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Immediate-early gene expression dynamics in human MSCs in response to a single mechanical loading event

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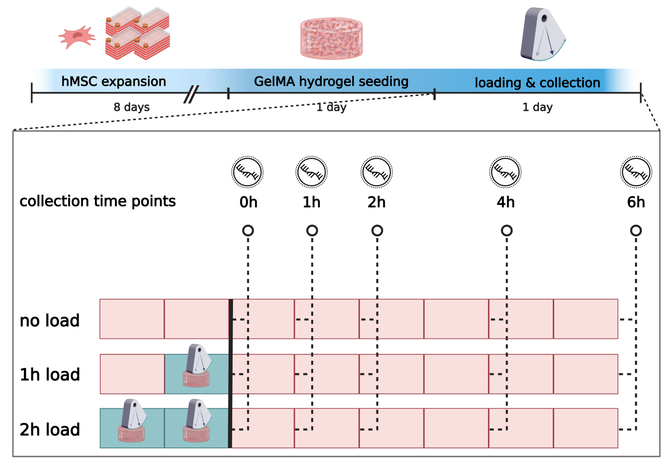
Objective

Osteoarthritis affects millions worldwide and lacks curative treatment. Microfracture surgery is used in an attempt to repair focal cartilage defects by recruiting mesenchymal stem cells (MSCs). However, the resulting repair tissue often lacks native cartilage functionality. Mechanical loading during postoperative rehabilitation is a critical but insufficiently understood regulator of MSC fate. Mechanical stimulation can activate latent TGFβ, initiate downstream signalling through the TGFβ pathway and thus promote MSC chondrogenesis. In parallel, mechanical cues act as zeitgebers for the cartilage circadian clock, a key regulator of tissue homeostasis, matrix turnover and cellular function. Yet the immediate-early responses of naïve MSCs to physiologically relevant loading remain unclear.

This study aimed to determine how a single mechanical stimulus of different durations can prime hMSCs toward chondrogenesis and modulates cell fate-determining, circadian, mechano-transductive, and TGFβ-signalling gene expression over time.

Design

After 2D monolayer expansion, human MSCs (4 donors) were encapsulated in 3D gelatine methacrylate (GelMA) hydrogels ($5x10^6$ million cells per construct, 4mm height, 8mm diameter). The following day, constructs were either left unloaded (free-swell) or subjected to a single multiaxial loading event (10% static + 10% dynamic compression and shear) for either 1h or 2h. Load cessation was defined as time point 0h. Samples were collected at 0, 1, 2, 4, and 6h post-load and analysed by RT-qPCR for lineage-associated, mechanoresponsive, TGFβ-pathway and circadian clock gene expression (31 genes in total).



Statistics:
Gene expression was analysed using linear mixed-effects models with estimated marginal means for post hoc comparisons and false discovery rate correction applied across genes at both model and post hoc levels.

Results

LOAD EFFECT ON THE CIRCADIAN CLOCK

One-time mechanical loading of naïve hMSCs freshly encapsulated in GelMA significantly altered the expression of a subset of core circadian clock genes. Genes of the negative clock arm, **PER2**, **CRY1**, and **CRY2**, were upregulated, together with the circadian output gene **DBP** (Fig. 1).

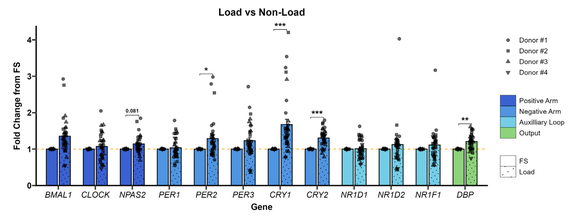


Figure 1. Mechanical loading modulates circadian clock gene expression in hMSCs. Relative gene expression of core circadian clock genes following a single mechanical loading event normalised to free-swelling controls. Data from all post-load time points were pooled. The loaded group includes data from both 1h and 2h loading durations.

When overall differences between 1h and 2h loading were compared independent of sampling time, load duration selectively affected the expression of **RUNX2** (osteo-genesis), **PPARG** (adipogenesis), and **MMP13** (matrix remodelling), which were elevated following 2h compared with 1h of loading (data not shown).

TEMPORAL DYNAMICS IN GENE EXPRESSION RESPONSES WITHIN LOAD CONDITIONS

Distinct gene expression dynamics within each load condition were observable when resolved for time. In **free-swelling controls**, significant temporal regulation of **COL6A1** (chondron formation), **PIEZO1** (mechanosensitive ion channel), **PAI1**, and **ACVRL1** of the non-canonical TGFβ-pathway (hypertrophy) indicated transcriptional adaptation to 3D encapsulation and culture, not mechanical load. Following **1h loading**, changes in gene expression levels were following similar trends as in the free-swelling control but were more pronounced for **COL6A1**. In contrast, **2h loading** induced a broader and more coordinated transcriptional response over time, including modulation of **SOX9**, **COL1A1**, **COL6A1**, **PIEZO1**, **TRPV4**, and multiple **TGFβ** pathway components.

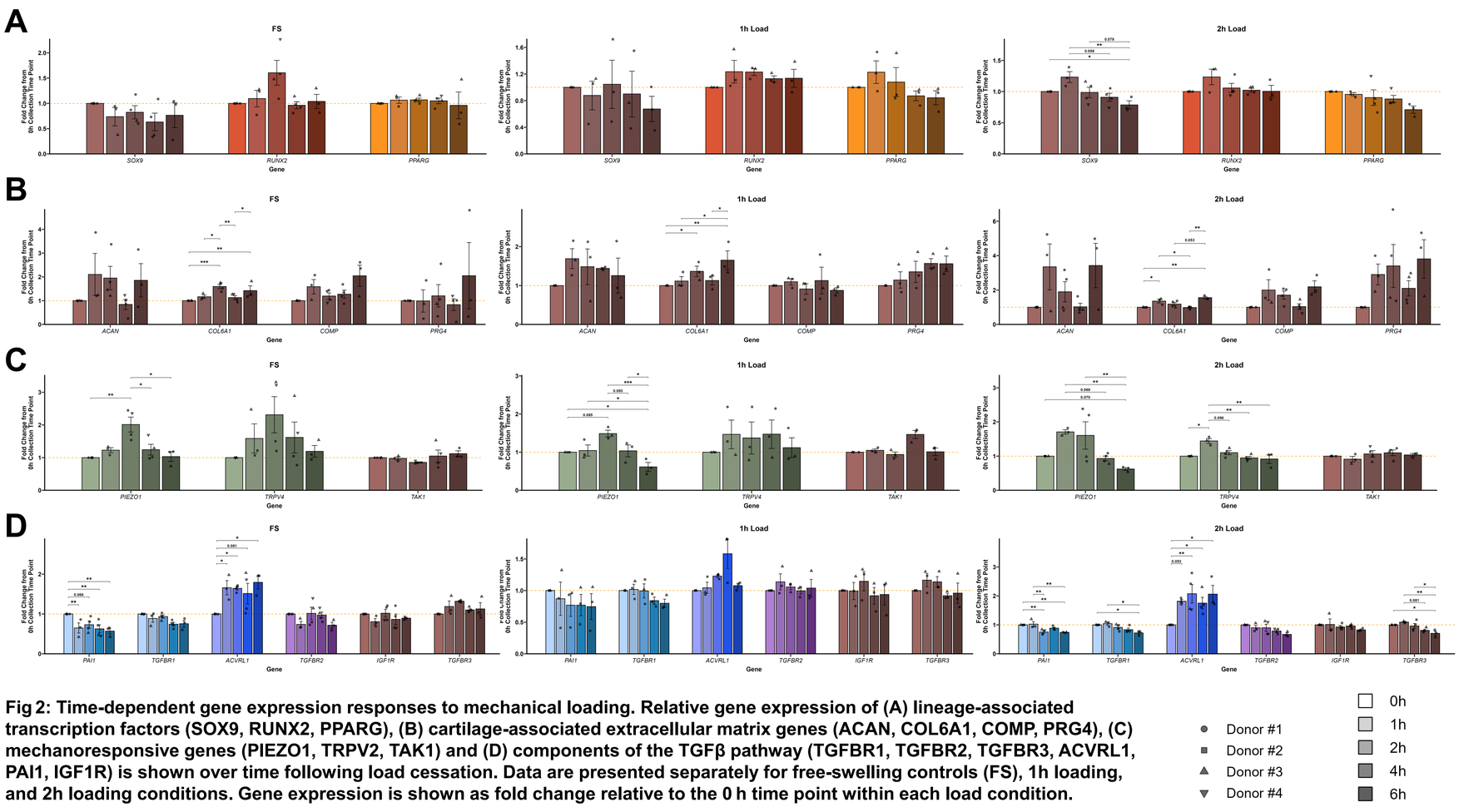


Fig 2: Time-dependent gene expression responses to mechanical loading. Relative gene expression of (A) lineage-associated transcription factors (SOX9, RUNX2, PPARG), (B) cartilage-associated extracellular matrix genes (ACAN, COL6A1, COMP, PRG4), (C) mechanoresponsive genes (PIEZO1, TRPV2, TAK1) and (D) components of the TGFβ pathway (TGFBR1, TGFBR2, TGFBR3, ACVRL1, PAI1, IGF1R) is shown over time following load cessation. Data are presented separately for free-swelling controls (FS), 1h loading, and 2h loading conditions. Gene expression is shown as fold change relative to the 0 h time point within each load condition.

Discussion & Conclusion

Mechanical loading rapidly modulated the expression of circadian clock genes in naïve hMSCs, indicating that these cells can sense and respond to mechanical cues at the level of the circadian gene circuitry. Notably, load predominantly affected components of the negative limb of the clock (**PER2**, **CRY1**, **CRY2**), together with the circadian output gene **DBP**, supporting the role of mechanical stimulation as a zeitgeber for cartilage progenitor cells. Time-resolved analysis revealed load-duration-dependent transcriptional dynamics, with 2h loading eliciting a more robust and coordinated gene expression response than free-swelling or 1 h loading. Together, these findings highlight mechanical load duration as a critical determinant of early MSC transcriptional priming relevant to cartilage repair.

Acknowledgements

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