body reactions with multinucleated giant cells and chronic inflammatory infiltrates were found (Asikainen et al. 2007). The foreign body reactions are unlikely to be associated with the chitosan component, since it has shown an anti-inflammatory effect both in vitro (Yoon et al. 2007) and in vivo (Qiao et al. 2011).

On the cellular and tissue levels, the complex systems controlling cell functions can be triggered or altered by changes in ion concentrations, pH, and the topography of the extracellular matrix. The release of ions (Si, Ca, P, Na, K, B) from the BG surface induces specific intracellular and extracellular responses that affect cell metabolism, proliferation, differentiation, and cell cycle (Sun et al. 2007), as well as the gene expression level (Jell and Stevens 2006).

Physiological pH is crucial for normal cell function, and radical changes in pH cause apoptosis and necrosis. For bone formation to take place, alkaline pH is required at the bone formation site (Samachson 1969, Bushinsky 2001). However, an abnormally high pH value above 9 can disturb the formation of reaction layers at the BG surface, and it may ultimately lead to the dissolution of the BG silica network itself.

The limited osteoblastic differentiation of hMSCs cultured on discs and fibers of BG 1–98 could be a sign of radical pH and ion leach. Cells stained with ALP were mainly found in cell layers surrounding the BG and not directly on the glass discs or fibers, a phenomenon described earlier by Gough and coworkers (Gough et al. 2004b). Other studies using hMSCs have also reported low or limited ALP expression together with BGs (Leach et al. 2006, Ruuttila et al. 2006, Yang et al. 2006, Reilly et al. 2007) , or with dissolution products of BGs (Reilly et al. 2007, Haimi et al. 2009). Opposite to our results, Yao and coworkers showed that a PLGA/BG 45S5 composite supported MSC proliferation and promoted osteoblastic differentiation (Yao et al. 2005).

BG/PLGA composites have been developed to overcome the problems associated with decreased pH upon PLGA degradation. Ionic release of calcium and silicon from BG can neutralize the acidic degradation products from PLGA; hence the local pH becomes stabilized (Li and Chang 2004, Li et al. 2005). In the 12-hour pH measurements with the BG 1–98/$PLGA_{80}$ fabric, the pH increased radically within the first hour. In the cell culture with the BG 1–98/$PLGA_{80}$ fabric, the pH had dropped below 7.4 by 7 days, as an effect of PLGA degradation. With the 3D BG 1–98/$PLGA_{80}$ composite, no such drop in pH was seen, this finding indicating a much slower degradation, which was attributed to the more compact structure.

Calcium ions are vital in bone formation and resorption, and are one of the main components in biological apatite. Intracellular calcium homeostasis is crucial for cellular functions, and already minor changes in concentrations can have major effects on cell metabolism (Bootman et al. 2001). Especially in nerve and muscle cells, proper calcium is crucial for function and survival. Maeno and coworkers found that a calcium concentra-

tion above 10 mmol was cytotoxic to osteoblatic cells in vitro (Maeno et al. 2005). However, the calcium content in BG 1–98 is in the same range as that of many of the commercially available BGs (Table 1).

Silicon is essential in the early stages of bone matrix calcification. It is excreted through the kidneys, and, in rabbit, has shown no adverse effect on kidney function with doses eightfold that of the silicon in BG 1–98/$PLGA_{80}$ fabrics (Lai et al. 2002).

The K_2O component was added to the BG 1–98 composition to slow down the rate of resorption. During the manufacturing process, there is, however, a risk that it will be exposed on a fiber surface with a P_2O_5 component. The fast release of these two components would lead to high concentrations toxic to tissues. Changes in the concentration of K, Na, and Ca ions directly influence the electric activity of nerve and muscle cells. An excess of potassium ions in the fluids surrounding the cells prevents essential flow outflux and therefore inhibits nerve impulses.

The rate and type of dissolution ions, and, consequently the biological response, depends not only on the BG composition, but also on the size and physical form. With BG of a smaller size (particles, fibers), the cellular reaction is more dependent on the surface area and chemistry, specific size, and the surface area-to-volume ratio (Chung et al. 2007, Motskin et al. 2009). It is speculated that the higher toxicity seen with nanoparticles of non-toxic bulk materials is due to the high surface area-to-volume ratio of nanoparticles and their surface chemistry (Labbaf et al. 2011), which may be the case also with thin BG fibers.

It is likely that the wide exposure in study II resulted in a large surgical dead space without dynamic tissue fluid circulation, resulting in an increase in the local pH and a rapid (within 24–72 hours) resorption of BG 1–98 fibers when compared with the dynamic environment of the femur study (I). With ion concentrations and pH exceeding the regulation capacity of the cells, apoptosis, necrosis, and resorption of the autograft were inevitable.

6.2. Bioactive glass S53P4 in spinal fusion for spondylolisthesis and trauma

Autogenous bone (AB) with its osteogenic potential, osteoinductive factors, and scaffold properties remains the gold standard for graft material used in posterolumbar spinal fusion. The rate of pseudoarthrosis using AB as graft material in spinal fusion has been reported to be 5%–43% (Miyazaki et al. 2009). Advances in instrumentation have diminished the rate to 10%–15% (Miyazaki et al. 2009). In this long-term study, a 100 % fusion rate was observed for the AB side based on the CT in both trials (III&IV).

BG S53P4 was used on the opposite side as a stand-alone bone graft substitute, resulting in a solid fusion in the CT of 12 of 20 patients and a partial fusion in 5 of 16 patients. The total fusion rate of all fusion sites (n=41) was 88% (88% for level L4/5 and 88% for level L5/S1) in the spondylolisthesis group (III).

In the trauma trial (IV), solid fusion was observed in the CT scans of five patients, and partial fusion was found for five patients. The subjective outcome was, however, good or excellent for 70% of the patients (IV). This finding is in accordance with previously reported results showing that unilateral fusion and instrumentation are adequate for the achievement of successful clinical results. Furthermore, no significant association between the radiological appearance of fusion and patients' clinical outcome has been found (Acharya et al. 2008).

Compared with the preoperative situation, the general subjective long-term outcome of the spondylolisthesis patients improved in 15 of 17 patients. The mean ODI score at the 11-year follow-up was 21 (range 0–52), when compared with the preoperative 49 (range 32–64). The median VAS score for back pain at the 11-year follow-up was 3.5 (range 0–8), compared with the preoperative situation, which was 7.3 (range 4–9). At the 12-month follow-up, the ODI score was reduced

to 74% and the VAS was 68% when compared with the preoperative scores. The long-term clinical outcome of improvement in the ODI and the VAS is similar to earlier published data on instrumented spondylodesis for degenerative patients (Andersen et al. 2008, Pearson et al. 2010).

CT has become the standard method for assessing posterolateral fusion and was the most sensitive method with which to determine whether or not BG granules were still present in the fusion bed. The fact that remnants of glass granules were still present in 50%–72% of the patients at the 11-year follow-up is not surprising. Similar findings have been shown for patients with benign bone tumors treated with BG S53P4 as a bone graft substitute. Glass granules have also been observed to be incorporated in cancellous bone that is harder than normal (Lindfors et al. 2009).

MRI is generally not optimal for the assessment of small calcifications, and the detection rate for bone fusion and small granules was also found to be low in these studies (III&IV). Varying degrees of metal artefacts caused by the steel osteosynthesis instrumentation in part of the patients (III) was also present, which affected the interpretation of the MRI scans.

Osteostimulation has been described as a phenomenon related to the implanting material properties of a bony environment. The characteristic features of the implanted material induce a stimulation of the recruitment and differentiation of osteoblasts, the activation of osteoblasts to produce new bone, and the activation of specific osteoblast genes in response to ion dissolution from the material. Experimental and clinical studies have revealed that BG S53P4 is osteostimulative (Virolainen et al. 1997). In an experimental bone healing model that used AB in rabbit, a combination of AB and BG S53P4 (70/30 vol%) and BG S53P4 as bone graft materials for spinal fusion, new bone formation between the transverse processes was found in all of the cases, and solid fusions had occurred in 50%–75% of the cases at 12 weeks (Lindfors et al. 2002).

Several other synthetic bone substitutes have been used for spinal fusion surgery. A combination of BG S45S5 (Novabone) and AB has been compared with AB in the treatment of adolescent idiopathic scoliosis, with good clinical results. The loss of correction of the main thoracic curve was slightly less for the BG group. Moreover, the blood loss and the complication rate were also significantly lower for the BG group (Ilharreborde et al. 2008). One has, however to consider that in scoliosis fusions, there is a continuous bony fusion bed. This is a more favourable environment for successful fusion if compared to intertransverse posterolateral fusion where a gap has to be bridged by the fusion mass. Local AB mixed with an apatite- and wollastonite-containing glass ceramic resulted in an 80% fusion rate for two-level spinal fusion in the absence of any spinal instrumentation (Kasai et al. 2003).

Contradicting results showing a high resorption rate was reported for a HA-BG composite (Chitra-HA-BG) used as stand-alone graft material on one side in an instrumented posterolateral fusion for the treatment of degenerative spondylolisthesis or spinal stenosis. The outcome of was excellent on the AB side, but 95% of the HA-BG composite cases showed poor consolidation. Chitra-HA-BG contains 80% HA and 20% of an unknown BG. HA is known to be relatively inert, and it has poor biodegradation properties that may hinder bone remodeling (Acharya et al. 2008).

Vascularization plays a crucial role in successful bone formation. Good vascularization in the presence of BG S53P4 has been reported by Peltola and coworkers. They reported that vascularization and new bone formation was faster in rabbits for BG S53P4 than for HA-filled bone defects (Peltola et al. 2001).

Allograft bone is highly osteoconductive, but its osteoinductive capacity is weak and it lacks osteogenic capacity (Zimmermann and Moghaddam 2011). Concern also exists about the possible spread of viral transmission diseases caused by, for instance, HIV and HCV (Simonds et al. 1992, Tomford 1995). Furthermore, the production processes, which include freezing, freeze-drying, or sterilization of allograft bone, reduce the osteoinductive and osteoconductive capacities of the graft material.

No postoperative deep wound infections were observed in the S53P4 clinical trials. BG S53P4 has antibacterial properties, which have been suggested to be caused by the initial high pH and the subsequent osmotic effect caused by the dissolution of ions from the glass. Furthermore, BG S53P4 has been shown in vitro to have effective bacterial-growth-inhibiting properties towards 17 anaerobic bacteria and 29 clinically important aerobic bacteria (Leppäranta et al. 2008, Munukka et al. 2008). Moreover, BG S53P4 was successfully used as a bone substitute in the treatment of osteomyelitis of the spine and lower extremities (Lindfors et al. 2010a).

6.3. Limitations and strengths of the study

Study I lacked a sham control group, the use of which could have shown the real significance of bone grafting in the induction of periosteal new bone formation. In addition, the use of a control group with a non-absorbable polyester fabric could have made it possible to separate the impact of resorbable material on bone formation from the possible effects of mechanical stress and unintentional manipulation of the periost when the fabric was being wrapped around the femur. It remains unclear whether the close proximity of the two fabrics interfered with each other. To lower this risk, the narrow volume of interest for the pQCT analysis was selected from the central part of each fabric. The model did not allow for measurements of the interfacial strength between fabrics and the attached new bone. Previously, Marcolongo and coworkers have shown that BG fibers in a polymeric composite may improve the strength of the bond (Marcolongo et al. 1998). The use of bone-seeking in vivo labels could have provided useful additional information on the nature of cortical bone remodeling. In the future, the use of several time points would give exact information on the rate of new bone formation and bone remodeling in relation to fabric degradation. The model of autograft attachment with an investigational material to the femur seemed to be a feasible method with a very low complication rate.

Study II had several limitations. The animal experiment was not designed to evaluate adverse soft tissue responses; therefore the readiness for histopathological and toxicological evaluations was limited. The fact that several specimens were lost before the pattern of the BG-related adverse effect was evident weakened the objective results. Obviously there is a need for a systematic toxicological study, which would give a completely different perspective for this issue. In addition, the in vitro studies were designed to confirm that the investigated composites support the osteoblastic differentiation of hMSCs. In cell cultures with fibers, it was found that the pH followed the same trend with both the BG and polymer fibers, and there were no differences during the follow-up. However, with the thin fibers used, the fast ion exchange (Na^+ and K^+ with H^+) that affected the pH probably took place early, and measurements during the first hours would most likely have shown more radical pH changes. Without a doubt well-planned, biocompatibility, in vitro testing will easily provide answers to the questions raised regarding the safety of BG fibers of the Na_2O-K_2O-MgO-CaO-B_2O_3-P_2O_5-SiO_2 system.

The prospective clinical trial (III & IV) was set up at a time when BG S53P4 was approved only for investigational use in a clinic setting. This is one of the reasons for the use of BG only on one side of the fusion. Each patient served as his or her internal control, making the evaluation of the outcome measures difficult. With the experience gathered in my institution after using BG granules being used for several years in spinal fusion surgery, it has been found beneficial to mix BG with AB. This practice is due to the observation that extra-osseal bone formation around BG granules appears to be slow.

The high level of fusion achieved in both studies on the AB side may affect the outcome of the fusion potential observed on the BG side. Therefore, a randomization using one bone substitute in one patient on both sides can be debated. However, the subjective outcome of four patients with a partial fusion was excellent or good. This finding is in accordance with previously reported results that unilateral fusion and instrumentation

are adequate for achieving successful clinical results (Andersen et al. 2008). Furthermore, no significant association between the radiological appearance of fusion and patients' clinical outcome has been detected.

The strengths of the clinical trials were that a prospective cohort study was used, and there were no patients lost to follow-up during the first year. In the spondylolisthesis study only three patients were lost during the 11-year follow-up, two patients having died of unrelated causes and one patient having chosen not to participate. The result was a follow-up rate of 85%, which is considered high for long-term studies. The follow-up rate in the trauma study was lower (63%). This is a common problem in clinical, long-term follow-up studies, and it somewhat decreases the power of the conclusions.

6.4. Future aspects

Although BG fibers of different compositions have been studied for their in vitro and in vivo properties, no head-to-head comparisons have been made of the in vivo behavior of fibers and solid forms of the same BG composition. Such a comparison whould be important since the increased surface-to-volume ratio of BG in the form of fibers results in drastic changes in the reactivity and degradation pattern. The results suggest that, under certain conditions, the physical form of BG can be more critical than its chemical composition when a clinical application is being designed.

The use of BG in a composite structure requires an understanding of the properties of the BG, the polymer, or other composite material, and the effect that they pose on each other during degradation. The great opportunity to design specific degradation profiles for different uses of a bioactive implant is unique for BG. Nano technology can offer a solution to some of the obstacles of composites when combinations of biodegradable polymers and BG result in unpredictable degradation. The polymer component can be catalyzed by its own acidic degradation products, resulting in a scaffold breakdown. This problem can be overcome by nanotechnology that brings the BG and polymer closer to the bone cells, resulting in a tough material and a more linear degradation (Jones 2011). Further advances will be seen in BG with drug releasing properties, delivering active molecules in a controlled fashion. In order to decrease the potential side effects of delivering the high local doses of rhBMP needed to generate a fusion, new bioactive glasses could be developed for the purpose of only delivering the required amount of rhBMP into the fusion area.

The use of BG as a bone graft extender can be considered to be a good alternative for use in spine surgery in the future. Its use would warrant further investigations to define the ultimate granule size and mixture with AB.

7. CONCLUSIONS

On the basis of these experiments and clinical prospective trials, the following conclusions can be drawn:

1. BG fibers of the BG 1–98/$PLGA_{80}$ fabrics did not introduce any additional benefit to bone formation when compared with that of the polymer control. (**I**).

2. In a rabbit model of spinal fusion, unexpected serious adverse events (intractable pain, paralysis and death) occurred in an experiment aimed at testing biodegradable woven BG 1–98/$PLGA_{80}$. In vitro cultures showed growth inhibition of human mesenchymal stem cells next to BG 1–98 fibers and radical pH changes. (**II**).

3. The first long-term results concerning the clinical use of BG S53P4 as bone graft material in instrumented posterolateral spondylodesis to treat degenerative spondylolisthesis indicates that it seems to be a safe procedure, that is associated with a very low complication rate. BG S53P4 used as a stand-alone bone substitute resulted in a solid fusion in only 12 of 17 degenerative spine patients and cannot, therefore, be regarded being as efficient as AB in promoting solid fusion. (**III)**

4. The first long-term results concerning the clinical use of BG S53P4 as bone graft material in instrumented posterolateral spondylodesis to treat unstable lumbar spine fractures indicates that it seems to be a safe procedure, that is associated with a very low complication rate. BG S53P4 used as a stand-alone bone substitute resulted in a solid fusion in only 5 of 10 trauma patients and cannot, therefore, be regarded being as efficient as AB in promoting solid fusion. (**IV**).

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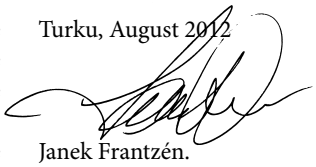

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