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Bioactive glass S53P4 as bone graft substitute in treatment of osteomyelitis

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ABSTRACT

Bioactive glass (BAG)-S53P4 is an osteoconductive bone substitute with proven antibacterial and bone bonding properties. In a multicentre study 11 patients with verified chronic osteomyelitis in the lower extremity and the spine were treated with BAG-S53P4 as a bone substitute. The cavitary bone defect and the surrounding of a spinal implant were filled with BAG-S53P4. The most common pathogen causing the infection was *Staphylococcus aureus*. The mean follow-up was 24 months (range 10–38). BAG-S53P4 was well tolerated. Nine patients healed without complications. One patient who achieved good bone formation sustained a superficial wound infection due to vascular problems in the muscle flap, and one patient had an infection due to a deep haematoma. This study shows that BAG-S53P4 is a good and well-tolerated bone substitute, and can be used in treatment of osteomyelitis with good primary results.

Introduction

Osteomyelitis, first described by Chassaignac in 1852, is caused by infecting micro-organisms and defines a destructive inflammatory process in bone that is often accompanied by bone destruction [1]. The infection can arise from a variety of aetiologies [2]. Most often it is caused by trauma, but any kind of bone or soft tissue surgery where pathogens can enter the bone, may cause the infection. In diabetic patients, osteomyelitis may appear as a secondary manifestation due to vascular insufficiency and soft tissue infection [3]. Haematogenous osteomyelitis has been found in children, as well as in elderly patients [4].

Acute osteomyelitis is an infection characterized by oedema, locally decreased blood supply and pus formation. Untreated or due to treatment failure, the infection can progress to a more chronic phase, with formation of a large area of devascularized dead bone, a sequestrum. In treatment of chronic osteomyelitis, adequate debridement is mandatory. Unfortunately, this treatment often results in a poorly vascularized large bone defect, a dead space. Bacterial infection can also cause local acidosis, leading to dissolution of bone matrix mineral [5]. Many different methods have been used to treat the bone defect and the infection, including free vascularized bone grafts, local muscle flaps, antibiotic-impregnated polymethyl methacrylate (PMMA) beads, granulation formation according to the technique of Papineau and the Masquelet technique [6] or bone reconstruction based on Ilizarov technology [7].

Staphylococcus aureus and Gram-negative bacilli are the pathogens most commonly involved [7]. The bacterial colonization of host tissue or implanted materials is promoted by the ability of the bacteria to produce protein-specific adhesins on their surfaces, which is followed by interactions with host protein components, such as fibrinogen, fibronectin and collagen. Bacteria also have sophisticated methods for communication through hormone-like compounds in biofilms, making treatment with antimicrobial agents difficult [2]. Use of synthetic bone graft substitutes in treating osteomyelitis is, therefore, generally not recommended.

Bioactive glasses (BAGs) are synthetic biocompatible osteoconductive bone substitutes, with bone bonding capacity and documented antibacterial and angiogenesis-promoting properties [8–16]. Previous studies on atrophic rhinitis, a chronic purulent disorder often caused by *Klebsiella ozaenae* and difficult to treat, have shown that BAG-S53P4 does not favour adhesion or colonization of *K. ozaenae* on its surface. In addition, *K. ozaenae* cannot form biofilms on BAG-S53P4 [17].

The aim of this study was to apply the experimentally known antibacterial properties of BAG-S53P4 to clinical practice, evaluating the operative outcome using BAG-S53P4 as a bone graft substitute in treating osteomyelitis.

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Patients and methods

This is a multicentre study on patients with verified osteomyelitis in 2007–2009. Eleven patients (nine males, two females) with a radiologically diagnosed osteomyelitis participated. Osteomyelitis was verified on MRI (nine patients), or on CT scans (two patients). Osteomyelitis was localized in the lower extremity in ten cases and in the spine in one case (Figs. 1A, 2A–B). Seven of the patients had sustained a fracture: in the distal tibia (three patients), in the calcaneus (two patients), in the distal fibula (one patient) and in the distal femur (one patient). Nine patients had undergone previous operative treatments, including revisions, osteotomies and artrodesis. Autologous bone grafts had been used in two patients and a bone substitute (Norian®) in one patient. Kanamycin granules had been used in one patient and Garamycin granules (Septocol®) in two patients. Antibiotic therapies had been given to all patients. One patient had been treated for osteomyelitis for 64 years, four patients for 7–16 years and six patients for ~ 1–2 years. Data including the predominating aetiology of osteomyelitis, previous treatments and methods for verification of osteomyelitis are shown in Table 1.

In 2007–2009, all of the patients were operated on due to chronic infection and verified osteomyelitis. In the operation, the infected bone and the soft tissue were removed, and the cavitary bone defects were filled with BAG-S53P4 (BonAlive^{R}, Bonalive Biomaterials Ltd., Finland). The whole cavitary defect was filled and, therefore, the amount of glass used was depending on the size of the cavity. In four patients, muscle flaps were used as part of the treatment. A patient with verified spondylitis was treated using a metal implant which was covered with BAG-S53P4. The most common pathogen causing the infection was *S. aureus* (six patients). The outcome of the treatment was evaluated by the surgeon as excellent (no complications), good (a small complication) or a temporary stable situation.

Data for operative treatment, BAG-S53P4 used, pathogens, postoperative treatment and complications are provided in Table 2.

Patients were seen at the outpatient departments at 1, 2, 3–4, and 6–15 months postoperatively. Five patients had a follow-up of 2–6 months and six patients of 8–15 months. Patient data were also obtained from hospital patient records until March 2010, resulting in a mean follow-up period of 24 months (range 10–38).

Results

BAG-S53P4 was well tolerated; no BAG-related adverse effects were seen in any patient. The use of BAG-S53P4 as a bone graft substitute resulted in a fast recovery, i.e. patients that had been treated with long-lasting therapies responded well to the treatment. Clinical outcome was good or excellent in nine of eleven patients. The clinical and radiological findings are summarized in Table 3.

Postoperative complications needing treatment were seen in two patients. In one patient, vascular problems occurred in the muscle flap, with a subsequent wound infection. No sign of osteomyelitis was, however, observed on X-rays. The BAG was well incorporated into the bone and the bone cavity healed well.

In another patient, a postoperative complication was observed one month after treatment. This patient had been in the Second World War in 1944, where he had a shell splinter accident and sustained a mutilated tibial fracture. Over the years, he had undergone numerous treatments. Postoperative X-ray verified that the evacuated cavity had not been properly filled with BAG. During arthroscopic revision the empty part of the treated cavity was observed to be filled with a haematoma, which was considered to be the cause of the re-infection.

According to patients' records, no relapses or other complications were observed.

The postoperative radiological appearance of the treated bone cavity and the spine is shown in Figs. 1B–C and 2C.

Discussion

Despite advances in antibiotic therapies and operative techniques, treatment of osteomyelitis remains challenging, expensive and time-consuming for both the doctor and the patient.

Debridement in combination with local administration of antibiotics, e.g. gentamicin-loaded PMMA beads, has for years been the method of choice in treating osteomyelitis. However, in a long-term follow-up study of 100 patients treated with gentamicin-PMMA beads, relapses were observed for 8.8% of patients with acute osteomyelitis and for 21.2% of patients with chronic osteomyelitis [18]. PMMA is also known to provide a favourable environment for proliferation of bacteria [19].

Biodegradable antibiotic-impregnated implants have also been used to treat chronic osteomyelitis. Five patients treated with calcium sulphate tobramycin impregnated pellets and one patient treated with calcium sulphate tobramycin–vancomycin impregnated pellets have shown excellent osseous repair [20]. However, an increase in antibiotic-resistant bacteria, such as gentamicin- or methicilline-resistant *S. aureus,* has been observed [21].

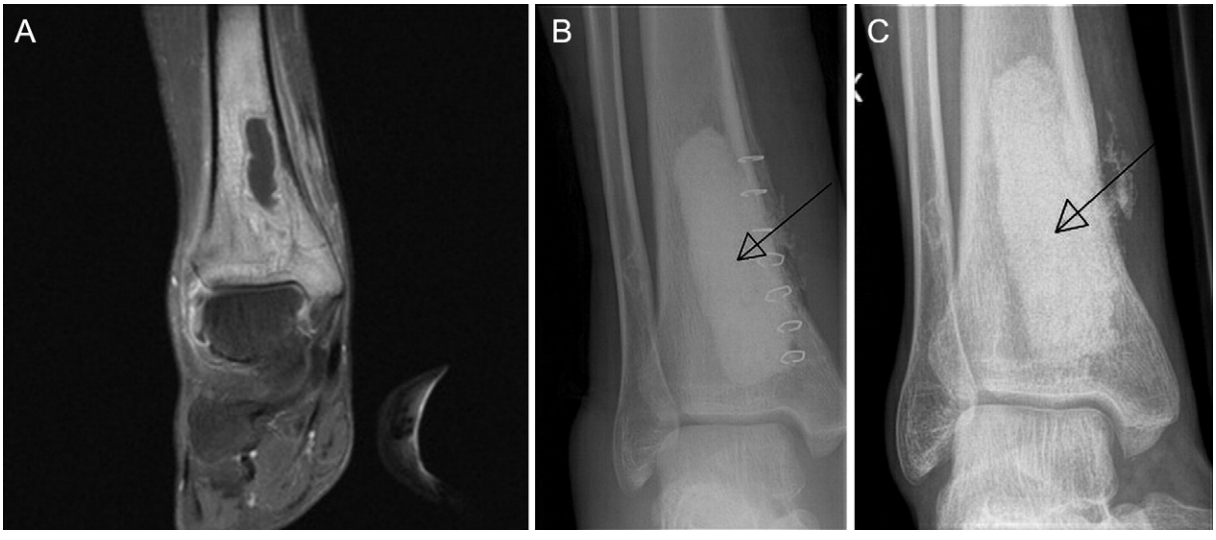

Fig. 1. (A–C) Osteomyelitis caused by *S. aureus* in distal tibia treated with BAG-S53P4 as bone graft substitute: (A) preoperative MRI showing osteomyelitis in tibia, (B) postoperative X-ray showing BAG-S53P4 in the treated bone cavity (arrow), and (C) X-ray at five months follow-up showing the treated region (arrow).

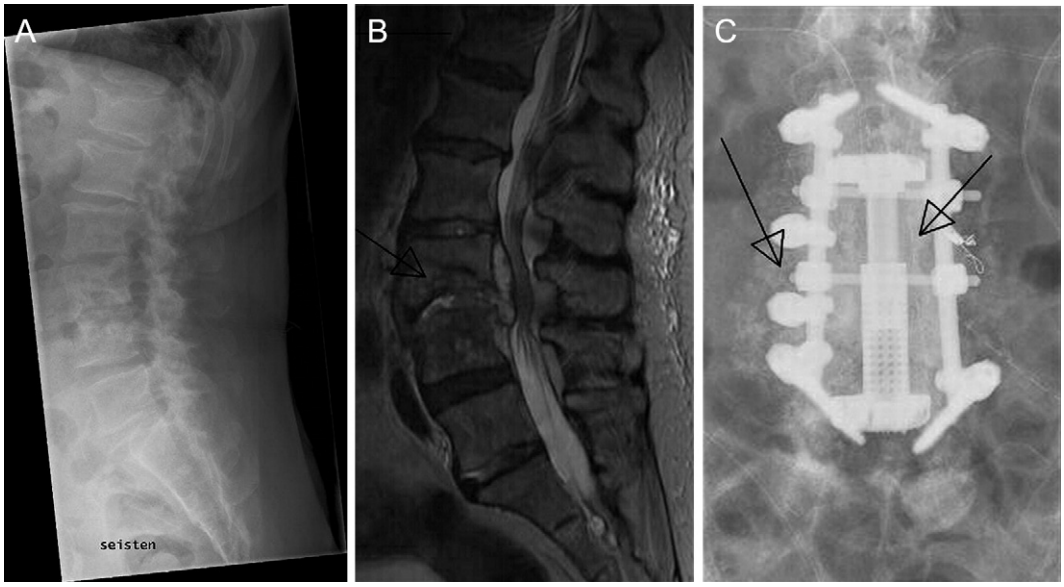

Fig. 2. (A–C) Osteomyelitis caused by *Mycobacterium tuberculosis* in the spine treated with BAG-S53P4 as bone graft substitute: (A) preoperative X-ray and (B) MRI scan showing abscess formation in L III (arrow) and (C) postoperative X-ray showing implant covered with BAG-S53P4 (arrow).

BAGs are bone substitutes with bone bonding and antibacterial properties. The bioactive process leading to bone bonding has been described as a sequence of reactions in the glass and at its surface. Implantation of the glass is followed by a rapid exchange of Na^+ in the glass with H^+ and H_3O^+ from the surrounding tissue, leading to the formation of silanol (SiOH) groups at the glass surface. After repolymerization, a SiO_2-rich layer is formed. Due to migration of Ca^{2+} and PO_4^{3-} groups to the surface and cystallization, a $CaO\text{-}P_2O_5$ hydroxyapatite (HA) layer is formed on top of the Si-rich layer. Finally, cell interactions with the HA layer subsequently initiate the bone forming pathway [8,11].

The initial leaching of alkali and alkaline earth ions lead to a rapid increase in pH around the glass; the basicity depends on the composition of the glass. BAG-S53P4 has in a simulated body fluid shown an increased pH_{max} value of 11.65 [22]. The high pH and the subsequent osmotic effect caused by dissolution of the glass have been suggested to partly explain the antibacterial properties observed for BAGs [23]. This is confirmed by the observation that neutralization of a highly alkaline solution with BAG eliminates the antibacterial effect [24]. Prevention of bacterial proliferation due to ion release has also been demonstrated for glass polyalkenoate cements [25]. Comparing bactericidal effects of different BAGs, BAG-S53P4 has been shown to be the most effective, with the fastest killing or growth inhibitory effect. This antibacterial effect has been observed *in vitro* for all pathogens tested, including the most important aerobic and anaerobic pathogens, as well as very resistant bacteria [14,15].

The effectiveness of a degradable and bioactive borate glass as a carrier for vancomycin has been compared with calcium sulphate in treatment of osteomyelitis of rabbits. At 8 weeks, vancomycin-loaded borate glass was found to be effective in eradicating osteomyelitis caused by methicillin-resistant *S. aureus* (MRSA), and the treated region was mostly reabsorbed and replaced with new bone. Treatment with pure borate glass was significantly less effective in eradicating MRSA [26].

Vascularization of the poorly vascularized dead space of the bone cavity and the surrounding tissue is vital in treating osteomyelitis. Vascular endothelial growth factor (VEGF) has been successfully used in preclinical models to enhance and promote the development of collateral blood vessels in ischaemic tissue [16,27,28]. 45S5 Bioglass™ has been shown to stimulate release of angiogenetic growth factors and to promote angiogenesis. In an *in vitro* model, addition of fibroblast-conditioned medium, produced in the presence of 45S5 Bioglass™, resulted in a visible increase in tubule branching and formation of complex networks of interconnected tubules. These are known to be essential for angiogenesis, cell migration, cell proliferation, vessel branching and anastomosis [16,29]. The proangiogenic potential of soluble products of 45S5 Bioglass™ has been determined by examining the capacity to induce endothelial cell proliferation and up-regulation of VEGF production. The results indicated that 45S5 Bioglass™ possesses a robust proangiogenic potential, and may provide an alternative to recombinant inductive growth factors [30]. A material approach to achieve an angiogenetic response has also been demonstrated in an *in-vivo* rat critical-size defect model. Significantly enhanced mitogenic stimulation of endothelial cells with an additive effect with VEGF release was observed in the presence of a BAG coating [31]. Vascularization and new bone formation have been observed to be faster in defects filled with BAG-S53P4 than in hydroxyapatite-filled defects. Initial fibrous tissue formation related to a considerable amount of blood vessels was also more rapid in the BAG filled defects [32].

In a prospective randomized study using BAG-S53P4 and autograft bone (AB) as bone graft substitutes in benign bone tumour surgery, no difference in cavity volume between BAG-S53P4 and AB was observed postoperatively either on X-rays or on CT scans at 24 months (small cavities) or 36 months (large cavities). The volume of large bone cavities filled with BAG-S53P4 started to diminish after 12 months, indicating that BAGs dissolve slowly and the bone graft area will remodel to bone over time [33]. This phenomenon may be beneficial in treatment of osteomyelitis, as the antibacterial and angiogenesis-promoting properties observed for BAGs may remain over a long period.

In a prospective, randomized 14-year follow-up study on BAG-S53P4 as bone graft substitute in benign bone tumours, the filled cavity had a dense appearance on X-rays. A mainly or partly fatty bone marrow was, however, observed on MRI. BAG-S53P4 has in long-term studies shown to be a safe and well-tolerated bone substitute [34], and it does not disturb the growth of bone in children [35]. The bacterial growth-inhibiting properties observed exclusively on BAGs, and the favourable outcome of the treatment of osteomyelitis in this study, suggest that BAG-S53P4 is suitable as an antibacterial treatment as well.

No correlation between age, the amount of BAG or granule size used was observed in this study. The most important factor affecting the outcome is a properly filled cavity. In one case (patient 5) only a

Table 1
Predominating aetiology, previous treatments and methods for verification of osteomyelitis.

Patient	Age	Sex	Aetiology/year	Previous treatment/year	How preoperative osteomyelitis was verified/year
1	16	Male	Brodie abscess/2000	Evacuation + autologous bone graft/2000	Osteomyelitis on MRI in proximal tibiae/2007
2	44	Male	Distal fibular fracture/2006	Operative treatment of non-union fracture. Five revisions during 6 months in combination with antibiotic therapy. Cement spacer 12/2006	Osteomyelitis on MRI in distal fibula/2006
3	36	Male	Distal femoral/shotgun accident 1991	A huge number of antibiotic therapies since 1991. Revision of infected area 6.2.2008 and 11.2.2008	Osteomyelitis on MRI and fistular formation to epidermis in femur/2008
4	53	Male	Distal tibial fracture/2007	Operative treatment with locking plate/2007. A huge number of antibiotic therapies over 4 months.	Postoperative wound infection Fistular and pus formation, diffuse osteolysis of bone on CT/2007
5	84	Male	Mutilated tibial fracture from grenade explosion in second world war/1944	Many and long therapies during decades. Shell splinter causing wound infections. Acute wound infection in 2006, antibiotic therapies.	Osteolysis on CT in proximal tibiae and fistular formation. MRI not possible because of metal shell splinter causing thoracic pain/2006.
6	38	Male	Comminuted calcaneus fracture 2006	Calcaneus osteotomy + arthrodesis 12/07 Collapse of subtalararhtrodesis, screw perforation in cystic area 2/08 Two revisions due to pus formation 2,3/08, Septocol® (Garamycin) in cavity, osteocyntes material removed Oxacillin, Clindamycin therapy	Abscess formation in calcaneus on CT/2008
7	62	Female	Stepped on a nail 8/2008	Penicillin, Cefalexine	Chronic infection after accident Osteomyelitis on MRI in distal first metatarsal bone/2008
8	33	Male	Stepped on a nail 1985 no treatment	Sulfa trimetoprime 1992 Revision and treatment with Kanamycin granules 1994 Fluorokinolone, Metronidazole 2006 Vancomycin, Linetsolid during 2 months 2006	Swelling, cystic formation in lateral cuneiforme bone, fistulografia shows fistular formation to os cuneiforme 10/1992 Acute infection with pus formation in operated region. Cystic formation in os cuneiforme laterale on MRI/2006. Despite antibiotic treatment abscess and pus formation. Cystic formation on MRI/2007.
9	53	Male	Comminuted calcaneus fracture due to 2 m fall 3/2008	Operative treatment of calcaneus fracture, postoperatively wound infection, Clindamycin. Osteosynthes material removed, revision of bone, cavity filled with Norian®. Vancomycin.	Open wound with visible osteosynthes material/2008. Postoperative wound infection, fistular formation to bone, pus formation/2008.
10	75	Female	Back pain and fever several months 2007, suspected urinary infection	Cefalexine, Pivmecillinam, Amoxicillin, Kefuroxime, Rifampicin	Spondylitis LIII-IV and epidural and psoas abscess formations on MRI/2007–2009, resistant to antibiotic treatment
11	72	Male	High energy open dislocation of talocrural joint. Severe contamination with soil and foreign materials/1999	External fixation. Several revisions due to persistent wound infection. Artrodesis and use of autograft bone. Stable situation 2003–2009. Fistulectomy and curettage of bone, Gentamicin beads/2009. Cefuroxime, Vancomycin, Rifampicin.	Pus formation and destruction of TC joint, osteomyelitis on MRI/1999. Fistular formation in affected joint. Pus formation and cavitary defect, osteomyelitis in fused TC joint on MRI/2009

Table 2
Operative treatment using BAG-S53P4 as bone graft substitute in treatment of osteomyelitis.

Patient no.	Date of operation with BAG	Operative treatment	Glass granule size (mm)	Glass granule volume (ml)	Post-operative antibiotic therapy	Pathogen(s)/ obtained from	Postoperative complications/ Need of reoperations	Result of treatment according to surgeon
1	9.1.2007	Evacuation of infected avascular bone and pus from abscess formation	1.0–2.0	24	Kefuroxime Rifampicin Fluconazole	*Staph aureus*/bone cavity	No/No	Excellent
2	26.1.2007	Evacuation of soft bone, extensor digitorum muscle flap and free skin graft	1.0–2.0	5	Vancomycin	*Strept magnus* *Corynebact*/bone cavity *Staph epiderm*/wound	Lung embolus/No	Excellent
3	14.2.2008	Evacuation of bone	2.0–3.15	16	Tazocin Vancomycin Glazidim	*Pseudomonas*/bone cavity	No/No	Excellent
4	22.1.2008	Revision and evacuation of bone, rectus abdominis muscle flap.	2.0–3.15	4	Vancomycin Tazocin Ciprofloxacin	*Staph aureus* *Staph epiderm*/wound	Five revisions due to circulatory problems and infection in muscle flap	Good, despite postoperative complication, no sign of infection in bone
5	18.6.2008	Revision, Gastrocnemicus muscle flap	2.0–3.15	20	Kefuroxime Glazidim Oxazolidinone Sulfa trimetoprime	*Staph aureus* *Gram neg bacilli* (very resistent)/ bone cavity	Postoperative infection and cavity formation below muscle flap/ arthroscopic revision, evacuation of haematome from bone and tissue cavity, application of Gentamycine-collagen	Temporary stabile situation
6	14.3.2008	Septocol® removed, revision	0.5–0.8	3	Clindamycin	*Staph aureus* *Staph epiderm*/wound	No	Fast recovery excellent results
7	25.5.2009	Revision	0.5–0.8	2	Kefuroxime 14 days	-	No	Excellent and surprisingly fast recovery although the antibiotic therapy was considered to be too short by mistake of the doctor
8	29.5.2007	Revision, pressure wash, hydrogenperoxide			Vancomycin Kefuroxime Oxazolidinone Fusidic acid Rifampicin	*Staph epiderm* *Staph aureus*/wound	No	After 9 months with heavy antibiotic therapy fast recovery after operative treatment with BAG
9	25.7.2008	Revision, reconstruction with stylo abductor digiti minimi muscle flap.			Clindamycin Levofloxacin	*Staph aureus* *Enterobact cloachae*/wound	Wound secretion muscle flap not completely vital. No new treatment needed.	Good recovery despite of postoperative wound secretion
10	28.4.2009	Posterior decompression LII/ III-LIII/IV, spondylodesis LII-V, lumbotomy, canalisation of paravertebral abscess, resection of LIII, IV, anterior decompression and reconstruction	0.8–1.0	32	Meropenem Vancomycin Rifampicin Levofloxacin	*Mycobacterium tuberculosis*, postoperatively from abscess formation in psoas muscle	6/2009 paravertebral abscess formation on MRI	Good recovery, despite observed abscess formation in psoas muscle (no treatment needed)
11	20.4.2009	Gentamycine beads removed.		8	Vancomycin Rifampicin	*Staph epidermidis*/wound		Good recovery

temporary stable situation was achieved. This was considered to depend on the fact that the cavity had not been properly filled, which resulted in a formation of a haematoma and a subsequent infection.

Patients who suffer from osteomyelitis differ in illness pattern. This study shows that BAG-S53P4 can successfully be used as a bone substitute in treating osteomyelitis independent of aetiology, pathogens, localization or previous treatment of the infection.

Defining the outcome of osteomyelitis is, however, not easy, as recurrences may appear months after treatment. According to the Infectious Disease Society of America guidelines on requirements of human trials on osteomyelitis, a favourable outcome is achieved when the patient is clinically free of disease at the end of the follow-up. According to these guidelines, clinical follow-up trials should last for a minimum of 1 year [36]. In this study, the clinical follow-up at the outpatient department varied between 2–6 months (4 patients) and 8–15 months (6 patients). However, according to patient´ records, no recurrences have been observed for any of the patients after the follow-up period. Thus all patients, except the one in whom a postoperative haematoma was observed, have had a favourable clinical outcome until March 2010, lasting for a mean of 24 months (range 10–38 months).

The operative treatment of osteomyelitis is usually a two-stage procedure, including antibiotic-impregnated beads, which are later

Table 3
Post-operative clinical and radiological outcome using bioactive glass S53P4 (BAG) as bone graft substitute in treatment of osteomyelitis.

Patient	Clinical outcome/ 2 wk	Clinical outcome/ 1 month	Radiological findings/ 1 month	Clinical outcome/ 2 months	Radiological findings/ 2 months	Clinical outcome/ 3–4 months	Radiological findings/ 3–4 months	Clinical outcome/ months	Radiological findings/ months
1	No sign of infection	No sign of infection	BAG incorporated in bone on X-ray	No sign of infection	BAG incorporated in bone on X-ray	No sign of infection	Callus formation on X-ray	No sign of infection/ 9 months	Bone bridge on X-ray in treated region/ 9 months
2		No sign of infection, small necrosis on border of skin graft	Ca-formation in treated defect on X-ray	No sign of infection	BAG resorption and bone growth on X-ray	–	–	No sign of infection/ 8 months	New bone formation seen on X-ray in treated region/ 8 months
3		No sign of infection	–	No sign of infection	BAG well incorporated in bone on X-ray	No sign of infection	BAG well incorporated in bone on X-ray	No sign of infection, painless/ 9 months	
4	Wound infection		–	No sign of infection	BAG well incorporated in bone on X-ray	No sign of infection	BAG well incorporated in bone on X-ray, good bone formation		
5	No sign of infection	No sign of infection	X-ray shows that treated cavity not properly filled with BAG	Avascular soft tissue under muscle flap, cavity formation	PET-CT shows osteomyelitis in untreated proximal tibiae				
6		No sign of infection	No cystic lesions on X-ray	No sign of infection Painless	Subtalar bone formation Callus formation in calcaneus Sclerosis in region treated with BAG on X-ray				
7	No sign of infection	No sign of infection	BAG well incorporated in cavity on X-ray			No sign of infection	BAG well incorporated in cavity on X-ray		
8	Fast recovery			No sign of infection	No sign of lytic formation on X-ray	No sign of infection	No sign of lytic formation on X-ray	No sign of infection/ 15 months	No sign of osteomyelitis on X-ray/ 15 months
9	Wound secretion	Muscle flap not completely vital		Wound granulation	No sign of oedema or infection around cavity filled with BAG. Small oedema around adjacent previously filled cavity with Norian on MRI.			No sign of infection/ 11 months. Sustained a distal tibial fracture, osteoporotic bone.	No sign of osteomyelitis on CT 11/ months
10	Good	Good		Good	Abscess formation in psoas muscle on control MRI,TT-biopsy	No sign of infection	No sign of infection, bone formation on X-ray	No sign of infection excellent recovery/ 6 months	
11	Good	No sign of infection	No sign of infection	No sign of infection	BAG incorporated in bone on X-ray	No sign of infection	No sign of infection on X-ray	No sign of infection/ 9 months	BAG granules incorporated in bone on CT/ 9 months

removed and replaced with cancellous bone grafts or a vascularized bone graft [7]. In this study, BAG-S53P4 was used with good results in a one-stage procedure in six patients, although pus was observed in some of the patients' surrounding tissues. Using BAG-S53P4 as a bone substitute in an one-stage procedure, with no second operation required and no harvesting of AB from the iliac crest, makes BAG-S53P4 a cost-effective, as well as, a rapid method in treating osteomyelitis.

In conclusion, these primary results indicate that BAG-S53P4 can be considered as a good, effective and usable material for the treatment of osteomyelitis. Longer follow-ups are, however, needed to verify the long-term beneficial outcome of the treatment.

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