INTRODUCTION:
Adult stem cells from adipose tissue can potentially be used in cell-based therapies for cartilage repair. We have previously demonstrated that functional cartilage tissue can be engineered using porcine infrapatellar fat pad (IFP) stem cells (FPSCs), however it remains unclear if such grafts can be engineered using stem cells isolated from human osteoarthritic IFP tissue. The objective of this study was to firstly compare chondrogenesis of FPSCs in pellet culture to that in agarose hydrogels which are commonly used to engineer functional cartilaginous grafts. We next explored how biochemical (serum) and biophysical (hydrostatic pressure) cues would influence the functional development of cartilage tissues engineered using human FPSCs.

METHODS:
Human infrapatellar fat pad was harvested during total knee replacement following ethical approval. FPSCs were expanded under 20% or 5% O2. After expansion to P2, FPSCs were cultured as pellets (250,000 cells/pellet) or seeded into agarose hydrogels (10 million cells/ml) and maintained at 5% O2 in a chemically defined chondrogenic media supplemented with 10ng/ml of TGF-β3 with or without 10% fetal bovine serum for 3 weeks. In a second experiment, FPSCs were seeded into agarose hydrogels at a density of 20 million cells/ml and subjected to 10MPa hydrostatic pressure for 2 hours/day, 5 days/week for 4 weeks. Constructs were analyzed by DNA, GAG, collagen assays and histological staining.

RESULTS:
The chondrogenic potential of FPSCs is diminished in agarose hydrogel culture compared to pellet culture, as evident by lower levels of GAG and collagen synthesis on a per cell basis, see Fig. 1. Expansion of cells in 20% or 5% oxygen did not appear to affect chondrogenesis in either pellet or hydrogel culture. Supplementation with serum was observed to enhance chondrogenesis in hydrogels but not in pellets. Hydrostatic pressure was found to enhance the dynamic modulus of FPSC-seeded hydrogels that were not supplemented with serum (Fig. 2A), despite the finding that it had no effect on overall levels of GAG and collagen accumulation (Fig. 2B). Histological analysis revealed that hydrostatic pressure resulted in increased matrix staining within the hydrogels, with less evidence of tissue outgrowth from the engineered constructs (Fig. 2C).

DISCUSSION:
Diseased human FPSCs displayed a diminished chondrogenic potential upon encapsulation in agarose hydrogels despite the attainment of a round cellular morphology. This suggested that the condensation and cell-to-cell communication that exists within the pellet culture system is critical to initiating robust chondrogenesis in human FPSCs. Serum stimulation appeared to partially recover the diminished chondrogenesis in hydrogel culture. While the application of hydrostatic pressure did not increase total levels of matrix synthesis, it did appear to result in greater accumulation within the hydrogel and less tissue outgrowth. This may explain the enhanced mechanical properties observed in tissues that were subjected to HP.

SIGNIFICANCE:
We have demonstrated that the inherent chondrogenic capacity of FPSCs is diminished upon encapsulation in agarose hydrogels, and that this capacity can be partially restored through application of the appropriate biochemical or biophysical cue. Recreating the environmental conditions that exist within pellet culture in either a scaffold or hydrogel would appear to be critical step towards engineering functional cartilaginous grafts using stem cells isolated from osteoarthritic patients.

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