**BIOMECHANICS AND BONE INTEGRATION ON INJECTABLE CALCIUM SULPHATE AND HYDROXYAPATITE IN LARGE BONE DEFECT IN RAT**

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**Introduction**

Injectable calcium sulphate (CaS) has been used as a filler for bone defects, voids related to fractures and neoplastic processes in metaphyses. In the ideal material a rate of resorption should be balanced by new bone formation. Calcium sulphate is a soluble osteoconductive material. The drawbacks are rapid resorption and low strength, making this material less useful in larger defects (1). Hydroxyapatite (HA) acts as a matrix for long-term osteoconductivity. The combination of the two materials may be of benefit for bone integration (2,3). The mechanical properties of resorbable materials in bone defects are important to allow load bearing and stability during healing. The aim of the study was to investigate development of mechanical strength in defects implanted with CaS, and CaS with HA, and to compare this with histological changes, in an experimental rat model.

**Materials and Methods**

Medical grade α-calcium sulphate hemihydrate (CaSO4 1/2H2O; CSH) as control and CSH mixed with 40 wt % sintered hydroxyapatite (Ca10(PO4)6(OH)2; HA) named CERAMENT® (both materials from Bone Support AB, Lund, Sweden) were used in this study. The sterilised materials were prepared by mixing the powder with OmnopaqueTM (Amersham Health AS, Solna, Sweden), for increased radiopacity, at a materials were prepared by mixing the powder with OmnopaqueTM (Amersham Health AS, Solna, Sweden), for increased radiopacity, at a liquid-to-powder ratio (L/P) of 0.40 ml g⁻¹.

**Bone defect filling**

Thirty one male Sprague-Dawley rats (300-320 g) were used. Under general anaesthesia, the anterior medial aspect of the tibia and lateral distal femur were exposed and holes with 3 mm diameter and 4 mm deep were drilled both in the tibia and femur. The materials were randomly injected into the bone defects. The hole was covered by a silicon cap fixed by a “u” pin. For each time point (1, 7, 21 and 42 days), six rats received material, and a sham operation was performed on one animal, with drilling and leaving the hole empty. In order to know the strength of cancellous bone, the cortex on the tibia was removed by the same hole-cutter in a further 6 tibiae (3 rats).

**Indentation test**

The tibiae were harvested for large area indentation testing. The resected tibiae were fixed in gypse and were placed on an Instron 8511 load frame with a 500 N load cell and an MTS TestStar II controller. A 2 mm diameter metal rod was positioned above the centre of the hole and moved down at 1 mm min⁻¹. The indentation load was recorded. The data was analysed by selecting the load starting from 0.1 N as a base, and then taking the data of the load from every 0.3 mm displacement until 3 mm. The mean of each point in all 6 samples was calculated and shown in the figures.

**Histology**

The implanted and control femora were prepared for histology. The specimens were fixed in formalin, decalcified and embedded in paraffin and stained with H&E. Bone integration and ingrowth were analyzed using light microscopy and an image analysis system. Area of new bone and material remaining in the bone defect were analysed blindly using three sections for each sample.

**Results**

**Indentation test**

Compared to cancellous bone, the bone defect area without filling showed no strength at days 1 and 7; after day 21, a force of about 20 N was generated after a small displacement (0.3 to 0.6 mm), which then decreased rapidly with further displacement (Fig. 1,2). In the bone defect with CaS, a lower force than cancellous bone was developed at days 1 and 7. After day 21, the load was increased to 12 N at a displacement of 0.6 mm, and continued increasing (Fig. 3). At days 21 and 42, their load curves during displacement were similar to the cancellous bone. In the bone defect with CaS + HA, the load was increased after day 21 and 42. The load curve for the CaS+HA during displacement was higher than the equivalent curve for cancellous bone (Fig. 4). Analysis of the load data at the displacements of 0.6, 1.5 and 2.1 mm showed statistically significant differences between the materials (p<0.01, two way Anova).

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**References**