INTRODUCTION:
Although chondrocytes have been used with some success for autologous implantation in localized defects of articular cartilage there remain issues in relation to their clinical application. In particular, limited availability, donor site morbidity and limited capacity for extended expansion in vitro have led to the search for alternative cell sources. Mesenchymal stem cells (MSCs) appear to represent a viable option and multiple tissues have been suggested for harvest. The infrapatellar fat-pad (Hofmann) has been proposed previously as a promising source [1-4]. Advantages include low morbidity from loss of fat-pad tissue, relative ease of harvest and ex vivo evidence of good chondrogenic capacity.

Expansion of MSCs derived from human fat-pad in the presence of fibroblastic growth factor-2 (FGF-2) has been shown to enhance subsequent chondrogenesis in pellet culture [3]. Such studies demonstrate how the differentiation capacity of MSCs are strongly dependent on environmental conditions during expansion. To further elucidate this process, this study will assess the role of transforming growth factor β3 (TGF-β3) and Fibroblast Growth Factor-2 (FGF-2), in addition to an altered oxygen tension environment, on the growth kinetics of these cells during expansion and their subsequent chondrogenic capacity.

METHODS:
Ethical approval was obtained from the review board of the Mater Misericordiae University Hospital. Infrapatellar fat-pads (IFP) were obtained with informed consent from the knees of 3 patients undergoing total knee arthroplasty for primary osteoarthritis. The tissue was finely dissected and cells isolated via collagenase digestion (4 hours).

Isolated cells from each donor were expanded in various media formulations- (STD, FGF, TGF and FGF/TGF at both 20% and 5% oxygen tensions (8 expansion conditions). Standard media (STD) consisted of high glucose Dulbecco’s modified eagle medium (hDMEM) supplemented with 10% fetal bovine serum (FBS) and 100U/ml penicillin/streptomycin. FGF-2 was added to STD media at a concentration of 5ng/ml and TGF-3 at a concentration of 0.5ng/ml. Colony forming unit fibroblast (CFU-F) assays were performed for each expansion group with an initial seeding density of 100 cells/cm² and assessed through crystal violet staining.

Passage 2 (P2) cell aggregates of 250,000 cells were formed by centrifugation at 650g and pellets from each group underwent chondrogenic differentiation in a chemically defined medium (CDM) with TGF-β3 (10ng/ml) supplementation at 37°C and 5% CO2 in both low oxygen tension (5%) and atmospheric oxygen tension (20%) (Total of 16 experimental groups). Pellets were assessed after 21 days and biochemically analyzed for DNA, sulfated glycosaminoglycans (sGAG) and collagen content. Histological analysis was also performed to assess the distribution of GAG and collagen (types I and II).

RESULTS:
Oxygen tension during expansion was observed to influence the colony size for both FGF and FGF/TGF groups, with larger colonies which stained more intensely being formed at 5% PO2. Smaller colonies were observed in STD and TGF groups (Fig 1).

hMSCs expanded in FGF/TGF supplemented medium proliferated more rapidly than other groups with significant differences observed in the number of population doublings per day (Fig 2A). hMSCs expanded in media containing FGF had the highest sGAG synthesis rates, while those expanded in FGF/TGF produced the least sGAG (Fig 2B). This result was observed for all donors. Cells expanded in FGF/TGF combination also produced less collagen (Fig 2C). Expansion of MSCs at 5% PO2 had little effect on subsequent chondrogenic capacity in pellet culture. While differentiation at 5% PO2 lead to higher mean levels of sGAG for most expansion conditions, its effect was generally less potent compared to expansion in FGF-2.

DISCUSSION:
In agreement with previous findings [3], expansion of IFP derived MSCs isolated from human osteoarthritic tissue in FGF-2 resulted in enhanced chondrogenesis in pellet culture. FGF-2 expansion was also observed to increase the colony forming capacity of plated MSCs, but unexpectedly this did not lead to a significant increase in the number of population doublings per day (Fig 2A). The higher cell plating density used for passaging MSCs compared to that used for CFU-f assays may be mitigating the effects of environmental factors during the expansion phase. Supplementation with both FGF-2 and TGF-β3 during the expansion phase dramatically decreased the population doubling time compared to other groups but reduced cell survival during differentiation and lead to lower levels of matrix synthesis. Expansion in low oxygen tension resulted in larger colony diameters but had a variable effect on subsequent chondrogenesis. We did not observe any notable positive effects of expansion in a low oxygen environment, although differentiation in low oxygen did have a beneficial effect on subsequent chondrogenesis. In conclusion, compared to the other factors investigated, addition of FGF-2 during the expansion phase was the most potent promoter of the subsequent chondrogenic potential of hMSCs isolated from the infrapatellar fat pad.

REFERENCES:

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