

Hacking mechanical memory

Jeroen Eyckmans^{1,2,*}

¹Department of Biomedical Engineering and the Biological Design Center, Boston University, Boston, Massachusetts and ²Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, Massachusetts

Autologous chondrocyte implantation (ACI) is a surgical procedure used to treat cartilage defects in articular joints. It involves removing healthy cartilage cells called chondrocytes from a patient, cultivating them in a laboratory for several weeks, and then reimplanting them into the patient's joint to regenerate the damaged cartilage (1). Although ACI has been heralded as a promising technique for cartilage repair, expanding primary chondrocytes in conventional tissue culture vessels results in the irreversible loss of the chondrogenic phenotype and the emergence of a fibrotic one (2). This phenomenon, known as chondrocyte dedifferentiation, has hindered the clinical translation of ACI in patients. In this issue of *Biophysical Journal*, a study from Scott, Neu, and colleagues (3) provides new insights into how culturing chondrocytes on stiff substrates leads to changes in chromatin architecture and concomitant loss of the chondrocyte phenotype, which can be partially prevented by treatment with chromatin modifying inhibitors.

Cells detect and process biophysical cues in order to direct cellular processes such as migration, proliferation, and differentiation (4). New evidence

in the last decade suggests that cells not only directly respond to their mechanical environment but also retain information about their previous mechanical environment. Hinz and colleagues initially described mechanical memory in the context of myofibroblast differentiation (5). In their experiments, fibroblasts from a lung explant differentiated into myofibroblasts when cultured on a stiff (Young's modulus of 100 kPa) silicon substrate, whereas cells adhered to a soft (Young's modulus of 5 kPa) substrate retained their fibroblast phenotype. Surprisingly, when fibroblasts were primed on stiff substrates for a short period of time, they developed a myofibroblast phenotype, which was reversible when the cells were replated onto soft matrices. In contrast, fibroblasts that had been mechanically primed for an extended period of time on stiff substrates retained their myofibroblast phenotype when transferred to soft matrices. Since this seminal study, mechanical memory has been demonstrated for the differentiation of bone marrow stromal cells into preosteoblasts (6,7) and myofibroblasts (5,8) and for collective migration of epithelial cells (9).

In this study, Scott and colleagues extend the concept of mechanical memory to chondrocyte dedifferentiation (3). To accomplish this, the researchers expanded freshly isolated bovine chondrocytes on tissue culture plastic for 8 and 16 population dou-

blings (PDs) and found that PD8 and PD16 chondrocytes lost their chondrogenic phenotype as indicated by a decreased expression of chondrogenic marker genes (*SOX9*, *COL2A1*, *ACAN*) and an increased expression of fibrotic-related genes (*COL1A1*, *VCAN*, *THY1*). PD8 and PD16 chondrocytes were then encapsulated in a soft hyaluronic acid-polyethylene glycol diacrylate hydrogel and cultured for an additional 10 days. PD8 chondrocytes, but not PD16 chondrocytes, regained the chondrogenic phenotype, suggesting that the duration of mechanical priming on a stiff substrate instills a mechanical memory in chondrocytes, which is consistent with findings from previous research (5,6,9,10).

To investigate how chondrocytes encode mechanical memory, the researchers zoomed in on chromatin architecture. Double-stranded DNA is wound around histone proteins (nucleosomes) in the nucleus, where it is densely packed into chromosomes. The winding and unwinding of chromatin, a process that is regulated by histone deacetylases and demethylases (unwinding) and histone acetyltransferases and methylases (winding), render DNA promoter sites either available or hidden from binding to transcription factors. In this study, the researchers investigated the role of the triple methylation modification on the ninth lysine residue of histone 3 (H3K9me3) in establishing mechanical

Submitted February 23, 2023, and accepted for publication March 7, 2023.

*Correspondence: eyckmans@bu.edu

Editor: Guy Genin.

<https://doi.org/10.1016/j.bpj.2023.03.012>

© 2023 Biophysical Society.

Eyckmans

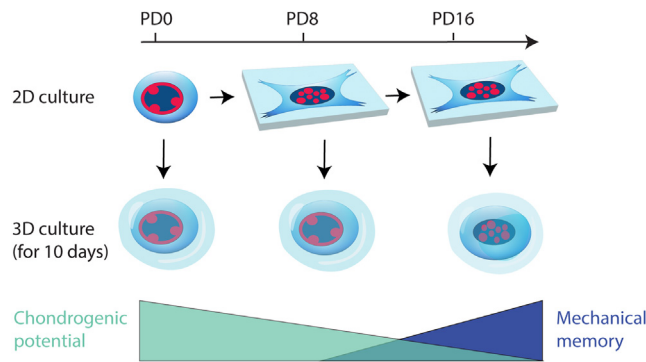


FIGURE 1 Mechanical memory in chondrocytes is encoded in chromatin architecture. Naive chondrocytes are expanded for 8 or 16 population doublings on tissue culture plastic. The stiff substrate drives condensation of H3K9me3 chromatin throughout the nucleus and loss of chondrogenic potential (red filled circles). Encapsulation in a soft hydrogel rescues chromatin architecture and chondrogenicity of PD8 chondrocytes, but not of PD16 chondrocytes, suggesting that mechanical memory is established between 8 and 16 population doublings. To see this figure in color, go online.

memory because H3K9me3 has been linked to chromatin condensation under mechanical load and is involved in stem cell differentiation. The researchers discovered that H3K9me3-marked chromatin was found adjacent to the nuclear envelope in PD0 chondrocytes but appeared as distinct foci dispersed throughout the nucleus in PD8 and PD16 chondrocytes. The nuclear architecture of PD8, but not PD16, chondrocytes was restored to that of PD0 cells after encapsulation in hydrogels, as measured by the localization and number of H3K9me3 foci, nuclear area, and aspect ratio of the nucleus, and the expression of chondrogenic genes in these cells was recovered (Fig. 1).

Because correlation does not imply causation, the researchers looked into ways to change the chromatin architecture by altering the expression of H3K9me3-marked heterochromatin. They started with chondrocytes isolated from H3K9M mice, which have an inducible histone H3.3 lysine-to-methionine mutation that acts as a global dominant negative inhibitor of H3K9 trimethylation. It was hypothesized that H3K9M chondrocytes would have a more open chromatin structure, making them less susceptible to dedifferentiation than wild-type cells. Surprisingly, despite the reduced trimethylation of H3K9, the chromatin architecture of PD16 H3K9M chon-

drocytes when primed on stiff mechanical substrates was comparable to that of wild-type cells. Furthermore, H3K9M chondrocytes dedifferentiated to the same extent as wild-type cells, indicating that decreasing levels of H3K9 methylation had no effect on stiffness-induced chromatin remodeling and dedifferentiation.

In contrast, treatment of chondrocytes with ML324, a chemical inhibitor of KDM4, a demethylase of H3K9me3, increased H3K9me3 levels and preserved the naive chondrogenic chromatin architecture during stiff substrate culture, resulting in increased *SOX9* and *COL2A1* gene expression while decreasing *COL1A1* expression. Interestingly, *ACAN*, *VCAN*, and *THY1* gene expression in PD16 chondrocytes remained unchanged when ML324 was used versus DMSO controls. Thus, increasing the levels of trimethylated H3K9 only partially protected the chondrogenic phenotype when cultured on stiff substrates. Together, these data suggest that remodeling of the chromatin architecture is an essential feature for encoding mechanical memory, and the native chromatin architecture can be preserved in chondrocytes by increasing the levels of H3K9me3.

These findings are consistent with previous research showing that chromatin remodeling in response to stiffness or mechanical loading instills

mechanical memory in marrow stromal cells (MSCs) (10,11). However, the devil, as is often the case in research, is in the details. MSCs, unlike chondrocytes, exhibit increased chromatin condensation when adhered to soft matrices, and stiffness priming prevents chromatin condensation, likely through the action of histone acetyltransferase 1 (11). Thus, how mechanical memory is encoded in the chromatin architecture may be cell-type specific. Despite these differences, mechanical memory shares significant similarities across cell types. For example, only stiff substrates, not soft substrates, appear to instill a mechanical memory in cells. And stiffness-induced changes in chromatin architecture often result in a loss of the naive cell state with a concomitant increase of fibrotic or osteogenic markers. Therefore, it is tempting to speculate that mechanical memory may be part of a pathological mechanism that drives fibrotic diseases. It remains, however, to be demonstrated if cells acquire a mechanical memory in *in vivo* settings, for example, in stiff tumors or fibrotic lesions.

Nevertheless, even if mechanical memory is a phenomenon that only manifest *in vitro*, then preventing or erasing mechanical memory could serve as a tool to mitigate undesired phenotypic changes that occur during cell culture. Hinz and colleagues demonstrated proof of concept by transplanting MSCs into a dermal wound, which improved wound healing by reducing scarring and suppressing myofibroblast formation and tissue contracture when the cells were either expanded on soft substrates or memory erased by knocking down miR-21 levels (8). Given that the chondrogenic phenotype is better preserved in chondrocytes that are expanded on soft matrices (5kPa) (12), and based on this work of the Neu group showing the potential use of KDM4 inhibitors to preserve the chondrogenic phenotype when cells are expanded on a stiff substrate (3), epigenetic resetting of mechanical memory prior to chondrocyte transplantation may hold promise

for regenerating stable cartilage in vivo. Although the field has yet to fully comprehend how stiffness and mechanical loading lock the chromatin architectures in a specific state, treatment of cells with chromatin-modifying agents may one day find its way to the toolkit of the 21st century cell culturist.

ACKNOWLEDGMENTS

This work was supported by NIBIB (R21 EB028491) and NSF (DMR-2036842).

DECLARATION OF INTERESTS

The author declares no competing interests.

REFERENCES

1. Brittberg, M., A. Lindahl, ..., L. Peterson. 1994. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N. Engl. J. Med.* 331:889–895.
2. von der Mark, K., V. Gaus, ..., P. Müller. 1977. Relationship between cell shape and type of collagen synthesised as chondrocytes lose their cartilage phenotype in culture. *Nature.* 267:531–532.
3. Scott, A. K., E. Casas, ..., C. P. Neu. 2023. Mechanical memory stored through epigenetic remodeling reduces cell therapeutic potential. *Biophys. J.* <https://doi.org/10.1016/j.bpj.2023.03.004>.
4. Eyckmans, J., T. Boudou, ..., C. S. Chen. 2011. A Hitchhiker's guide to mechanobiology. *Dev. Cell.* 21:35–47.
5. Balestrini, J. L., S. Chaudhry, ..., B. Hinz. 2012. The mechanical memory of lung myofibroblasts. *Integr. Biol.* 4:410–421.
6. Yang, C., M. W. Tibbitt, ..., K. S. Anseth. 2014. Mechanical memory and dosing influence stem cell fate. *Nat. Mater.* 13:645–652.
7. Wei, D., A. Liu, ..., H. Fan. 2020. Mechanics-controlled dynamic cell Niche guided osteogenic differentiation of stem cells via preserved cellular mechanical memory. *ACS Appl. Mater. Interfaces.* 12:260–274.
8. Li, C. X., N. P. Talele, ..., B. Hinz. 2017. MicroRNA-21 preserves the fibrotic mechanical memory of mesenchymal stem cells. *Nat. Mater.* 16:379–389.
9. Nasrollahi, S., C. Walter, ..., A. Pathak. 2017. Past matrix stiffness primes epithelial cells and regulates their future collective migration through a mechanical memory. *Biomaterials.* 146:146–155.
10. Heo, S. J., S. D. Thorpe, ..., R. L. Mauck. 2015. Biophysical regulation of chromatin architecture instills a mechanical memory in mesenchymal stem cells. *Sci. Rep.* 5:16895–16914.
11. Killaars, A. R., J. C. Grim, ..., K. S. Anseth. 2019. Extended exposure to stiff microenvironments leads to persistent chromatin remodeling in human mesenchymal stem cells. *In Transactions of the Annual Meeting of the Society for Biomaterials and the Annual International Biomaterials Symposium*, p. 670.
12. Schuh, E., J. Kramer, ..., N. Rotter. 2010. Effect of matrix elasticity on the maintenance of the chondrogenic phenotype. *Tissue Eng. Part A.* 16:1281–1290.