INTRODUCTION Mesenchymal stem cells (MSCs) are a promising cell-source for cartilage tissue engineering given their ability to undergo chondrogenesis [1]. To date, however, MSC-laden constructs have failed to achieve mechanical properties akin to those of native tissue or donor-matched chondrocyte-based constructs [2]. MSC are most commonly selected via plastic adherence from bone marrow, yielding a mixture of cells with varying differentiation potentials [3, 4]. A recent study by Hardingham et al. showed that individual clonal populations cultivated as cell masses displayed different ECM forming capacities and phenotypic stability [5]. This underlying heterogeneity may limit formation of functional tissues. Of great importance to the development of functional ECM is the early establishment of a pericellular matrix (PCM). For chondrocytes in agarose, the PCM protects cells from deformation applied to the gel as early as one week after encapsulation [6]. We posited that a similar micromechanical system might be used to assess heterogeneity of MSC populations. To test this hypothesis, clonal populations from bovine bone marrow were derived via limiting dilution assays. We hypothesized that these clones would show varying degrees of deformation when seeded and deformed in 3D. We further hypothesized that early PCM deposition after chondrogenic induction would protect cells from deformation, but would do so to a different extent, depending on the degree to which an individual cell (in bulk or from clonal populations) fabricated cartilaginous PCM.

METHODS Bovine MSCs were isolated [7] by platting the initial aspirate for 72 hrs, washing with media, and culturing for an additional 72 hours. Cells were re-plated in 96-well tissue culture plates at a density of 1 cell per 3 wells, and each well evaluated for single colony formation indicative of successful clonal selection. After 14 days, cells derived from a single colony were selected, trypsinized, and expanded through passage 2. Three clonal populations (C1 and C2), and the heterogeneous marrow population at passage 2, were encapsulated in 2% agarose at 250,000 cells/mL. Gels (4mm thick) were maintained in pro-chondrogenic media with 10ng/mL TGF-β3 [7]. Constructs were evaluated on day 1 (before induction) and at day 7. For analysis, constructs were halved, stained with calcine-AM, and placed in a custom microscope-based deformation device (Fig. 1) similar to [8]; cells were imaged using a 20X objective. Grip-to-grip compressive strains of 0, 10, 20, and 30% were applied, and cells were imaged at equilibrium after a 2 minute period of stress relaxation in 12 fields of view per construct and strain level. A minimum of 55 cells (all in the same focal plane) per condition were analyzed via a custom MATLAB script to determine aspect ratio (AR) and cross sectional area (CSA). Gels from each group were processed for histology and stained with Alcian Blue and H&E. Histograms of AR were created using MiniTab and normal distributions fit to experimental data. Significance (p<0.05) was established by ANOVA with Tukey HSD post-hoc tests.

RESULTS Two clonal populations with disparate morphologies (C1: small, spindly; C2: large, irregular) were isolated and seeded into agarose hydrogels along with the parent ‘Heterogeneous’ (H) bone marrow population. Upon encapsulation, all cells adopted a rounded morphology (Fig. 1). Under free swelling conditions, cell CSA varied, with H and C1 not different from one another, while C2 was larger than both (Fig. 2A, p<0.05). After one week in CM+, H and C1 increased in CSA, while C2 decreased markedly, such that the three populations were no longer different. With the application of step-wise compression, cell AR increased with each step on day 1 (Fig. 2B, Population H, p<0.05). After one week in CM+, cells appeared stiffer, with less change in AR for each strain increment (p<0.05, 0-30%). Histograms of the unstrained (0%) and strained (30%) AR for all MSC populations are shown in Fig. 3, before (day 1) and after (day 7) chondrogenic induction. Notably, compression markedly increased AR for all populations on day 1. This change in AR was substantially attenuated by day 7, particularly for C1, where no increase in AR occurred with 30% gel deformation. This suggests that clone C1 (and its associated PCM) had become stiff enough to resist bulk deformation in the gel at this time. Of further note, it appears that differentiation itself, even in unstrained gels, increases the dispersion of MSC AR; on day 1, 50-80% of cells within a clone fell within one bin, while on day 7, at most 25% of cells fell in the same bin. Consistent with these findings, histological analysis showed that most cells in H and C1 had generated a proteoglycan-rich PCM by day 7; in contrast, little staining was observed in C2, confirming reduced chondrogenesis in this clone (not shown).

DISCUSSION In this study, we interrogated MSC micromechanical heterogeneity in bulk marrow and clonally derived cell populations. Findings show that MSC deformation changes both as a function of population heterogeneity, as well as over the time course of chondrogenic induction. In whole marrow populations, deformation of the gel led to large increases in AR on day 1, with a reduced (but still positive) change in AR after differentiation and elaboration of PCM. For one clone, C1, we observed very little change in AR after 7 days, while clone C2 began with a large change in AR that was only modestly reduced with differentiation. The larger initial cell size, decreased stiffness upon encapsulation, and failure to resist bulk gel deformation may indicate that clone C2 became senescent during expansion, or that it simply did not possess robust chondrogenic potential from the outset. As there are few surface markers specific for MSCs [8], and none related to mechanical potential, our micro-mechanical approach may aid in the identification and selection of MSCs with robust, and functional, chondrogenic potential. Future studies will further characterize the micromechanical properties of MSC clones, and couple micro-dissection and non-invasive imaging methods to select cells for the production of MSC-based constructs with improved bulk mechanical properties.

Figure 1: Left) Microscope-based compression device for visualizing MSC deformation. Right) MSCs at 0% (top) and 30% (bottom) strain. Scale = 50 μm.

Figure 2: A) CSA of MSCs before (day 1) and after (day 7) chondrogenic induction. *indicates p<0.05 vs. day 1 within group, **indicates p<0.05 vs. H and C1 on day 1. B) Change in AR with step-wise gel deformation for group ‘H’: note that error bars are standard error of the mean for this plot.

Figure 3: Normal distributions of MSC AR as a function of group, time in culture, and compression level; 0% (solid line) and 30% (dotted line).

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