The Molecular Basis for Functional Mesenchymal Stem Cell Chondrogenesis for Cartilage Tissue Engineering

Articular cartilage provides a low-friction bearing surface for transmitting forces across joints. The poor ability of cartilage to self-repair has motivated efforts to engineer functional replacement tissues. While chondrocyte-laden constructs have been generated with near-native mechanical properties, limitations in chondrocyte availability may preclude their clinical use. Therefore, mesenchymal stem cells (MSCs), which can undergo chondrogenesis in 3D culture, have emerged as promising alternative. Recent studies in hydrogels have shown, however, that while MSCs deposit a extracellular matrix (ECM), mechanical and biochemical properties are lower than those achieved with chondrocytes (**Figure 1**). Using microarray analysis, we recently showed that limitations in functional MSC chondrogenesis may stem from incomplete or incorrect molecular induction; molecular differences were observed between donor-matched differentiated chondrocytes and newly differentiated MSCs over 8 weeks of 3D culture (**Figure 2**). Representative results for proteoglycan 4 (PRG4) and TNFRSF6 demonstrate the patterns of this transcriptional misregulation during MSC chondrogenesis. We further observed differential deposition of two matrix molecules, PRG4 and TGF-• -induced 68 kDa protein (TGFBI) (**Figure 3**). While it is currently unclear whether the expression levels of these genes relative to the observed sub-optimal functional differentiation by MSCs is causative or correlative, the identification of these genes serve as a starting point for future molecular intervention. If causative, the manipulation of their expression may enhance the functional capacity of MSCs for regenerative medicine. On the basis of these findings, future and ongoing work will elucidate new mechanisms of modulation, by identifying novel biochemical mediators of chondrogenesis using high-throughput screening, or by enhancing differentiation through tailored mechanical loading regimens.

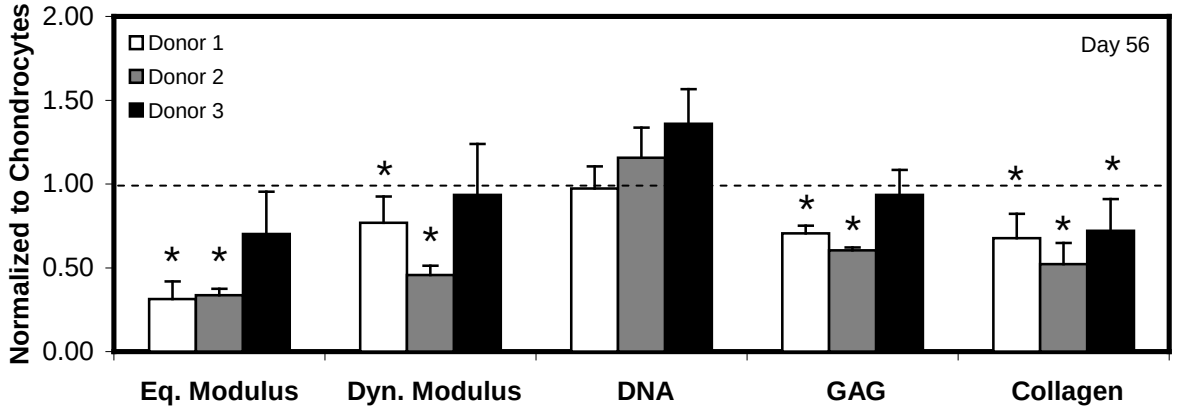

Figure 1: Mechanical properties and biochemical content of MSC-laden constructs normalized to their donor-matched chondrocyte gels (day 56). * lower than donor-matched chondrocyte ($p<0.05$), n=4-5 samples/group.

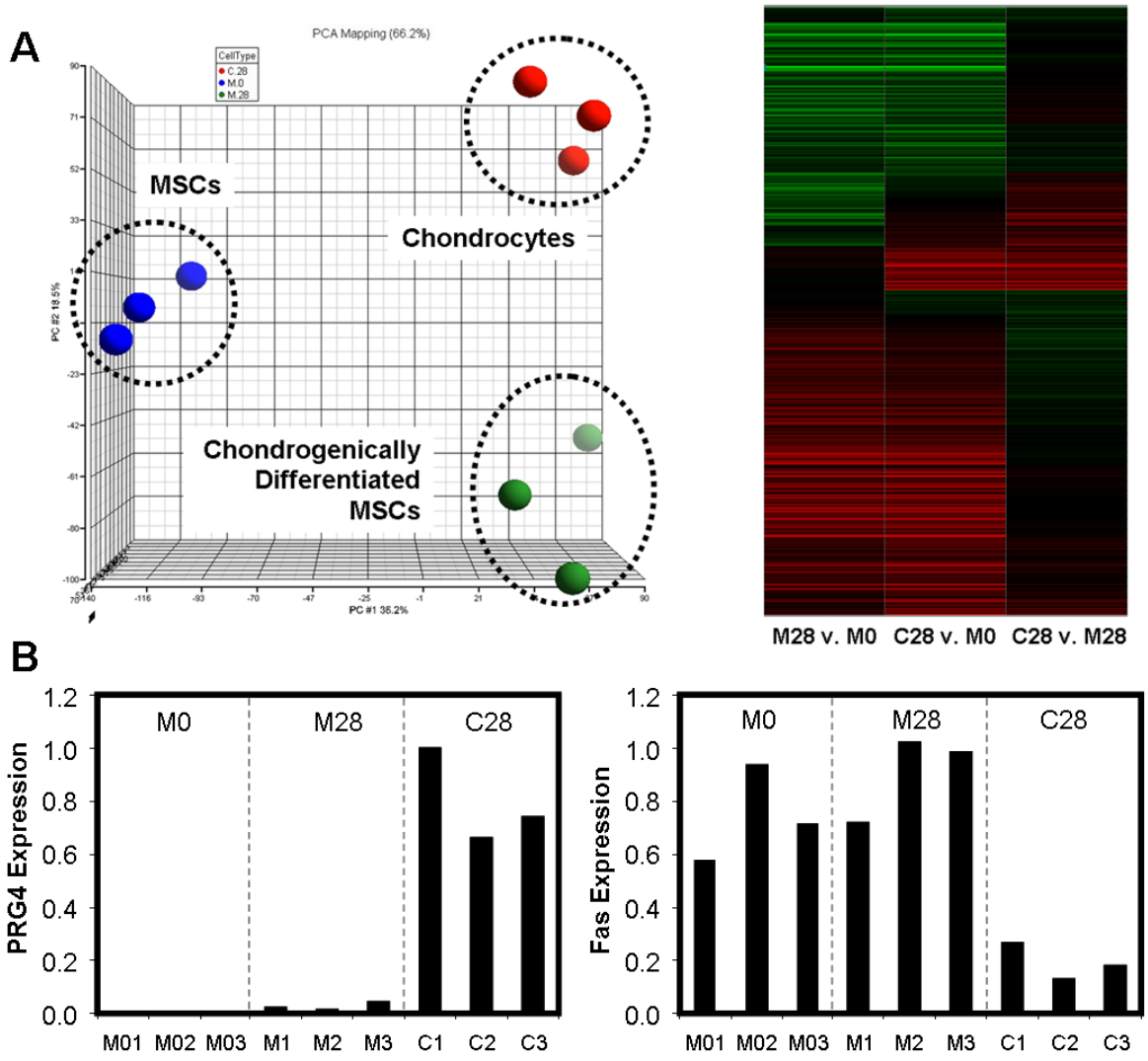

Figure 2: **(A)** Principle component analysis and heat map generated from microarray data representing gene expression of undifferentiated MSCs at day 0 (M0), chondrogenically differentiated MSCs at day 28 (M28), and chondrocytes at day 28 (C28). **(B)** Gene expression of PRG4 and TNFRSF6, illustrating genes that were under- or over-expressed in MSCs relative to chondrocytes. Data from three separate donors are shown.

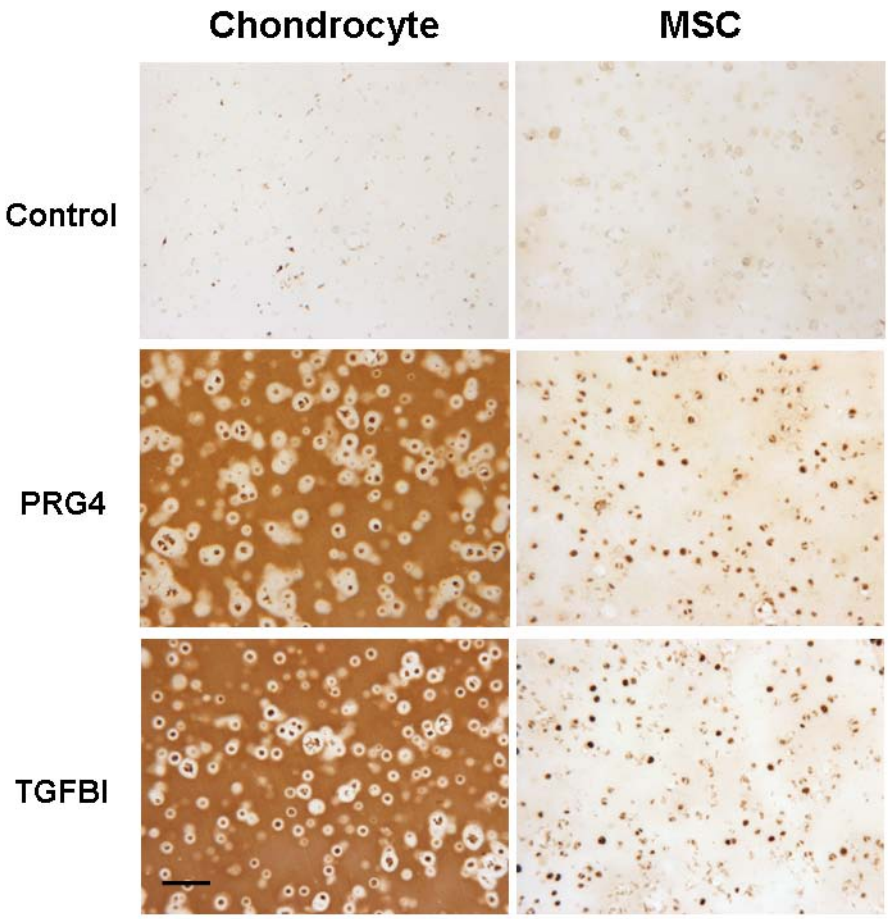

Figure 3: Immunohistochemical detection of PRG4 and TGFBI for day 56 cell-seeded constructs show robust staining in chondrocyte-seeded constructs and weak staining in MSC-seeded constructs. Scale Bar: 100 µm.

Recent Publications:

1. Huang AH, Stein A, Mauck RL. "Evaluation of the Complex Molecular Topography of Mesenchymal Stem Cell Chondrogenesis for Cartilage Tissue Engineering," 2009, *Tissue Engineering*, in review.
2. Huang AH, Stein A, Tuan RS, Mauck RL. "Transient Exposure to TGF-B3 Improves the Mechanical Properties of Mesenchymal Stem Cell-Laden Constructs in a Density Dependent Manner," 2009, *Tissue Engineering: Part A*, 15(11): 3461-3472.
3. Erickson IE, Huang AH, Chung C, Li R, Burdick JA, Mauck RL. "Differential Maturation and Structure-Function Relationships in MSC and Chondrocyte Seeded Hydrogels," 2008, *Tissue Engineering*, 15(5):1041-1052.
4. Huang AH, Yeger-McKeever M, Stein A, Mauck RL. "Tensile Properties of Engineered Cartilage Formed From Chondrocyte- and MSC-Laden Hydrogels," 2008, *Osteoarthritis and Cartilage*, 16(9):1074-1082.

5. Huang AH, Motlekar NA, Stein A, Diamond SL, Shore EM, Mauck RL. “High Throughput Screening for Modulators of Mesenchymal Stem Cell Chondrogenesis,” 2008, *Annals of Biomedical Engineering*, 36(11):1909-21.
6. Mauck RL, Yuan X, Tuan RS. “Chondrogenic Differentiation and Functional Maturation of Bovine Mesenchymal Stem Cells in Long-Term Agarose Culture,” 2006, *Osteoarthritis and Cartilage*, 14(2):179-189.

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