Dynamic Stretch Rapidly Alters Nuclear Structure and Increases Chromatin Condensation in Mesenchymal Stem Cells

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Introduction

Mesenchymal stem cells (MSCs) are an attractive cell type for regenerative therapies in orthopedics given their multipotent nature.[1] Exogenous mechanical stimuli modulate the lineage specification of these cells.[2,3] Our group and others have shown that dynamic tensile strain enhances functional growth by MSCs seeded on aligned nanofibrous scaffolds.[2,4] Within the nucleus of differentiating cells, the distribution of the Lamin A/C (LMAC) network and the condensation state of chromatin are strongly correlated with transcriptional activity.[5] In previous work, we showed that MSC differentiation induced by soluble chondrogenic factors, or dynamic tensile loading (DL) in the absence of these factors, resulted in a marked reorganization of the LMAC, as well as increases in heterochromatin (HTC) content.[6] It has recently been suggested that mechanical perturbation, transmitted through the cytoskeleton, can reach to nucleus more rapidly than soluble signals.[7] In the current study, we investigated the early remodeling of MSC nuclei in response to mechanical stimulation, and compared the rate of these changes to differentiation mediated by soluble factors applied over the same time course.

Methods

Aligned poly(ε-caprolactone) nanofibrous scaffolds were fabricated via electrospinning.[2] Bovine bone marrow derived MSCs (200,000 cells) were seeded onto scaffolds (5×60 mm2) and pre-cultured in a chemically defined media without TGF-β3 [CM(−)] for 2 days. After pre-culture, 10ng/ml TGF-β3 was added to the CM(−) media [to produce CM(+)] or samples were exposed to dynamic tensile loading (DL, 3%, 6 hrs/day, 1 Hz) using a custom bioreactor[2] in CM(−) media. LMAC organization (Thermo) and HTC levels (Abcam) were visualized by immunofluorescence. HTC staining intensity was measured using MetaMorph® (Molecular Devices Inc.). To assess chromatin condensation, nuclei on scaffolds were stained with DAPI, and scanned across their mid-section using a confocal microscope (Zeiss, LSM 510). To generate a chromatin condensation parameter (CCP) describing internal nuclear structure, a gradient-based Sobel edge detection algorithm was employed using MATLAB to measure the edge density for individual nuclei.[8] Gene expression levels were determined by real time RT-PCR, and normalized to GAPDH. Statistical analysis was performed by ANOVA with Fisher's post-hoc tests.

Results

Consistent with our previous findings, LMAC was distributed throughout the nucleus in undifferentiated cells. Addition of TGF-β3 did not change LMAC organization over the first 3 days of culture (Fig. 1A). Interestingly, after 2 days of DL in CM(−), LMAC started to become restricted to the nuclear periphery, with full reorganization to the periphery evident after 3 days of DL (Fig. 1A). LMAC gene expression did not change with the addition of TGF-β3, while DL increased expression at each time point (Fig. 1 B). Additionally, HTC formation occurred much more rapidly with DL than with CM(+), with increased HTC staining apparent as early as day 1 for DL compared to day 3 with CM(+) (Fig. 1C). The intensity of HTC staining in DL conditions was higher than both CM(−) or CM(+) conditions at day 1 (Fig. 1D), indicating that mechanical stimulation rapidly increases HTC formation in the absence of exogenous growth factors. To verify induction of true chromatin restructuring, a novel image based technique was applied on day 1. Chromatin condensation was evident in DL nuclei as distinct, chromatin free spaces within the nucleus, with this change leading to an increase in the number of visible edges (Fig. 2C). This was shown through a significant increase in the chromatin condensation parameter for DL nuclei (Fig. 2D). Similarly, gene expression analysis revealed an early and robust response to dynamic loading in comparison to TGF addition. While most markers increased on day 1 for both treatments, SOX9 and BMP2 expression decreased to baseline in CM(+) conditions, but were maintained at high levels in DL conditions through day 3 (Fig. 3). TGF-β3 gene expression

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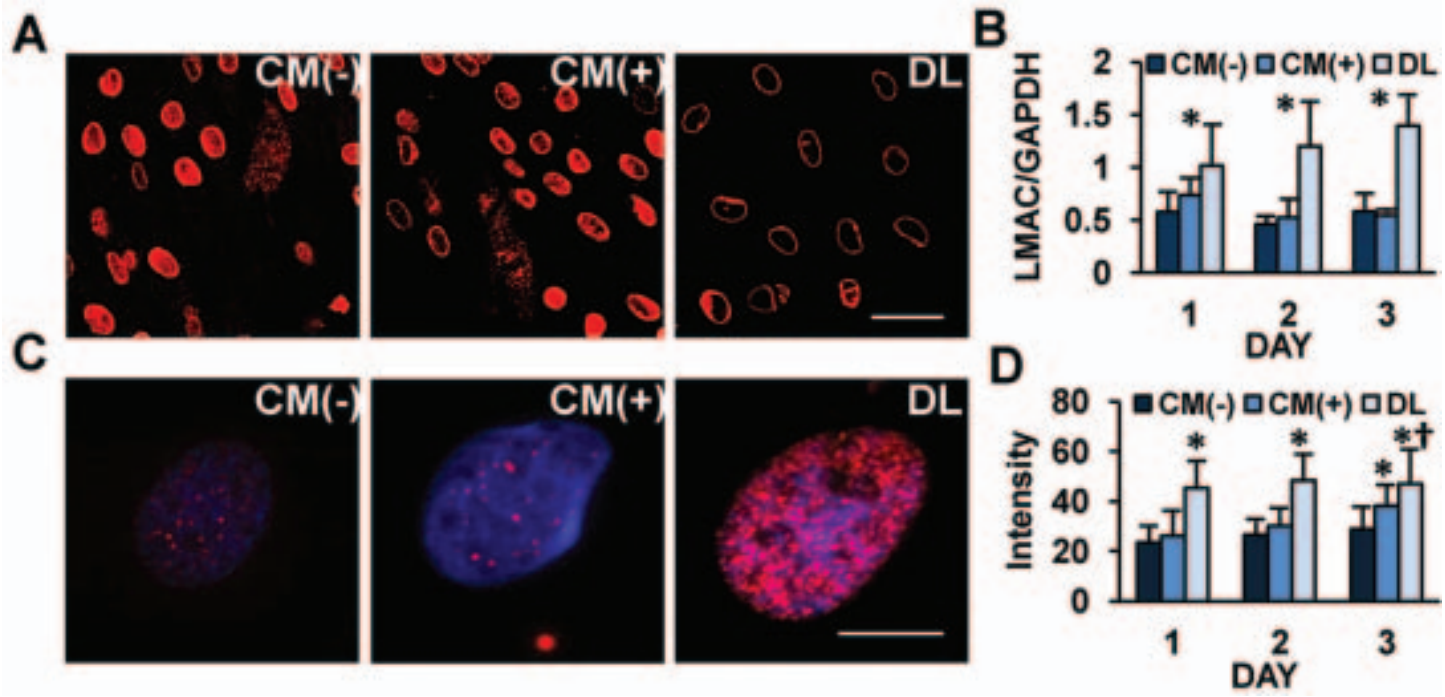

Figure 1A-D. (A) LMAC staining on day 3 with treatment (bar = 20 μm, (B) LMAC gene expression over time (n=3, *:p<0.05 vs. CM(−)). (C) HTC staining on day 1 with treatment (red: HTC, blue: DAPI, bar = 10 μm) and (D) quantification of staining intensity (n=20, *:P<0.05 vs. CM(−), †:P<0.05 vs. CM(+)).

did not change in CM(+) conditions, but increased with DL (Fig. 3). AGG expression increased gradually in both CM(+) and DL conditions through day 3, with expression in DL higher than that in CM(+) (Fig. 3).

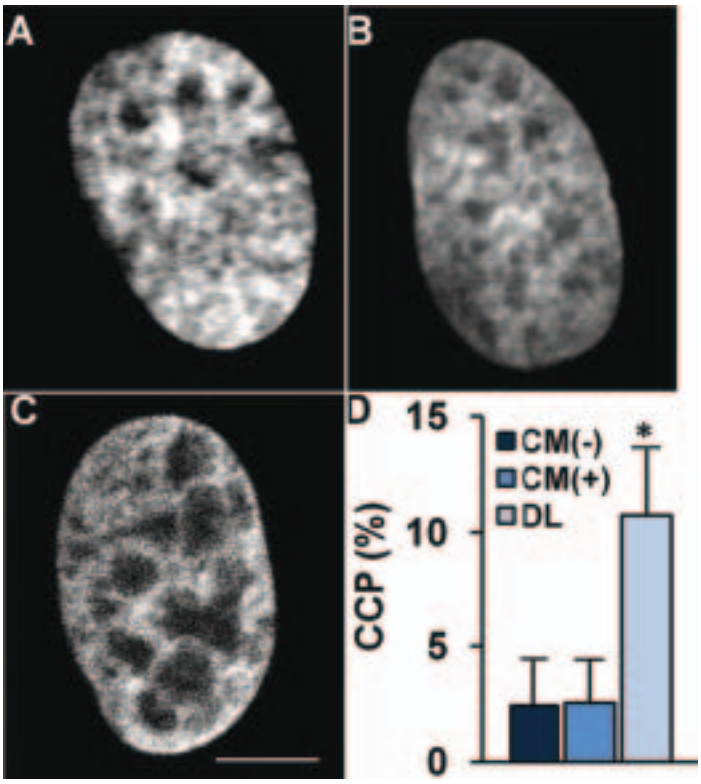

Figure 2. Representative DAPI stained nuclei on day 1 with treatment (A: CM(−), B: CM(+) and C: DL, bar = 5 μm), (D) Chromatin condensation parameter (CCP) on day 1 (n=45-50 cells per condition, *: p<0.001 vs. CM(−) and CM(+)).

Discussion

In this study, we demonstrated that, in the absence of exogenous differentiation factors (TGF-β3), short term DL of MSCs altered their LMAC distribution, and causes rapid chromatin condensation. These responses to DL occurred after only one day of loading, several days in advance of similar changes wrought by the addition of soluble TGF. This suggests that the mechanical input can invoke a rapid change in nuclear structure, and is consistent with the notion that mechanical forces, transmitted to the nucleus through the contractile cytoskeleton via the LINC complex, can more rapidly regulate signaling events in cells.[7] These rapid changes likely have physiologic consequence, as LMAC organization and its interaction with chromatin can influence transcriptional activity.[9] Indeed, along with acute LMAC reorganization and chromatin condensation, DL up-regulated fibro-chondrogenic and TGF-β and BMP2 gene expression in the absence of exogenous growth factors. These expression levels were generally higher than that induced by soluble growth factors, and persisted for a longer period of time. Future work will focus on elucidating the mechanism by which these changes are mediated, as well as the consequence of the mechanically induced changes in nuclear structure on subsequent responses to exogenous mechanical perturbation.

Significance

Mechanical stimuli are important for driving MSC lineage specification. However, the mechanism by which these stimuli influence fate decisions is still poorly understood, and the consequence of changing cell and nuclear structure and mechanics in this process is not fully defined. Here, we show that

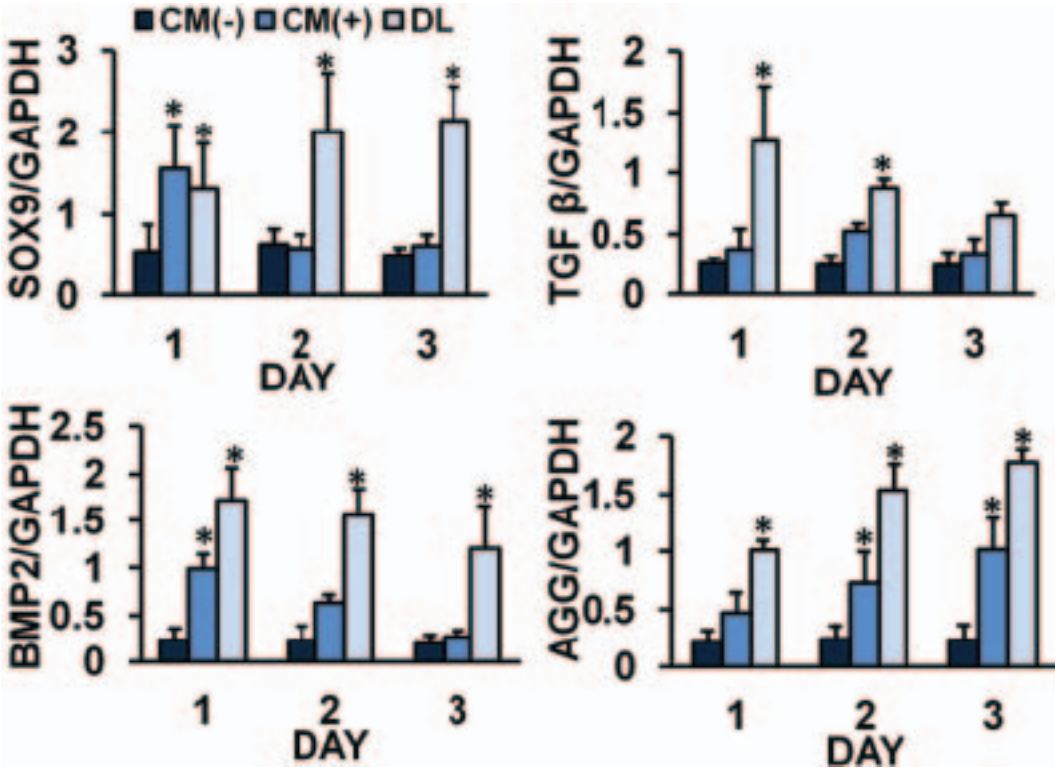

Figure 3. SOX9, TGF β, BMP2, and aggrecan (AGG) gene expression (normalized to GAPDH) with treatment (n=3, *: $p<0.05$ vs. CM(-)).

mechanical stimulation rapidly alters LMAC organization and promotes chromatin condensation, along with transcriptional activity indicative of an advanced differentiated state.

Acknowledgements

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