A Carboxy-Methyl Cellulose Carrier Reduces Bone Formation within a Silicate-Substituted Calcium Phosphate Scaffold.

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Introduction:
Bone substitute materials are increasingly being used, especially in oncologic surgery, traumatology, revision prosthetic surgery and in spinal surgery. Over two million bone grafting procedures are performed every year, and bone substitute materials are often used due to their excellent biocompatibility, improved safety profiles, low cost, time advantages and adaptability to a variety of clinical challenges. Replacement of autograft by bone graft substitutes reduces donor site morbidity. However a limitation with current calcium phosphate (CaP) bone substitute materials is that they exist in a dry granular form, limiting its handling ability during surgery. Recently, injectable and moldable forms of bone substitute material, such as pastes and putties, have been developed as they offer many advantages including increased handling ability and are able to completely fill contained defects of complex geometric shapes. In addition and with the development of minimally invasive surgical methods, the requirement to treat bony defects with directly injectable biomaterials is increasing. This study investigated the effect of using carboxy-methyl cellulose (CMC) as the binding agent on bone formation within a porous silicate-substituted CaP (SiCaP) scaffold implanted in an ovine femoral condyle critical-sized defect. Our hypothesis was that CMC would have no negative effect on bone formation and osteoconduction within the scaffold.

Methods:
Twenty-four 8 x 15 mm deep defects were created in the medial femoral condyles of 6 female, skeletally mature commercially cross-bred sheep. Ethical approval was granted and all procedures were carried out in compliance with the UK's Home Office Regulations (Animal Scientific Procedures Act 1986). Defects were (1) empty, or filled with either (2) SiCaP granules, (3) SiCaP Putty or (4) a SiCaP press-fit block. Implants remained *in vivo* for 28, 56 and 84 days (n=6). Scaffolds in each group were identical in composition and consisted of a phase pure SiCaP (0.8 wt % Si) with a total macroporosity of 80%, a microporosity of 22.5% and mean pore size of 300 µm. The SiCaP granules used in groups 2 and 3 were identical and the groups differed only by the addition of CMC. Fluorochrome markers (oxytetracycline and calcein green) were administered in order to measure bone apposition rates and following retrieval, specimens were processed for undecalcified histology. A histological section was made through the centre of each defect and image analysis techniques were used to quantify bone apposition rates, bone area within the graft, bone-implant contact and the amount of graft area remaining at 28, 56 and 84 days post surgery. Mann Whitney U tests were used for statistical analysis where $p<0.05$ was considered significant.

Results:
No new bone growth was measured within any of the empty defects at the 28, 56 and 84 days post-operative time points. ***Bone Apposition Rates:*** At 28 days, although the greatest bone apposition rates were measured in the SiCaP granules group (1.02 ± 0.49 µm/day^{-1}), no significant differences were found when each of the groups were compared. At 56 days post surgery, significantly increased bone apposition rates were measured in the SiCaP putty group (2.22 ± 0.22 µm/day^{-1}) when compared with the SiCaP granules group (1.79 ± 0.17 µm/day^{-1}; $p = 0.046$). In addition, significantly greater apposition rates were measured in the SiCaP block group (1.91 ± 0.39 µm/day^{-1}) when compared with the SiCaP granules group. At 84 days post operatively, no significant differences were observed when the granules, putty and block groups were compared.
Bone Area: At 28 days significantly increased bone area was measured within defects containing SiCaP granules (3.97 ± 1.11%) when compared with both SiCaP Putty (0.09 ± 0.06%; $p = 0.028$) and SiCaP block scaffolds (0.15 ± 0.08%; $p = 0.028$) (Figure 1). This trend continued with significantly increased bone formation measured within the SiCaP granules group at 56 days (20.14 ± 4.91%) when compared with both the SiCaP Putty (5.47 ± 1.46%; $p = 0.046$) and SiCaP block (6.69 ± 2.10%; $p = 0.046$) groups. At 84 days post surgery, significantly more bone was measured in the SiCaP granules group (40.56 ± 3.73%) when compared with SiCaP blocks (15.38 ± 5.30%; $p = 0.028$). No other significant differences were found. Longitudinal analysis showed that in all groups, apart from the empty defect bone area significantly increased from 28 to 84 days

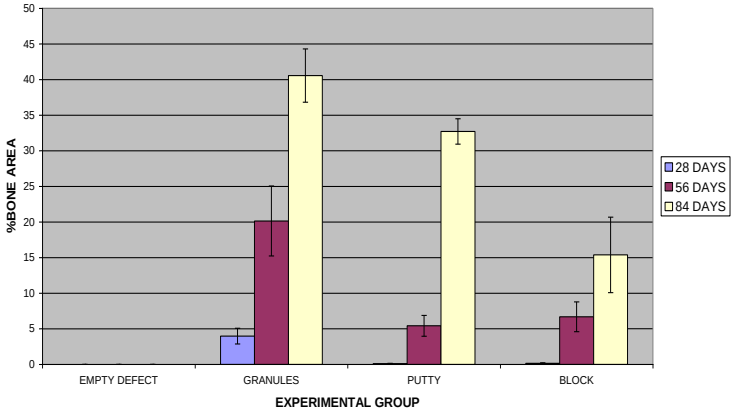

Figure 1: %Bone area measured within scaffolds in each of the experimental groups at 4, 8 and 12 weeks.

Bone-Implant Contact: At 28 days, results showed significantly increased bone-implant contact in the SiCaP granules group (10.25 ± 3.05%) when compared with both the SiCaP Putty (0.63 ± 0.63% $p = 0.028$) and SiCaP block group (1.05 ± 0.45%; $p = 0.028$) (Figure 2). At 56 days post surgery, significantly increased bone-implant contact was measured in the SiCaP granules (42.03 ± 9.46%) group when compared with defects containing SiCaP Putty (17.22 ± 3.61%; $p = 0.046$). At 84 days significantly increased bone-implant contact was measured in the SiCaP granules group (70.84 ± 6.66%) when compared with the SiCaP block specimens (37.53 ± 7.73%; $p = 0.028$). No other significant differences were found. Longitudinal analysis showed that in all groups, significantly increased amounts of bone-implant contact was measured over each time point (except in the empty defects and SiCaP blocks between 56 and 84 days time points).
Graft Area: Significantly reduced graft resorption was measured within defects in the SiCaP granules group when compared with the SiCaP Putty and SiCaP block groups at 28,56 and 84 days post surgery ($p < 0.05$ in all cases). Results from this study showed a trend where implant area gradually decreased in all groups over time, however, no significant differences within groups was observed. The highest rate of implant resoprtion was measured in the SiCaP granule group.

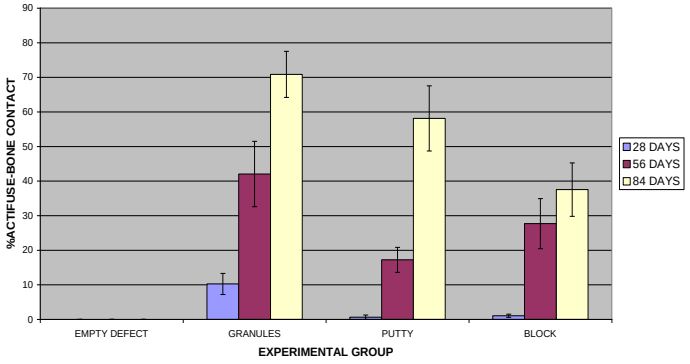

Figure 2: %Bone-Implant Contact measured within scaffolds in each of the experimental groups at 4, 8 and 12 weeks.

Discussion
Greatest amounts of bone formation and bone-implant contact was seen in the SiCaP granules group at all of the time points investigated. Results showed that CaP granules augmented increased amounts of bone formation when compared with a press-fit block composed of the same material. This was possibly due to

the increased surface area associated with the granules and subsequent increased scaffold resorption. Results from this study also showed the detrimental effect that carboxy-methyl cellulose has on bone growth and osteoconduction to a SiCaP scaffold. Further research is necessary to optimise CMC when used to bind SiCaP granules in the augmentation of bone.

Significance: Using CMC as a carrier for the application of bone graft substitute resulted in significantly reduced bone formation and bone-implant contact. CMC putty for bone graft substitutes should be used with caution.