INTRODUCTION:
Autologous cancellous bone grafting is the gold standard treatment for bone defects. Clinical settings deficient in osteogenic progenitors benefit from the additional use of bone marrow derived cells (Matsuda and Sakayama, 1998). These cells have excellent osteogenic potential but show age-related changes (Muscler et al, 2001); reduced lifespan, proliferation and osteogenic potential (Stenderup et al, 2003).

Mesenchymal stem cells are present in almost every adult human tissue including the infrapatellar fat pad (IPFP) (Wickham et al, 2003). Monolayer culture of IPFP derived stem cells with β-glycerophosphate supplemented medium has been shown to induce osteogenic differentiation. The osteogenic potential of adipose tissue derived mesenchymal stem cells is maintained with aging in mice (Shi et al, 2005) but no detailed study has explored their potential in humans. A potential cell source that does not show age related decline in proliferation and osteogenic differentiation is important in determining the optimal cell therapy for bone defects in an aging population. Compared to bone marrow derived stem cells, IPFP derived cells are easy to harvest, are greater in number, grow well in culture and can be cryopreserved (Aust et al, 2004).

In this study IPFP derived cells from two different populations were compared for their surface epitope profile, proliferation rates and osteogenic differentiation potential.

MATERIALS AND METHODS:
The infrapatellar fat pad was obtained from two groups of five patients undergoing total knee replacement for osteoarthritis following ethical approval and informed consent. Group one had a mean age of 57 years (SD 3 years) and group two had a mean age of 86 years (SD 4 years). Cells were isolated with 0.2% collagenase I digestion and expanded in monolayer in DMEM with 20% FCS. Cells from passage 2 were placed at a concentration of 5,000 cells/cm² in a 6-well plate. The cells were cultured either in osteogenic medium or in control basic medium for 21 days. Osteogenic medium consisted of DMEM with 10% FCS, β-glycerophosphate (10mM), dexamethasone (10mM) and L-ascorbic acid-2-phosphate (0.1mM).

Cells from both groups were stained for surface antigens (CD13, 29, 34, 44, 56, 90 and 105, 3G5, LNFGR, STRO1, a-SMA and IgG control) at confluence for epitope profiling. The cells were incubated with the primary antibodies (1:100 dilution) followed by FITC conjugated secondary antibody (1:40 dilution). The cells were then incubated with 4′, 6-diamidino-2-phenylindole stain.

Cell proliferation rates were measured for cells from the two groups plated at 1,000 cells/cm² in a 6-well plate. Cells were trypanosised and counted at day 2, 4, 6, 8 and 10 after plating and the cell number was determined by counting with a hemacytometer.

Total RNA was extracted with Tri Reagent (Sigma, UK). cDNA was generated using reverse transcription followed by poly-A PCR global amplification. Gene specific primers were designed within 300 base pairs of the 3′ region of the relevant gene using primer express software (Applied Biosystems). Quantitative real-time PCR was performed on a MJ Research Opticon using a SYBR Green Core Kit (Eugentec, Belgium). Gene expression analysis for alkaline phosphatase and osteocalcin was performed.

The cells were also rinsed, fixed in 4% formaldehyde and then stained with 1% alizarin red (pH 4.2) for 5 minutes. Alkaline phosphatase enzyme activity was measured in cell lysate with p-nitrophenyl phosphate as a substrate measuring the rate of p-nitrophenol formation. The absorbance was read at 405nm on a microplate reader and the amount of enzyme determined by comparison with a standard curve. The amount of protein was determined using a Bio-rad protein assay reagent kit and the absorbance was read at 595nm on the Dynex plate reader.

All experiments were performed in triplicates. An unpaired student’s t-test was used to compare the two groups. A p value of <0.05 was considered statistically significant.

RESULTS:
Most of the IPFP derived cells at passage 2 stained positively for CD13, 29, 44, 90 and 105. Occasional cells stained positively for 3G5 and LNFGR. No cells stained positively for STRO1, a-SMA, CD34 and 56. The cells from both groups proliferated well in monolayer culture and the total number of cells rose steadily up to day 10 reaching almost 4 times the initial cell number. There were no significant differences between the surface epitope profile and proliferation rates at any measured time point for the two groups.

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DISCUSSION:
Our findings show that stem cells derived from the IPFP have osteogenic potential and show osteogenic differentiation using β-glycerophosphate supplemented medium in vitro. The similar surface epitope profile and proliferation rates suggest that the two populations contain a comparable number of precursor cells and that the IPFP cell source is not significantly altered with age. Our findings also show that the osteogenic differentiation is not affected by age in later life since the gene expression, alizarin red staining and alkaline phosphatase enzyme activity showed no significant differences between the two groups.

The findings of this study have important implications for future tissue engineering applications of fat pad derived cells in the management of bone fractures or defects for an increasingly elderly population. These cells also have the potential advantage of being an attractive tissue-engineering alternative to autologous bone grafting. The amount of autologous bone graft that can be harvested is limited and it is associated with significant morbidity: surgical scars, blood loss, pain, prolonged surgical time and rehabilitation, unpredictable graft absorption and structural failure (Younger and Chapman, 1989). The use of IPFP derived mesenchymal stem cells for osteogenic applications may not therefore be hindered by any age-related decrease in their abundance, or decline in their osteogenic potential.

REFERENCES:

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