Do Differentiated Osteoblasts and Allogeneic Mesenchymal Stromal Cells Augment Bone Formation?

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Statement of Purpose: Fixation of massive bone tumour endoprosthetic replacements is not as successful as conventional joint replacement surgery and aseptic loosening is the major cause of implant failure. Increased bone growth and contact to the implan shaft by bony bridging has been shown vital in reducing implant failure. Our previous work has shown that spraying autogenic bone marrow derived mesenchymal stromal cells (MSCs) within fibrin glue onto the grooved hydroxyapatite (HA) collars of segmental bone tumour implants significantly increased bone formation in an ovine model[1]. In this study we investigated the hypothesis that allogeneic MSCs and MSC's differentiated in an osteogenic supplemented media (ogMSCs) will improve bone growth equal to undifferentiated autogenic MSCs in an *in vivo* massive tumor implant ovine model. This study also hypothesized that bone growth is dependent on cell concentration.

Methods: Four mls of bone marrow was obtained from the iliac crest of 20 sheep and autogenic and allogeneic MSC's isolated and expanded *ex vivo*. Forty-two mid-tibial tumour prostheses were cemented into the right hind limb of skeletally mature commercially cross-bred sheep and cell therapy treatment randomly assigned. Ethical approval was granted and all procedures were carried out within UK Home Office regulations (Animal Scientific procedures at 1986). In all groups, cells were sprayed suspended in fibrin glue and sprayed at 1 atm onto the proximal and distal HA collars. Implants remained *in vivo* for 6 months. Six animals were investigated in each group. Implants in group 1 were sprayed with fibrin glue only. HA collars in group 2 received $2x10^6$ autogenic MSC's; group 3 received $10x10^6$ autogenic cells; group 4, $2x10^6$ ogMSC's; group 5, $10x10^6$ ogMSC's; group 6, $10x10^6$ allogeneic MSC's and implants in group 7 were control and collars were HA coated only. Prior to surgery, 10mls of blood was taken and lymphocytes isolated. Mixed Leucocyte Reaction tests were performed to ensure that allogeneic cells given were from immunologically distinct animals. New bone area was quantified from radiographs and on retrieval specimens were processed for undecalcified histology. Transverse thin sections (60µm thick) were made through the centre of the HA collars and image analysis (Axiovision 4.5, Carl Zeiss) used to quantify bone area and bone-implant contact. Mann Whitney U tests were used for statistical analysis where $p<0.05$ was considered significant.

Results: Radiographic analysis showed that no bone had grown adjacent to the implant shaft in any of the specimens in the allogeneic group. Results also showed a trend with more bone present adjacent to implants in the cell given groups when compared with control, however there were no significant differences when remaining experimental groups were compared (Control=87.506±15.75mm^2; Fibrin only=82.01±32.21mm^2; Allogeneic=0.00mm^2; $2x10^6$ MSC's=171.94±29.66mm^2; $10x10^6$ MSC's =149.51±63.63mm^2; $2x10^6$ ogMSC's=80.63±31.08mm^2; $10x10^6$ ogMSC's=121.10±47.12mm^2). Histological analysis showed that most growth occurred adjacent to the $2x10^6$ and $10x10^6$ osteogenic differentiated MSC given groups and confirmed no bone growth adjacent to implants sprayed with allogeneic cells. Significantly less bone was observed in the allogeneic group when compared with all other experimental groups ($p<0.05$ in all cases). Results showedleast bone formation occurred in the allogeneic (0.00mm^2), control (21.07±7.40 mm^2) and fibrin only (11.24±4.75mm^2) groups with increased bone in the $2x10^6$ MSC (54.00±10.64mm^2), $10x10^6$ MSC's (58.23±17.47mm^2), $2x10^6$ ogMSC's (76.84±28.25mm^2) and highest in the $10x10^6$ ogMSC group (112.66±30.75mm^2) (Figure 1). No significant differences were found when all cell treated groups were compared with each other however significantly increased bone was measured adjacent to all cell given implants when compared with control and the fibrin only groups ($p<0.05$ in all cases). Highest levels of bone-implant contact was seen in both ogMSC groups when compared with all other groups however results were not significant. Lowest bone-implant contact values were measured in the allogeneic group. It appeared that increased cell concentration resulted in increased bone formation.

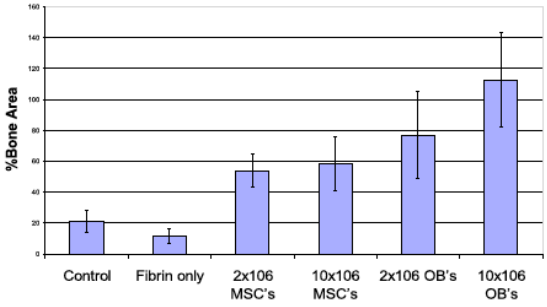

Figure 1: A Graph showing Total Bone Area between Groups.

Conclusions: Bone formation and bone-implant contact to massive endoprosthetic implants was significantly improved by spraying the implant surface with MSC's and ogMSC's suspended in fibrin glue. Enhanced fixation may help in preventing aseptic loosening and this method of applying stromal cells can also be used successfully for more conventional joint replacements[2]. Spraying tumor implants with bone marrow derived autogenic stromal cells could be used in humans however further work is needed to determine the role of allogeneic cells in bone augmentation *in vivo*. This work was funded by the BBSRC UK.

References:

1. Kalia P. J Tiss Eng, 2006 Jun;12(6):1617-26.
2. Kalia,P. Tissue Eng Part A. 2009 Dec;15(12):3689-96.