Introduction:
Bone graft is crucial in the repair and regeneration of large or critical-sized defects and hydroxyapatite has been widely used in many clinical conditions as a bone graft substitute material. However, in the older patient group the challenges faced by the surgeon to repair and regenerate defects with high quality bone can be difficult and improved materials are needed. Bone marrow aspirate (BMA) is the most practical source of multipotent stromal cells and some studies have reported enhanced bone ingrowth within hydroxyapatite (HA) scaffolds. This study investigated the effect of the binding agent Calcium/Sodium Alginate fibre gel and the addition of autogenic BMA on bone growth into a porous HA scaffold manufactured using novel patented techniques and implanted in an ovine femoral condyle critical-sized defect. Our hypothesis was that Alginate fibre gel would have no negative effect on bone formation and osteoconduction within the scaffold and that BMA would augment the bone turnover rate and incorporation of the graft with the surrounding bone at 6 and 12 weeks post implantation.

Methods:
Twenty-four 8mm x 15mm 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 carried out in compliance with UK’s Home Office Regulations (Animal Scientific Procedures Act 1986). Defects were filled with either porous HA granules, porous HA granules + Alginate fibre gel (HA putty) or porous HA granules + Alginate fibre gel + BMA (HA putty +BMA) and remained in vivo for 6 and 12 weeks (n=4). During surgery, bone marrow aspirate was collected from the iliac crest and 1ml of aspirate per cm² of graft used. Fluorochrome markers (oxytetracycline and calcein green) were given at weeks 3 and 5 in the 6 week group and at weeks 8 and 10 in the 12 week group respectively in order to measure bone turnover rates within the defect. Following retrieval, specimens were processed for undecalcified histology and a thin histological section was made through the centre of each defect. Image analysis techniques were used to quantify bone apposition rates, bone ingrowth into the graft, bone-implant contact within the defect and the amount of graft used.

Results
Bone apposition results showed that the highest rate of bone formation measured was seen in the 12 week HA putty+BMA group with a mean rate of 1.57±0.24μm/day. Reduced bone apposition was seen in the 6 week HA putty group when compared with the other two groups at this time-point however results were not significant. Overall, the 12 week specimens had a higher rate of bone apposition when compared with the 6 week groups. Results of bone ingrowth measured as the % of bone within the graft area showed that HA granules at 12 weeks encouraged the greatest increase in bone formation within the defect area (33.56±3.53%). The lowest amount of bone was measured in the 6 week HA putty+BMA group with a mean of 8.57±2.86% (Figure 1).

Discussion
Highest amounts of bone formation and bone-implant contact was seen in the porous HA granules group at both the 6 and 12 week time point. Results from this study showed the detrimental effect that Calcium/Sodium Alginate fibre gel has on bone growth and osteoconduction to a HA scaffold. Results from this study also showed that the inclusion of BMA did not augment bone growth to the scaffold or increase its osteoconductive capacity when combined with Calcium/ Sodium Alginate fibre gel. Further research is necessary to optimise Calcium/Sodium Alginate fibre gel when used to bind HA granules in the augmentation of bone and to investigate the effect of BMA with this type of HA alone.

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Figure 1: A Graph comparing bone area in all groups at 6 and 12 weeks.

Figure 2: Photomicrographs taken through HA putty+BMA[A] and HA granules[B] at 6 weeks showing increased bone formation in photo B. Photomicrographs C+D are sections taken through the HA putty and HA granules at 12 weeks respectively. There is notably increased bone present in the HA granules specimen.