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Exploring the roles of integrin binding and cytoskeletal reorganization during mesenchymal stem cell mechanotransduction in soft and stiff hydrogels subjected to dynamic compression

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ABSTRACT

The objective of this study was to explore how the response of mesenchymal stem cells (MSCs) to dynamic compression (DC) depends on their pericellular environment and the development of their cytoskeleton. MSCs were first seeded into 3% agarose hydrogels, stimulated with the chondrogenic growth factor TGF-β3 and exposed to DC (~10% strain at 1 Hz) for 1 h on either day 7, 14, or 21 of culture. At each time point, the actin, vimentin and tubulin networks of the MSCs were assessed using confocal microscopy. Similar to previous results, MSCs displayed a temporal response to DC; however, no dramatic changes in gross cytoskeletal organization were observed with time in culture. Vinculin (a membrane-cytoskeletal protein in focal adhesions) staining appeared more intense with time in culture. We next aimed to explore how changes to the pericellular environment, independent of the duration of exposure to TGF-β3, would influence the response of MSCs to DC. To this end, MSCs were encapsulated into either 'soft' or 'stiff' agarose hydrogels that are known to differentially support pericellular matrix (PCM) development. The application of DC led to greater relative increases in the expression of chondrogenic marker genes in the stiffer hydrogels, where the MSCs were found to have a more well developed PCM. These increases in gene expression were not observed following the addition of RGDS, an integrin blocker, suggesting that integrin binding plays a role in determining the response of MSCs to DC. Microtubule organization in MSCs was found to adapt in response to DC, but this effect was not integrin mediated, as this cytoskeletal reorganization was also observed in the presence of RGDS. In conclusion, although the PCM, integrin binding, and cytoskeletal reorganization are all involved in mechanotransduction of DC, none of these factors in isolation was able to completely explain the temporal mechanosensitivity of MSCs to dynamic compression.

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1. Introduction

Both extrinsic and intrinsic mechanical signals (i.e. those generated outside the cell and transmitted inwards via the pericellular matrix (PCM) and those generated within the cell in response to its substrate) have been well characterized as key regulators of mesenchymal stem/stromal cell (MSC) differentiation (Engler et al., 2006; Kelly and Jacobs, 2010; Reilly and Engler, 2010; Steward et al., 2011, 2012, 2013; Thorpe et al., 2012). Dynamic compression (DC) has been shown to enhance chondrogenesis of MSCs depending on the timing and duration of its application (Campbell et al., 2006; Haugh et al., 2011; Huang et al., 2004, 2005, 2010; Mouw et al., 2007; Thorpe et al., 2010); however, the underlying mechanisms responsible for this temporal mechanosensitivity are not well understood. The application of DC from the onset of TGF-β3 stimulation has been shown to suppress markers of chondrogenesis, myogenesis and endochondral ossification; however, the application of this mechanical cue following 3 weeks of TGF-β3 induced differentiation has been shown to promote chondrogenesis but still suppress markers of both myogenic differentiation (Thorpe et al., 2012) and progression along the endochondral pathway (Thorpe et al., 2013). Both adaption of a chondrogenic phenotype (by long term exposure to chondrogenic growth factors) and development of a mature PCM have been proposed as possible mechanisms to explain the observed temporal mechanosensitivity of MSCs (Haugh et al., 2011). Chondrocytes seeded in agarose hydrogels synthesize a more mature cartilaginous matrix at later time points than at earlier ones, and this more developed matrix may be necessary for the mechanotransduction of DC (Buschmann et al., 1995). Furthermore, blocking integrin-mediated binding to the PCM has been shown to alter chondrocyte response to DC (Kock et al., 2009). A similar temporal development of the PCM has been observed for MSCs embedded into agarose hydrogels (Haugh et al., 2011), however it is still unclear whether this is merely correlating with a more anabolic response to DC or if it is indeed causative.

As the PCM develops not only does its biochemical composition change but it also stiffens (Vigfúsdóttir et al., 2010). The intrinsic stiffness of the local matrix is known to modulate MSC differentiation in both 2D (Engler et al., 2006; Park et al., 2011) and 3D (Huebsch et al., 2010; Murphy et al., 2012; Parekh et al., 2011; Pek et al., 2010). Recently, studies have begun investigating the interplay between extrinsic and intrinsic mechanical cues and their subsequent effects on cellular differentiation. Denser and stiffer hydrogels were found to alter PCM organization, cytoskeletal organization, and integrin binding relative to less dense and softer hydrogels (Steward et al., 2013). These changes led to a more robust response to the application of extrinsic mechanical signals in the stiffer hydrogels, demonstrating the importance of the PCM for determining the response of MSCs to such cues (Steward et al., 2013). These changes to the PCM occurred in parallel with changes to the internal cytoskeleton, raising the possibility that the temporal response of MSCs to extrinsic mechanical cues could be related to changes in either the PCM or to the cytoskeleton during chondrogenesis.

The objective of this study was to first characterize changes in both the pericellular environment and cytoskeletal organization during TGF-β3 mediated chondrogenesis of MSCs in hydrogel culture, with the aim of potentially correlating such alterations with the temporal response of the cells to DC. Since both the pericellular environment and cytoskeleton may adapt as differentiation proceeds, decoupling the relative importance of such changes in determining the temporal response of MSCs to DC from other phenotypic changes that may occur in response to increasing exposure to TGF-β3 becomes difficult. Therefore we next aimed to determine how altering the pericellular environment of MSCs, by changing the stiffness/concentration of the hydrogel within which they were encapsulated, would impact their response to DC. Furthermore, we explored how blocking integrin-mediated binding of MSCs to their local pericellular environment would impact their response to DC. The final aim of the study was to elucidate whether cytoskeletal adaptation (i.e. changes in actin, intermediate filaments and microtubules post DC) is a feature of the response of MSCs to extrinsic mechanical loading.

2. Materials and methods

2.1. Cell isolation, expansion, and encapsulation

Bone marrow was harvested from the femoral diaphysis of 4-month-old pigs (~50 kg) under sterile conditions. MSCs were isolated and expanded according to a modified method developed for human MSCs (Lennon and Caplan, 2006). Briefly, bone marrow was removed from the femur, washed and centrifuged twice, and sieved through a 40 μm pore-size cell sieve (Falcon, Starstedt). The remaining cell suspension was counted by trypan blue exclusion and seeded at a density of 10×10^6 cells per 175 cm^2 T-flask in a humidified atmosphere of 37 °C and 5% CO_2. Non-adherent cells were removed after 3 days in culture to allow MSCs to attach to the flask. At each passage, cells were reseeded at a density of 875,000 cells per 175 cm^2 T-flask. Cultures were expanded in high-glucose Dulbecco's modified Eagle's Medium (hgDMEM GlutaMAX) supplemented with 10% fetal bovine serum (FBS), and penicillin (100 U/mL)-streptomycin (100 μg/mL) (all GIBCO, Biosciences). After expansion (third passage) MSCs were encapsulated in agarose (Type VII, Sigma-Aldrich) at a density of 15×10^6 cells/mL. Briefly, MSCs were mixed with 5% agarose at ~40 °C to yield final gel concentrations of 1% or 3% (with equilibrium moduli of 0.5 and 15 MPa, respectively). The agarose-cell suspensions were cast in stainless steel molds, and cored using biopsy punches to produce cylindrical scaffolds (Ø 5 × 3 mm thickness). Constructs were maintained in 2.5 mL/construct of a chemically defined media (CDM) consisting of hgDMEM GlutaMAX supplemented with penicillin (100 U/mL)-streptomycin (100 μg/mL) (GIBCO, Biosciences), 100 μg/mL sodium pyruvate, 40 μg/mL L-proline, 50 μg/mL L-ascorbic acid-2-phosphate, 1.5 mg/mL BSA, 1 × insulin–transferrin–selenium, 100 nM dexamethasone (all Sigma-Aldrich), and 10 ng/mL recombinant human transforming growth factor-β3 (chondrogenic, TGF-β3; ProSpec-Tany TechnoGene Ltd). Additional groups were also cultured with the addition of a 167 μM RGDS peptide (RGDS+, Tocris Bioscience) in order to inhibit integrin binding during the first week of culture. Cells that were to be cultured with the RGDS peptide were equilibrated in the chondrogenic media supplemented

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