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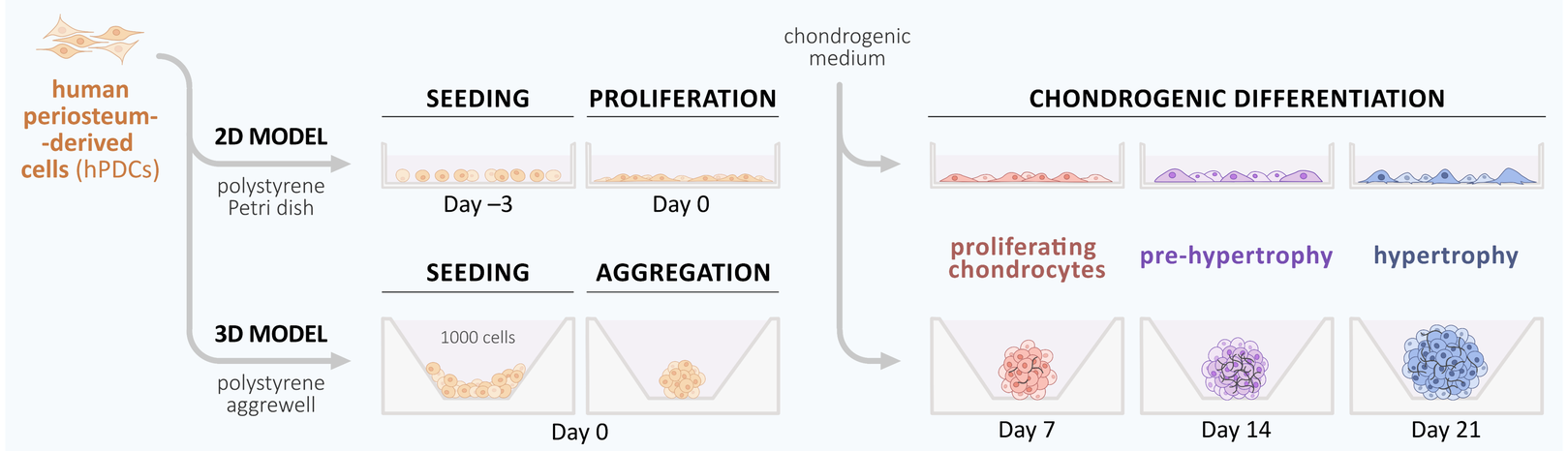
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From cells to living materials: dynamic mechanical and morphological changes of cartilaginous spheroids in 3D culture

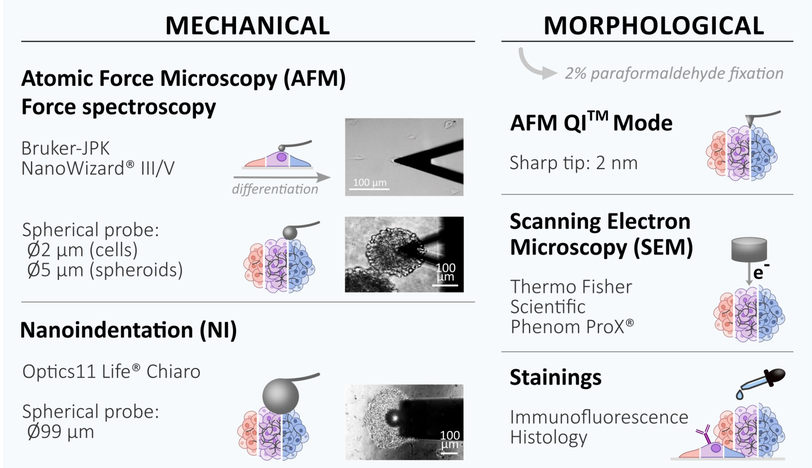


During healing, progenitor cells differentiate into chondrocytes, forming cartilage templates that are further remodelled into bone. Nowadays, tissue engineering aims to implement these developmental strategies using self-organised microtissues as living building blocks [1]. As cells undergo chondrogenic differentiation, the microenvironment within 3D cell-based spheroids evolves dynamically. Extracellular matrix (ECM) deposition and cytoskeletal reorganisation drive changes in cell behaviour and the mechanical properties of the constructs, highlighting the transition from individual cells to tissue-like materials [2-3]. In this work, we investigate the mechanical and morphological changes of differentiating human periosteum-derived cells (hPDCs) in both 2D (monolayer) and 3D (spheroid) culture systems.

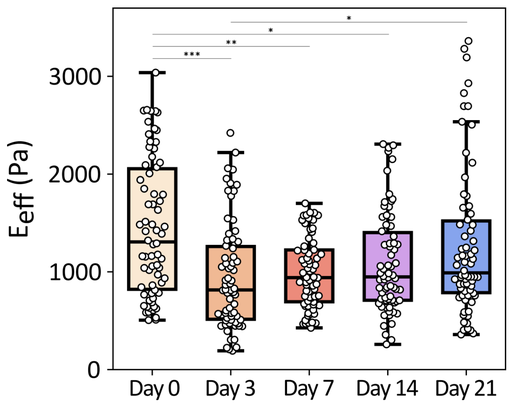
2D and 3D cell culture



Characterisation toolkit



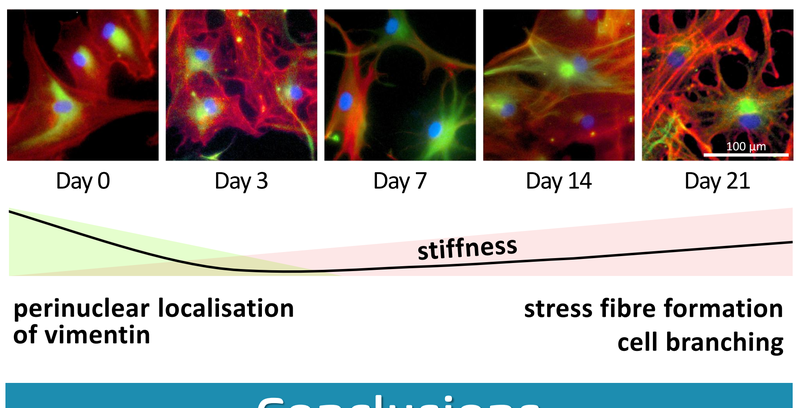
2D: hPDCs



↤ Mechanical changes of hPDCs are driven by dynamic reorganisation of the cytoskeleton

In 2D, softening at Day 3 is followed by a progressive stiffening through Day 21. The initial softening correlates with the relaxation of vimentin bundles, which are prominent at Day 0, but redistributed as the cells begin to differentiate. The formation of actin stress fibres provides a structural reinforcement.

Immunofluorescence staining
Vimentin / Actin / DAPI

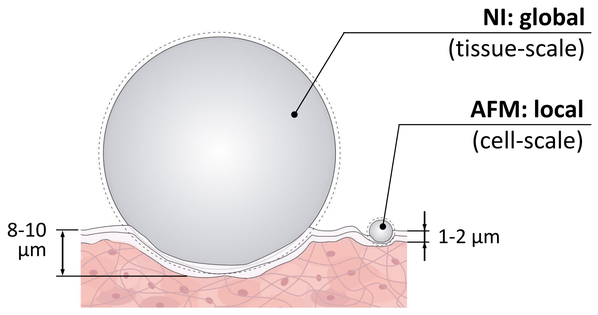


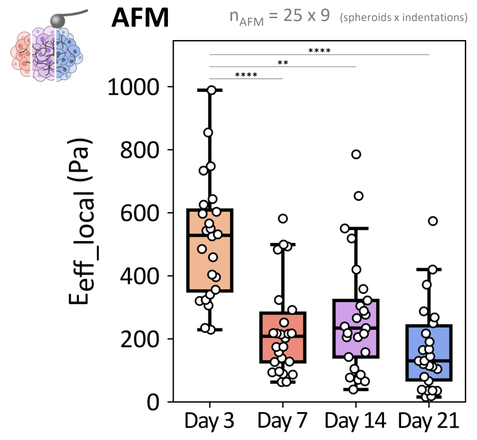
perinuclear localisation of vimentin

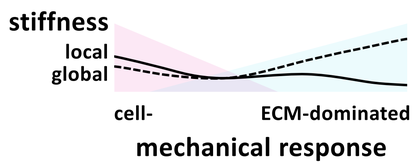
stress fibre formation cell branching

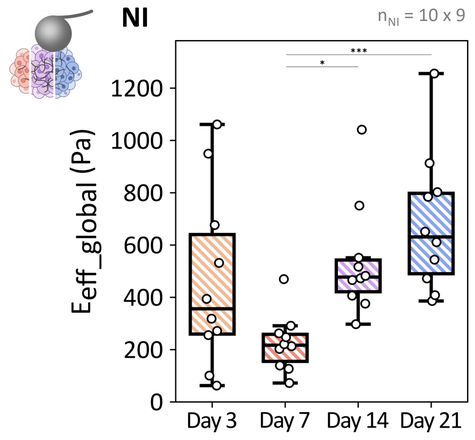
3D: hPDC-based spheroids

AFM and NI indentation can reveal scale-dependent mechanical changes. While AFM probes the surface of the aggregates, NI indents deeper, allowing to assess the stiffness of their core, as it changes over time with increasing ECM deposition.







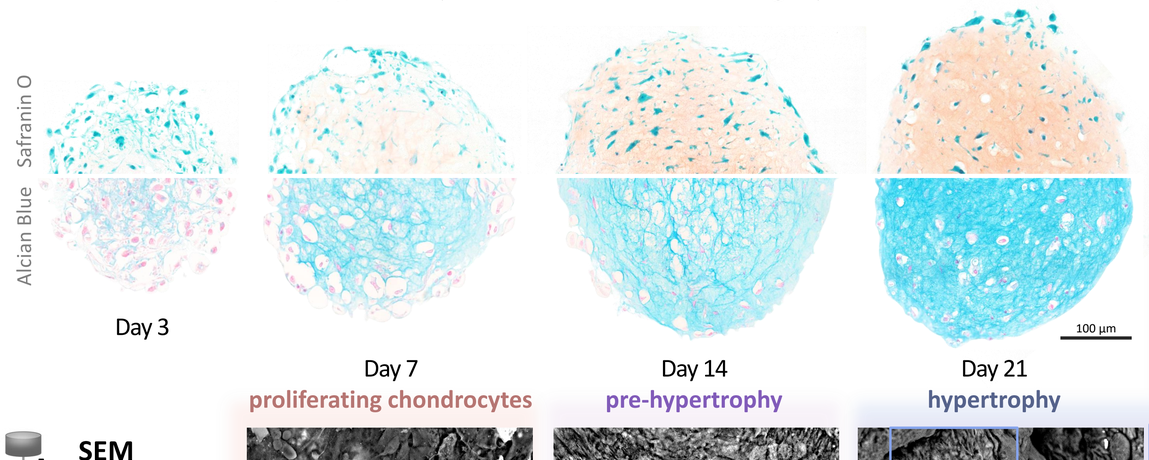


↖ **Surface properties:** cytoskeletal remodeling driven by cell differentiation

↗ **Tissue-scale properties:** ECM-driven progressive maturation

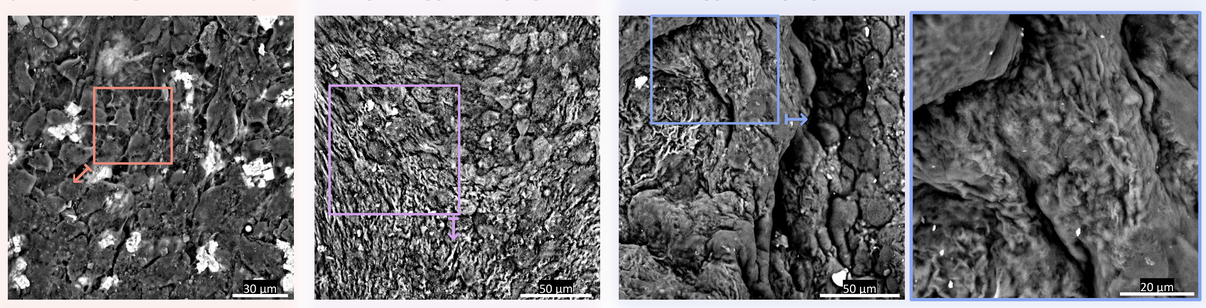
Mechanical properties of spheroids can be directly linked to changes in their morphology. As the aggregates mature, cells develop a rich extracellular matrix (ECM), which provides the structural integrity and influences their local and global properties.

Histological staining

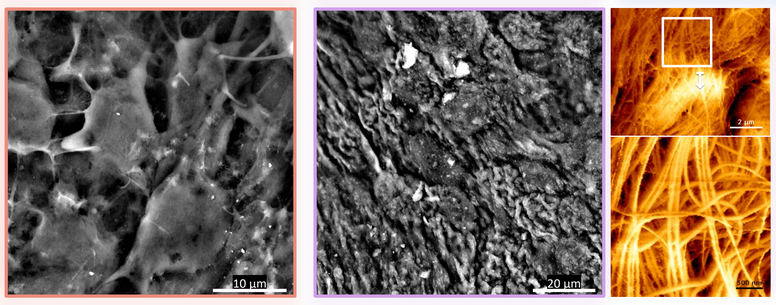


Upon hypertrophy, the secreted ECM becomes densely packed (Col X, XI), resulting in an increase in the overall stiffness of the construct.

SEM
non-coated
non-dehydrated



While proliferating, hPDCs start to secrete ECM components, such as collagens type I, II, VI, and proteoglycans, as aggrecan [4-6].



AFM
↤

In the pre-hypertrophic stage, irregular fibrous networks, formed by Col I and II, are present on the surface of the aggregates [5-6].

Conclusions

- Remodelling of actin and vimentin networks serves as the primary driver for the changing material properties of hPDCs [4] and governs the mechanical response of spheroids during early-stage differentiation.
- During differentiation, spheroids develop a hetero-geneous, zonated structure: a stiffer core for load-bearing (dense, fibrous ECM) and a softer, hydrated outer layer (cells embedded within a GAG-rich, amorphous ECM).
- The 3D spheroid model promotes ECM deposition and provides a tissue-like microenvironment, that may encourage cells to reach hypertrophy at an earlier stage.

References

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