Real-Time Deformability Cytometry Detects Mechanically Driven Osteogenesis in MSCs by Day 7

Nanovibrational stimulation produces a distinct, time-dependent mechanical phenotype in MSCs, resolvable at population scale.

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1 kHz · 30 nm sinusoidal

AIM To use high-throughput Real-Time Deformability Cytometry (RT-DC) to track the mechanical phenotype of nanostimulated MSCs over time and link cytoskeletal stiffening to differentiation commitment confirmed by RNA-seq - testing whether the mechanical readout precedes and supports transcriptomic confirmation.

Background

Nano-stimulation applies nanoscale vibration (sinusoidal, **1 kHz, 30 nm amplitude**) to drive osteogenic differentiation [1]

Mechanical stimulation reshapes the cell's own mechanical phenotype, yet this has mostly been probed with low-throughput tools (e.g. **atomic force microscopy**), limiting population-level insight.

RT-DC measures the deformation of thousands of cells per minute using microfluidic label-free mechanophenotyping at scale. [2]

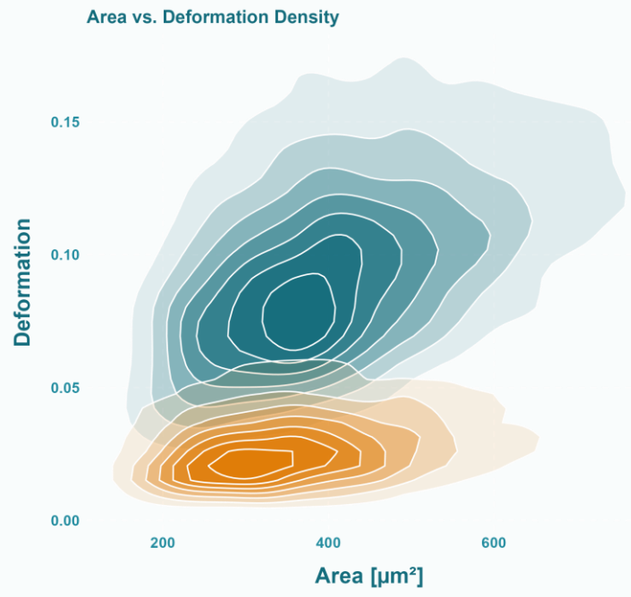
Methods

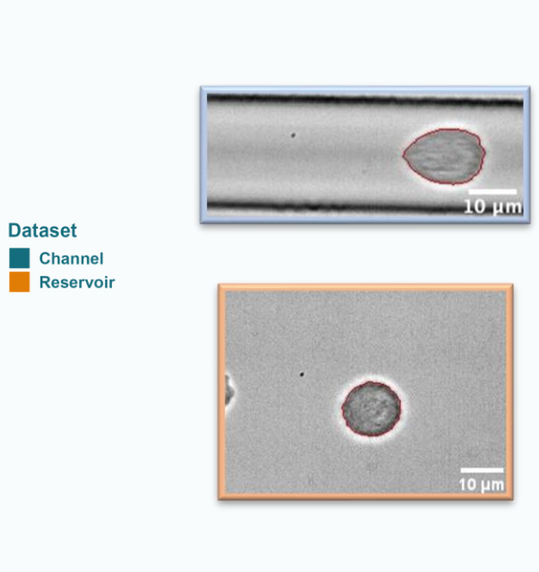
- **Optimisation:** MG63 osteoblast-like line used to establish RT-DC settings.
- **Model:** BM-MSCs from 3 donors - control (CT), nanovibration (NV), osteogenic media (OM).
- **RT-DC:** Deformation + apparent Young's modulus at days 7, 14, 21, 28.
- **RNA-seq:** Bulk sequencing.
- **Stats:** Kruskal-Wallis test using Dunn's (non-parametric). One/Two-way ANOVA (parametric testing).

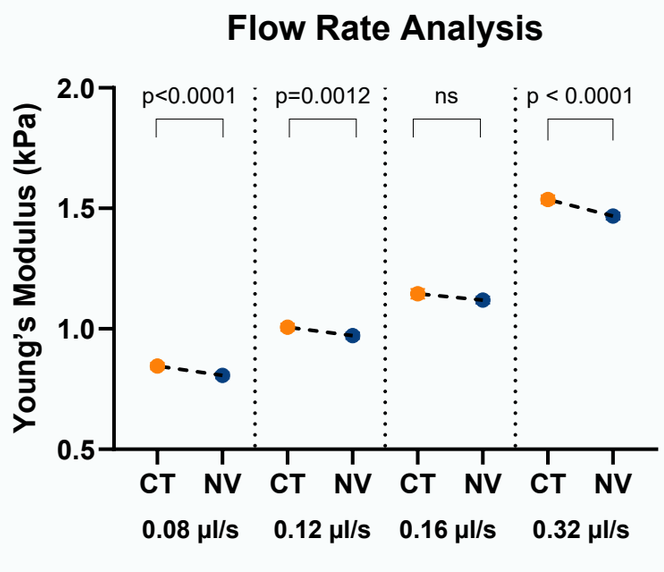
Results

CT Control **NV** Nanovibration **OM** Osteogenic media

FIGURE 1 - RT-DC optimization - a measurable mechanical phenotype – MG63 optimization (72 hours)







Note: Channel size: 30 µm; Flow rate: 0.32µl/s (1:3 ratio) chosen as the ideal testing parameters [1].

FIGURE 2 — MSC mechanical phenotype across days 7–28

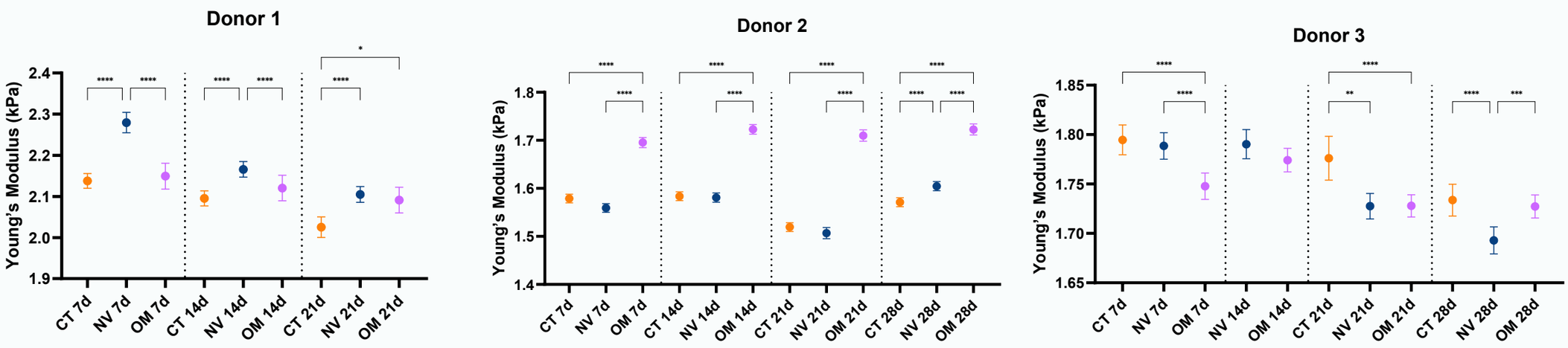


FIGURE 4 — RNA-seq – day 7

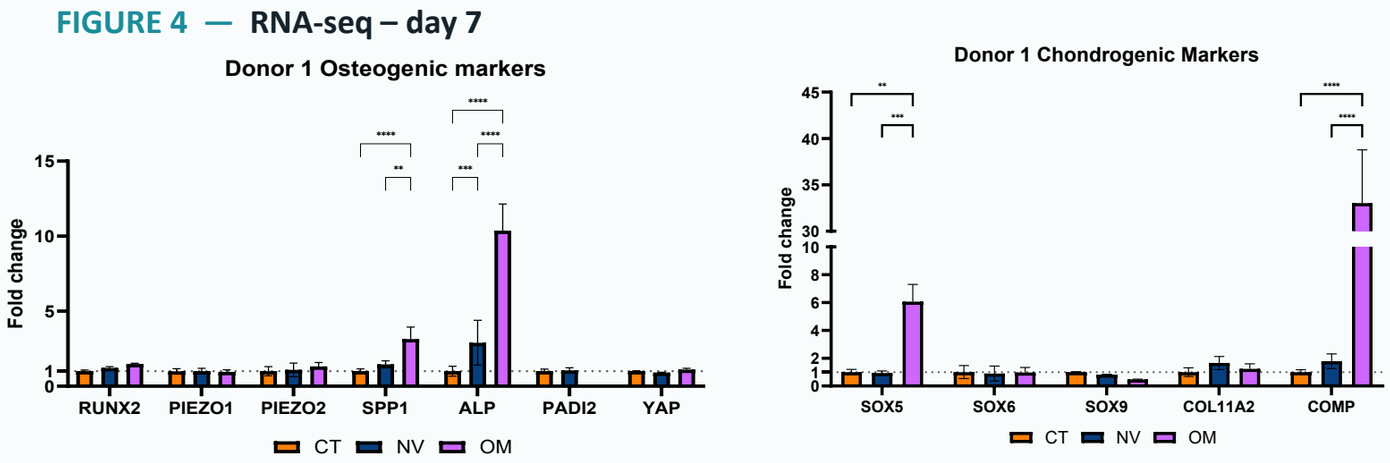
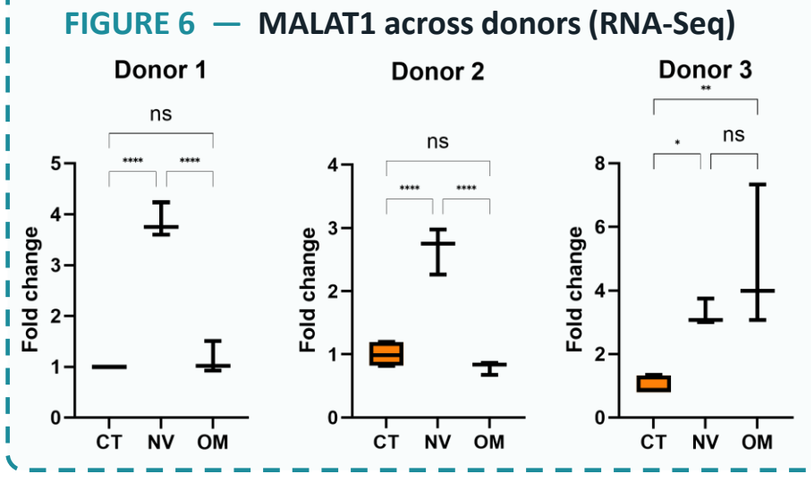


FIGURE 6 — MALAT1 across donors (RNA-Seq)



Discussion

- RT-DC distinguishes CT, NV and OM mechanical phenotypes **from day 7** - earlier than many transcriptomic readouts fully mature.
- NV gives a **weaker but more lineage-specific** osteogenic signature than OM.
- OM co-upregulates chondrogenic markers (**SOX5/6, COMP**), indicating less specific commitment.
- **MALAT1** rises across all mechanically stimulated donors - a candidate mechanoresponsive marker.

Conclusion

RT-DC, together with MALAT1, makes it possible to identify mechanically driven osteogenesis by day 7 of culture.

High-throughput mechanophenotyping is a viable, early, label-free readout of MSC differentiation commitment complementing, and in places preceding, transcriptomic confirmation.

References

[1] Pereira Sousa, R. P. et al. Mechanical Phenotyping of MG63s Following Vibrational Stimulation. FASEB, 2026.

[2] Otto O. et al. Real-Real-time deformability cytometry: on-the-fly cell mechanical phenotyping. Nature Methods 2015.

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