THE USE OF MESENCHYMAL STEM CELLS TO ENHANCE BONE FORMATION AROUND REVISION HIP REPLACEMENTS

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Materials and Methods
Fifteen adult sheep were divided into 3 groups with 5 sheep in each group: Group 1 sheep: MSCs + allograft; Group 2 sheep: osteoprogenitors + allograft; Group 3 sheep: allograft only (control).

The allografts from fresh frozen ovine femoral bones were morselized in a Bone Mill, washed in an ultrasonic bath and gamma irradiated at 25KGrays (Isotron, Reading, UK). Allograft was maintained in storage at -20°C before surgery.

Three millilitres of bone marrow was aspirated from sheep iliac crest and the mesenchymal stem cells were isolated using a Ficol gradient. The cells were seeded into culture flasks at 37°C with 5% CO₂ and maintained in growth media consisting of DMEM supplemented with 10% FCS, penicillin / streptomycin (1000iu/ml; 100µg/ml). The cells were stored at -20°C before surgery. 

A group of MSCs were differentiated into osteoprogenitors with media supplements at concentrations of: 10mM β-glycerophosphate (Sigma D1756), 50mM Ascorbic acid (Sigma A4544), and Dexamethasone 1x10⁻⁶ M (Sigma D-2915). The differentiation into osteoprogenitors was determined by measurement of ALP production, osteocalcin expression and deposition of mineral. The cells were harvested days for seeding onto allograft during surgery. Either MSCs or osteoprogenitors derived from MSCs were trypsinised from flasks, centrifuged and during surgery, resuspended into 10ml of plasma. The cells in plasma were seeded evenly onto allograft at a concentration of 1 x 10⁶ cells/cm³ graft. Plasma alone was added to allograft in the control group.

The hip stem for an ovine hemiarthroplasty was designed to fit the geometry of the proximal femur allowing for at least a 3mm gap between the implant and the cortical bone for impacted allograft. The stem was made of titanium alloy with a 25mm cobalt chrome head. The hip was fully hydroxyapatite coated.

All surgeries were carried out in accordance to the U.K.s Animals Scientific Procedures Act 1986. The hip joint was exposed through a 3mm gap permitting for at least a 3mm gap between the implant and the cortical bone for impacted allograft.

Bone formation within the graft and at the implant/bone interface was measured using histological methods. The Kruskal-Wallis Test for non-parametric data was carried out at 95% significant level followed by a post hoc, pair-wise Mann-Whitney U Test to examine any differences between groups.

Results
Compared with the pre-operative GRF, the post-operative GRF for sheep with MSCs, Osteoprogenitors and controls were 106 ± 11%SE, 60 ± 6%SE and 82 ± 6%SE respectively. The statistical analysis showed no difference between the MSCs and controls (p=0.1), and between osteoprogenitors and controls (p=0.086), but there was a difference between the MSCs and osteoprogenitors (p=0.034).

Allograft combined with MSCs produced more new bone compared with controls (p=0.003). New bone formation within the graft was significantly greater (p=0.00) when the graft containing the MSCs was compared with graft containing osteoprogenitor cells. However, no difference was found between the control group and the group with osteoprogenitor cells (p=0.724) (Fig. 1).

There were significant differences in bone formation at the endosteal/allograft interface and the implant/ bone interface in control (p=0.039) and osteoprogenitor (p=0.004) groups where more bone was produced on the endosteal surface. However bone formation was more evenly distributed throughout the graft for the MSCs group and there was no significant difference between bone formation at the endosteal surface and implant interfaces (p=0.462) (Fig. 2).

There was no difference in bone formation at the endosteal interface between all the groups. When comparing new bone formation at the implant interface, the group with MSCs generated significantly more new bone than the control group (p=0.015) and the osteoprogenitor group (p=0.00) (Fig 2).

Discussion
The ovine hemiarthroplasty model is appropriate for investigating the remodelling of allograft. The femora of sheep are tubular and sclerotic with little cancellous bone which is similar to that encountered in revision surgery. The forces and moments passing through the sheep femur are of the same magnitude and orientation as for humans. This study MSCs added to the allograft at the time of surgery produced significant more new bone compared with other groups. There was no effect on bone formation when osteoprogenitor cells were used. The reason is unclear. One assumption is that the primitive stem cells may be less affected by the process of graft impaction and the environment associated with impaction grafting. The clinical relevance of this animal study is that MSCs can play an important role in regenerating new bone in revision hip replacements and this technique can translated to the clinical study.

References

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