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ORIGINAL ARTICLE

# Tissue reaction and material biodegradation of a calcium sulfate/apatite biphasic bone substitute in rat muscle

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biodegradation;
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tissue reaction

**Summary** *Background/Objective:* A biphasic ceramic bone substitute consisting of calcium sulfate and hydroxyapatite has been reported to give good clinical outcome regarding bone regeneration and may serve as a carrier for antibiotics in the treatment of bone infections. Often, the overlying muscle is in direct contact with the synthetic graft. The dissolving bone substitute induces inflammation, which may be harmful to the surrounding soft and muscle tissue. The aim of the present study was to evaluate the surrounding soft tissue reaction and the biodegradation of the biphasic bone substitute.
*Methods:* Rods (3 mm × 6 mm) were cast and implanted in the rat abdominal rectus muscle. The rods were either soaked or not soaked in autologous bone marrow before insertion to induce bone formation. Thirty-two rats underwent bilateral operation. After 6 weeks and 12 weeks, the bone substitute material and the surrounding muscle were harvested. The right rod was evaluated by histology to study tissue reaction and the left rod was analysed with micro-computed tomography and scanning electron microscopy to study bone substitute degradation.
*Results:* The muscle tissue around the material was similar at 6 weeks and 12 weeks, with or without prior treatment with bone marrow. The remaining material showed close contact with the muscle, and blood vessels penetrated the material in both groups. Wide bundles of collagen were embedded around the apatite particles, more at the 12-week time point. No bone formation was found, either at 6 weeks or 12 weeks, and scanning electron microscopy showed that the calcium sulfate phase was resorbed after 6 weeks with the calcium phosphate phase remaining intact. Micro-computed tomography showed significantly more hydroxyapatite at 6 weeks than after 12 weeks.

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*Conclusion:* Calcium sulfate hydroxyapatite bone substitute can be used as a carrier for antibiotics or other drugs, without adverse reaction due to the fast resorption of the calcium sulfate. No bone formation was seen despite treating the bone substitute with autologous bone marrow.


## Introduction

Bone grafts are used to augment and enhance healing in a number of surgical procedures, for example in bone tumour resection, eradication in chronic osteomyelitis, nonunion, revision arthroplasty, and spinal fusions. Due to the well described limitations of both autografts and allografts [1,2], synthetic alternatives have been developed over the past 2 decades. An injectable synthetic bone substitute that contains calcium sulfate (CaS) with embedded hydroxyapatite (HA) has been investigated previously, alone as a void filler in noncritical defects [3] or as a carrier for antibiotics or other drugs. It has been shown to promote bone formation, both in clinical studies and in bone defect animal models [4—9]. However, few studies have focused on the soft tissue reaction. When a bone substitute is used it is often in direct contact with, or covered by, a muscle. Material leaking from the filled cavity, or during the resorption of the material could cause a harmful tissue reaction.

Clinical data have documented that pure CaS pellets can enhance bone formation in bone defects [10,11]. CaS pellets seem to function as a conductive scaffold that provides a structural framework for angiogenesis and osteogenesis [12,13], but lacks any intrinsic osteoinductive or osteogenic capacity [14]. The rapid resorption rate of pure CaS is a clear disadvantage [15,16] that may affect osteoconduction and cause an inflammatory reaction including short-term drainage from the wound [17—20]. When CaS is combined with embedded HA, the resorption of the CaS leaves an apatite matrix with porosity that encourages bone ingrowth.

Goodman et al [21—24] have tested different materials as particles and showed that cobalt, chromium, titanium, and polymer particles as well as polyethylene, all induce an inflammatory reaction. HA has the ability to promote early bone growth [25]. It is a biocompatible material with osteoconductive properties [26,27] but also has the ability to encourage a host tissue to bond and integrate with an implant. However, with a bone substitute consisting solely of loose particles or granules, the particles may migrate from the implant site before ingrowth of new bone tissue anchors them in place. Particularly in a joint cavity this may increase third body wear [28]. By combining HA particles and calcium sulfate, an injectable material with an initial containment and stability can be obtained since the calcium sulfate will set within 6—12 minutes and bind the apatite particles [8].

Based on a limited donor availability but also the morbidity at the donor site, there will be an increased need for synthetic bone substitute, either alone or in combination with allo- and autografts. In several clinical situations, the bone substitute will come in direct contact with an overlying muscle and may induce an adverse tissue reaction. It is therefore important to study the reaction of a synthetic material in the surrounding tissue, especially the muscles [29], but few studies have focused on the soft tissue reaction. Bone substitute combined with bone marrow may have *in vivo* osteogenic potential. In a subcutaneous implant rat model, bone marrow in combination with porous HA composites induced bone formation after 4 weeks [30]. In another study, no bone formation was found using a collagen membrane or bone marrow alone, but bone formation was observed when the two were combined [31].

The aim of the present study was to evaluate whether the fast resorption of the CaS phase and the induced inflammation would negatively influence the histologic appearance of the surrounding muscle and soft tissue in a rat rectus abdominal muscle model [32]. Additionally, the effect of soaking the bone substitute in aspirated bone marrow was studied.

## Materials and methods

### Materials preparation

The injectable bone substitute Cerament (Cerament Bone Void Filler; BoneSupport AB, Lund, Sweden) consists of 60 wt. % CaS and 40 wt. % HA [8,18]. This ratio results in a mixture with a compressive strength (wet) of 5—8 MPa, comparable to trabecular bone. The HA particles are around 5 μm. The HA is of medical grade and tested according to American standard (ASTM F1185). The bone substitute powder was mixed with iohexol (Cerament C-TRU; 180 mg I/mL), a low osmolar nonionic iodinated contrast agent to form an injectable paste. The ratio of liquid to powder was 0.43. The Cerament was mixed for 30 seconds and then injected into a sterilised Teflon mould with holes that were 3 mm in diameter and 6 mm deep. After 12 hours, the cement was cured and the rods removed and stored in a sterilised glass bottle prior to implantation.

### Animals and implantation

A total of 32 female Sprague—Dawley rats with body weight of 190—220 g (age 8 weeks) were operated on. Rats were obtained from Taconic (Ry, Denmark). The rats were housed in a temperature-controlled room (21°C) and fed a standard laboratory diet. Three rats were kept in each cage with free access to food pellets and water. All animal procedures were carried out according to the institutional guidelines and were approved by the Ethic Animal Research Committee (M45-09) at Skåne University Hospital, Lund, Sweden.

The experiment was divided into two groups. In Group 1, 16 rats were implanted with a pair of Cerament rods. In Group