physiological differentiation patterns and germ-layer organization.

Irrespective of the underlying mechanism, the finding of nuclear auxeticity for ESCs in the metastable T state represents a significant step towards the understanding of the nucleus as a material. The work of Chalut and colleagues will facilitate future exploration of nuclear mechanics and mechanotransduction, chromatin structure and organization, transcription-factor activity, gene regulation and, ultimately, cellular functions. ❐

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STEM CELL DIFFERENTIATION

Sticky mechanical memory

Physical cues from the extracellular environment influence the lineage commitment of stem cells. Now, experiments on human mesenchymal stem cells cultured on photodegradable hydrogels show that the cells' fate can also be determined by past physical environments.

Jeroen Eyckmans and Christopher S. Chen

Scientists have been fascinated for decades by the ability to drive differentiation of pluripotent or multipotent stem cells down Waddington's epigenetic landscape — a conceptual framework that explains how genes interact with their surroundings to produce a cell's phenotype. Major efforts have identified a number of soluble biochemical factors that control stem cell plasticity and differentiation. More recently, it has become evident that differentiation is also governed by physical cues (such as substrate stiffness[1], matrix anchoring[2] and cell shape[3]) that the cells sense in their culture environment. Writing in *Nature Materials*, Kristi Anseth and colleagues now show that lineage-commitment decisions of human mesenchymal stem cells (hMSCs) in response to soluble cues are also influenced by past mechanical environments[4].

Anseth and co-authors adapted a classical differentiation assay in which primary-bone-marrow-derived hMSCs cultured on substrates with different bulk stiffness were treated in a mixed osteogenic and adipogenic differentiation medium[3]. Under these culture conditions, hMSCs adherent to soft substrates (Young's modulus $E < 2$ kPa) typically commit to the adipogenic lineage, whereas those grown on stiff substrates ($E > 10$ kPa) prefer to differentiate into osteoblasts. The authors first added hMSCs to tissue culture polystyrene (TCPS, E ~3 GPa) for 10 days, and then transferred the cells to soft substrates before exposing them to mixed (osteogenic and adipogenic) media. Surprisingly, the cells spread on the soft substrates and stained positive for alkaline phosphatase (ALP), an early osteoblast marker. The authors then assessed the nuclear translocation of transcription factors known to be important for osteogenic differentiation[5], namely yes associated protein (YAP), transcriptional co-activator with PDZ binding domain (TAZ), and Runt-related transcription factor 2 (RUNX2). Even in the absence of differentiation factors, these transcription factors exhibited persistent activation (nuclear localization); indeed, the longer the cells had adhered to stiff surfaces before they were transferred to soft environments, the larger the number of cells with YAP/TAZ and RUNX2 in the nucleus, a situation that was maintained even when the actin cytoskeleton was temporarily disrupted with the small molecule latrunculin A. These experiments suggested that hMSCs retain mechanical memory of the stiffness of the substrates they have been cultured on.

Still, the enzymatic transfer of cells from TCPS to compliant substrates can introduce potential artefacts. To circumvent these, Anseth and co-authors took advantage of a previously developed photodegradable poly(ethylene glycol) (PEG) hydrogel[6] because it could be softened *in situ* by controlled exposure to ultraviolet light. This allowed the authors to expose hMSCs to temporal changes in stiffness (that is, to mechanically dose them; Fig. 1a) while the cells remained adhered to the substrate. These experiments proved that mechanical memory has even greater persistence: after softening for 10 days, YAP and RUNX2 remained for 10 additional days in the nucleus of the cells that had been conditioned on the initially stiff photodegradable hydrogels (Fig. 1b).

Moreover, Anseth and colleagues observed that, in their assays, RUNX2 transcription was dependent on YAP signalling, which led them to propose that YAP/TAZ may act as a mechanical rheostat that tempers cellular responses to dynamically changing mechanical microenvironments. Interestingly, mechanical pre-exposure of hMSCs to soft substrates did not prevent the nuclear translocation of YAP or RUNX2 when plated on stiff hydrogels, suggesting the presence of a mechanism for remembering past exposure to stiff, but not soft, environments.

However, it is unclear at present what element is responsible for such mechanical memory. Is it the persistence of a cytoskeletal structure (as evidenced by the persistent cell spreading), a downstream mechanotransduction process, or simply a long-lived signalling factor? Other classical mechanisms for stabilizing phenotype, such as epigenetic or genomic modifications found in the development of memory T-cells, or positive feedback in transcriptional networks[7], could also be at play.

The appreciation of the role of mechanics in stem cell differentiation has already fuelled practical applications. For example,

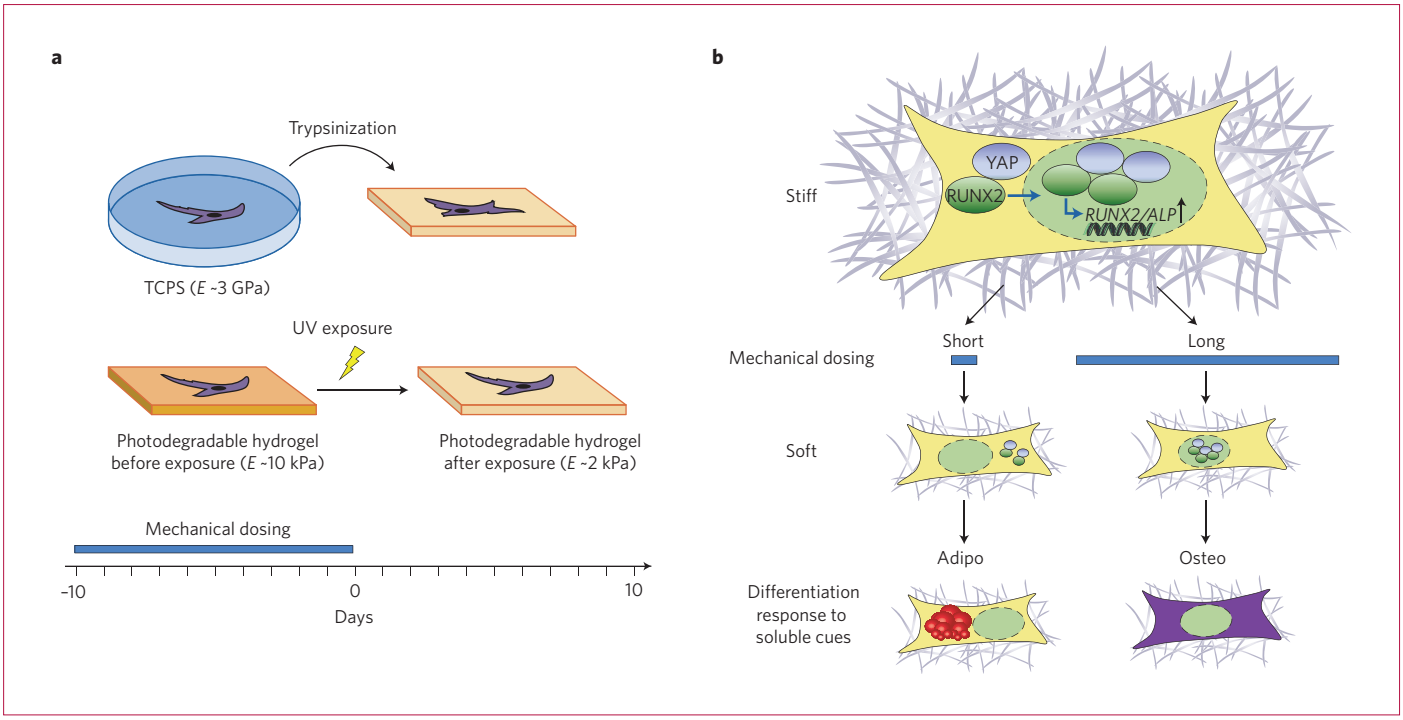

Figure 1 | Mechanical memory influences the fate of human mesenchymal stem cells[4]. **a**, hMSCs were seeded on stiff substrates (TCPS, tissue culture polystyrene) or photodegradable poly(ethylene glycol) hydrogels, and cultured in growth medium for different time spans (up to 10 days of mechanical dosing) before they were transferred to poly(ethylene glycol) hydrogels or the hydrogel was softened *in situ* by exposing it to ultraviolet (UV) light, respectively. **b**, hMSCs grown on stiff substrates display nuclear accumulation of the transcription factors YAP and RUNX2, which upregulate the transcription of the *RUNX2* and *ALP* genes. Depending on the previous culture time (mechanical dosing) on stiff substrates, YAP/RUNX2 in the hMSCs on the soft substrates either translocate to the cytoplasm (short duration) or persist in the nucleus (long duration), which determines the differentiation outcome in response to mixed osteogenic/adipogenic media, as indicated by Oil-Red-O-stained lipid droplets (red) and ALP-stained cells (purple).

altering substrate mechanics has been essential for expanding significant numbers of muscle satellite cells[8] and hematopoietic stem cells[9] that retained their function when implanted *in vivo*. The recognition that mechanical memory and dosing also matters is likely to further improve the engineering of cell fates. Tailoring dynamic substrate mechanics to 'erase' or 'trick' the memory may better facilitate differentiation or maintain stem cells in an undifferentiated state. Most likely, (photo)tunable hydrogels, as showcased in Anseth and co-workers' work, will play a prominent role in this endeavour. ❐

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STEM CELL DIFFERENTIATION

Yielding substrates for neurons

Soft culture substrates improve the yield of functional motor neurons derived from human pluripotent stem cells.

Emily Rhodes Lowry and Christopher E. Henderson

The surfaces of most tissues in mammalian organisms are textured and flexible. Yet in the laboratory smooth and rigid surfaces such as glass or plastic are widely used for cell culture. It is thus hardly surprising that cells on a dish often exhibit strikingly different behaviour compared with when *in vivo*. Now, Jianping Fu and colleagues report in *Nature Materials* that soft, micropatterned substrates significantly increase the yield of functional motor neurons (spinal cord cells that innervate muscles and initiate movement) differentiated from cultured human pluripotent stem cells (hPSCs) when compared with those cultured in the same differentiation conditions but on standard culture dishes[1].