Emergence of Tissue Organization of Multi-Organoid Cartilage Constructs

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INTRODUCTION

Articular cartilage has a limited capacity for self-repair, motivating the development of regenerative strategies that can restore the native tissue structure and function. Cartilage organoids have emerged as promising tissue-engineering building blocks due to their intrinsic self-assembly and incorporation of a native-like extracellular matrix (ECM)[1]. However, creating larger functional tissue constructs for clinical application requires a deeper understanding of how individual organoids, with a distinctly isotropic organization, fuse and mature into cohesive tissues. Therefore, this study aims to elucidate **spatiotemporal changes in ECM organization** of organoid-based cartilage tissue constructs and to explore strategies to actively guide collagen organization using **boundary constraints**.

METHODS

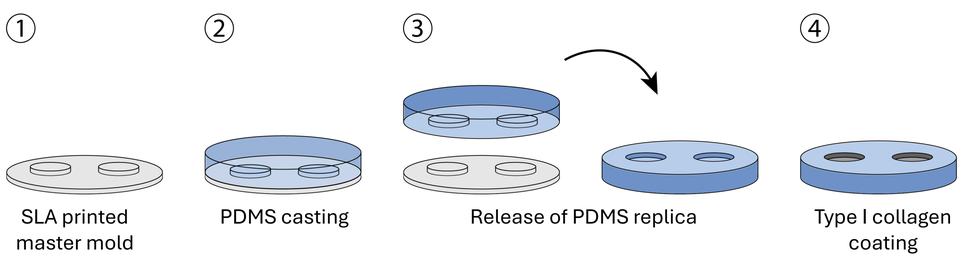


Figure 1 Mini-well arrays were fabricated by casting polydimethylsiloxane (PDMS) with stereolithography (SLA)-printed molds. PDMS walls were functionalized with type I collagen.

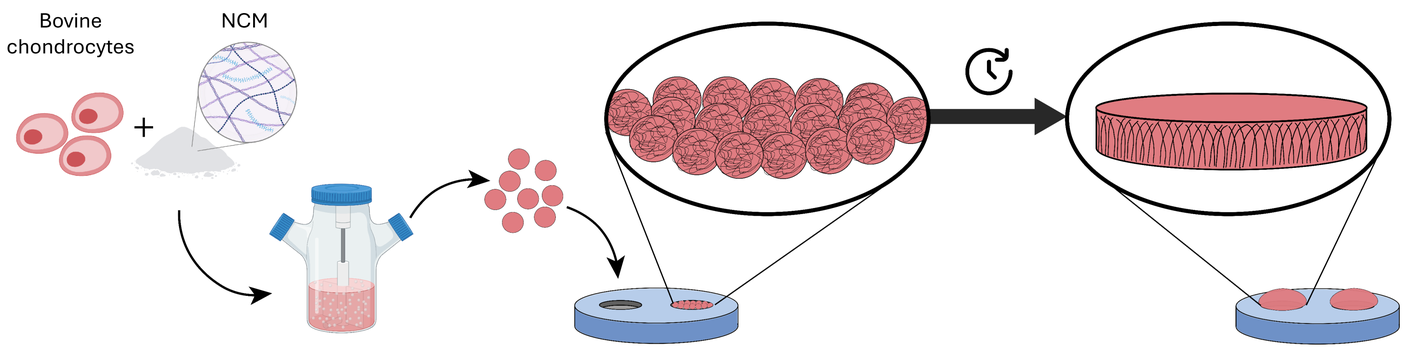


Figure 2 Cartilage organoids[2] were created from bovine chondrocytes and ECM-rich notochordal cell-derived matrix (NCM) and seeded into the mini-wells as a single layer. The disorganized organoids were allowed to fuse and create cohesive cartilage tissue constructs for up to 6 weeks. ECM production and organization were analyzed at several timepoints, using histological stainings and quantitative polarized light microscopy.

RESULTS

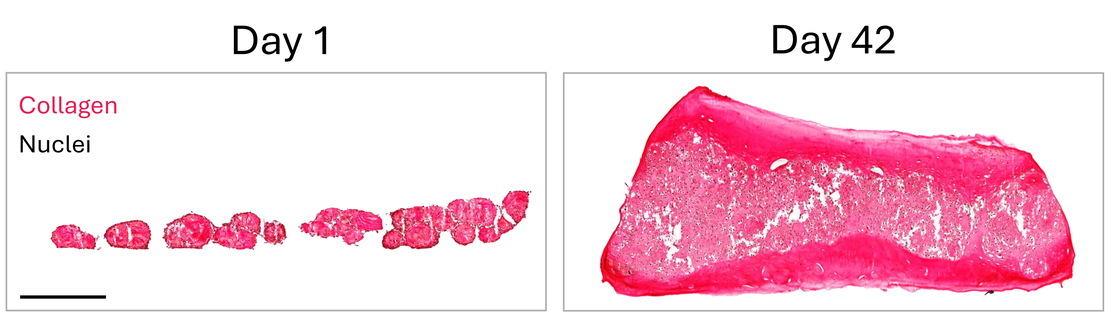


Figure 3 Cartilage organoids have a dense isotropic collagen network at day 1, but when allowed to fuse and grow for 42 days, cohesive cartilage tissues were formed. The tissues show a collagen distribution that is highly organized. Scale bar = 500 µm.

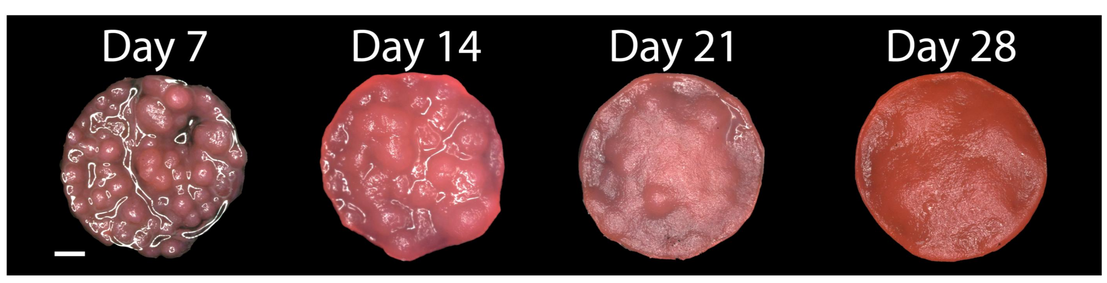


Figure 4 Over time, the top surface of the tissue constructs progressively smoothens and the individual organoids cannot be distinguished. Tissues were stained with eosin. Scale bar = 500 µm.

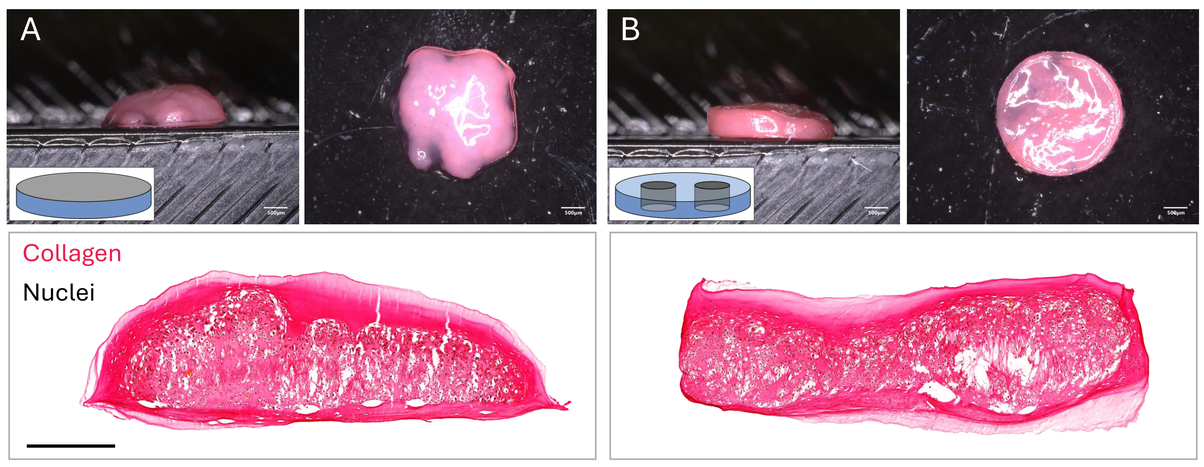
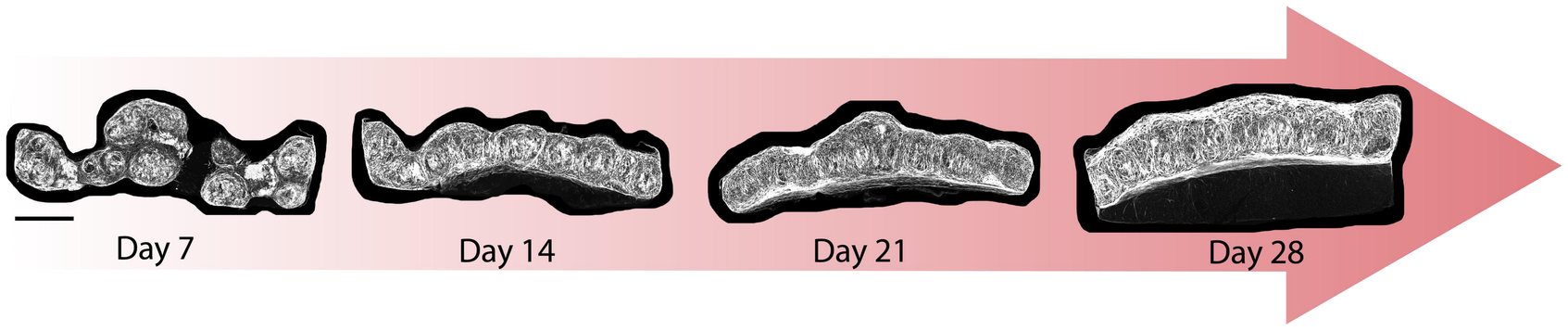


Figure 6 By varying the boundary constraints provided to the tissues, the morphology of the resulting constructs after 42 days was altered. Constructs cultured within defined walls (B) conform to the well geometry, resulting in a flat top surface. In contrast, constructs cultured without side boundaries (A) exhibit a dome-shaped top surface, and their planar morphology is largely determined by the initial shape of the seeded organoids. In both culture conditions, the construct consists of a core formed by the original organoids, surrounded by a collagen-rich layer at the top and bottom surfaces. Scale bar = 500 µm.

Figure 5 During the initial stags of fusion, new ECM forms in between the original organoids. With time, the organoid edges become less distinct, and the tissue re-organizes into a highly aligned network. Collagen fibers, as visualized by polarized light microscopy, in the core re-orient to obtain a preferred orientation perpendicular to the tissue surface. Fibers on the periphery commonly align along that surface. Scale bar = 500 µm.



CONCLUSIONS & OUTLOOK

- Dense and ECM-rich cartilage organoids fuse into uniform larger tissue constructs.
- Over time, collagen fiber network re-organizes from an isotropic to an anisotropic collagen organization and interfaces between organoids become less distinct.
- Boundary constraints can direct tissue surface morphology.
- Ongoing work is focused on both early and long-term growth behavior to deepen our understanding of how organoid building blocks assemble into mature cartilage tissues.



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REFERENCES

[1] J.R. Peters et al. *Sci. Rep., 2024.*

[2] J. F. Crispim and K. Ito. *Acta Biomater., 2021*

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