Human cartilage organoids and beyond

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In recent years, the field of organoid research has undergone a remarkable transformation, heralding a new era in biomedical science.[1,2] This progress is exemplified by the development of an array of sophisticated organoid models, including intestinal,[3-5] brain,[6,7] liver,[8,9] and osteo-callus[10] systems. These sophisticated *in vitro* systems have proven invaluable across a spectrum of applications, from drug screening and toxicology evaluation to unraveling disease mechanisms and advancing tissue regeneration studies. A groundbreaking study, recently published in *Cell Stem Cell*, has further expanded the frontiers of this field, particularly in the realm of cartilage regeneration.[11] The study, titled "A human organoid drug screen identifies α2-adrenergic receptor signaling as a therapeutic target for cartilage regeneration", was conducted by Wei et al.[11] from Southern Medical University. The researchers ingeniously developed a novel cartilage organoid system derived from human expanded pluripotent stem cells (hEPSCs). This system incorporates a dual-reporter mechanism, utilising type II collagen (COL2A1)-mCherry and type X collagen (COL10A1)-enhanced green fluorescent protein (eGFP), enabling real-time monitoring of chondrogenesis and hypertrophy processes.

Leveraging this innovative tool, the researchers implemented a meticulous two-stage screening process to analyse a comprehensive library of 2040 U.S. Food and Drug Administration-approved drugs. This rigorous approach led to the identification of a class of α-adrenergic receptor antagonists, with phentolamine emerging as a particularly promising candidate (**Figure 1A**). To validate their findings, the researchers employed a multi-faceted approach, utilizing an array of advanced models and techniques. These included mouse and minipig microfracture models, human xenograft implantation experiments, and human articular cartilage explant cultures. Through these comprehensive studies, they convincingly demonstrated phentolamine's dual capacity to enhance chondrocyte differentiation while simultaneously preventing hypertrophic differentiation.

In this groundbreaking study, the researchers combined two-dimensional and three-dimensional culture techniques to construct highly sophisticated cartilage organoids. These organoids demonstrated a remarkable capacity to mimic the complex developmental processes of natural cartilage formation. The temporal progression of the organoids' development was meticulously monitored. A significant milestone was observed at day 28 of culture, marked by the emergence of eGFP fluorescence. This fluorescent signal heralded the onset of hypertrophic differentiation within the cartilage organoids. By day 42, the researchers noted that approximately 21% of cells within the organoids expressed COL10A1, a key marker of hypertrophic chondrocytes.

Further analysis of the 42-day cultured cartilage organoids using immunofluorescence staining revealed a fascinating zonal architecture, reminiscent of native cartilage structure: 1) The hypertrophic zone exhibited the highest concentration of COL10A1$^+$ cells; 2) The middle zone was characterised by a predominance of COL2A1$^+$ cells; 3) The deep zone displayed intense labeling for aggrecan and SRY-box transcription factor 9 (SOX9), crucial components of cartilage extracellular matrix and chondrogenic transcription factor, respectively. This zonal distribution, illustrated in **Figure 1B**, underscores the organoids' ability to recapitulate the complex spatial organisation of natural cartilage tissue. Delving into the mechanistic underpinnings of their findings, the researchers made a crucial discovery: phentolamine, the α-adrenergic receptor antagonist identified in their drug screen, exerts its chondroprotective effects by inhibiting the production of secretory leukocyte protease inhibitor. This mechanism effectively prevents hypertrophic degeneration in the cartilage organoids.

The clinical implications of this study are particularly exciting. Phentolamine, being

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an already approved drug, presents a prime candidate for repurposing in cartilage regeneration therapies. This aspect significantly enhances the translational potential of the study, offering a streamlined pathway to clinical application with reduced development costs and risks. This comprehensive approach, combining innovative organoid technology with drug repurposing strategies, not only advances our understanding of cartilage biology but also paves the way for novel therapeutic interventions in cartilage-related disorders.

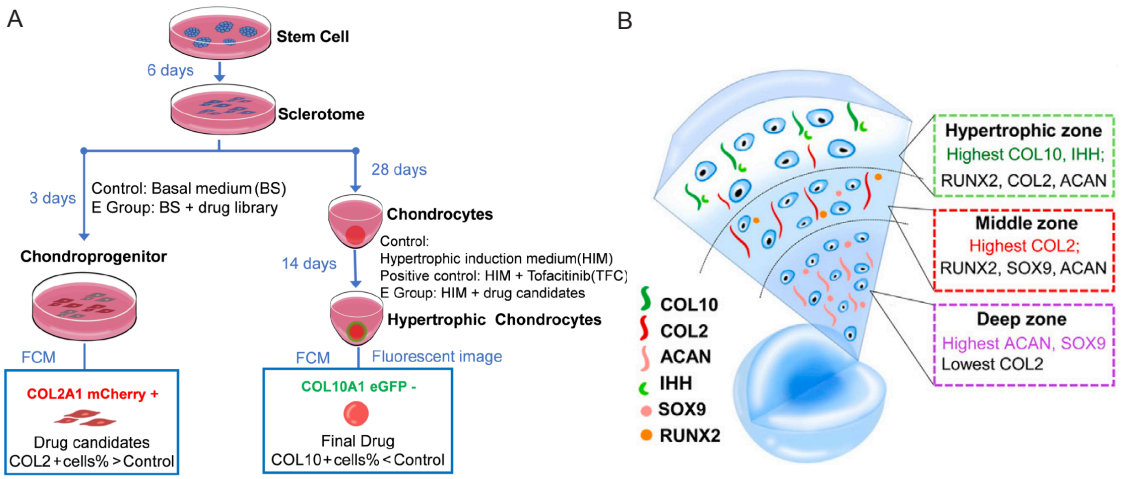

Figure 1. High-throughput screening strategy based on human cartilage organoids and schematic representation of the typical structure of cartilage organoids. (A) Flow diagram of the two-step high-throughput screening strategy. hEPSCs are cultured in two-dimensional for 6 days to induce sclerotome cell formation. The primary screening continues in two-dimensional mode, culturing sclerotome cells for an additional 3 days. Drug candidates promoting chondrogenesis are identified based on the increased proportion of COL2-mCherry$^+$ cells. The secondary screening transitions to three-dimensional culture, where sclerotome cells are grown for 28 days to establish cartilage organoids. These organoids are then cultured for an additional 14 days, during which the final drug candidates are selected based on their ability to reduce the proportion of COL10-eGFP$^+$ cells. (B) The cartilage organoid exhibits a distinct zonal distribution, comprising three layers with diverse cellular compositions. The outermost layer, designated as the hypertrophic zone, is characterised by COL10A1$^+$ cells, which predominantly co-localise with IHH expression. The middle zone contains the highest concentration of COL2A1$^+$ cells. The deep zone is distinguished by intense labelling of ACAN and SOX9. Reprinted from Wei et al.[11] under exclusive licence to Elsevier Inc. ACAN: aggrecan; BS: basal medium; COL: collagen; eGFP: enhanced green fluorescent protein; FCM: flow cytometry; hEPSCs: human expanded pluripotent stem cells; HIM: hypertrophic induction medium; IHH: Indian hedgehog; RUNX2: Runt-related transcription factor 2; TFC: tofacitinib.

The innovative double-reporter cartilage organoid model developed in this study has demonstrated remarkable potential in drug screening. However, there are several avenues for further enhancement and expansion of this groundbreaking technology:

1) Automation and high-content imaging: Future iterations of this model could benefit from increased automation, particularly through the integration of high-content imaging systems. This advancement would allow for automatic quantification of fluorescence signal changes and the proportion of specific cartilage subtypes, superseding the current flow cytometry techniques. Such improvements could significantly accelerate the identification of compounds that promote the formation of specific cartilage subtypes, thereby expediting the drug discovery process.

2) Mechanical stimulation integration: Given the crucial role of mechanical stress in cartilage tissue function *in vivo*, future cartilage organoid models could incorporate mechanical stimulation devices. This integration would better simulate the physiological conditions of cartilage development and regeneration, providing a more accurate representation of *in vivo* processes.

3) Multifactorial evaluation platform: The versatility of this system could be further exploited to evaluate the effects of various factors on cartilage regeneration, including: different growth factor combinations, biomaterial scaffolds, and physical stimuli (e.g., electromagnetic signals, electrical signals, oxygen concentration). This comprehensive approach would provide invaluable insights into the complex interplay of factors influencing cartilage development and regeneration.

4) Osteoarthritis modelling: By introducing inflammatory factors into the organoid system, researchers could observe chondrocyte responses and matrix degradation processes, effectively simulating the development of osteoarthritis. This application would create a powerful platform for screening potential anti-osteoarthritis drugs, addressing a significant unmet medical need.

5) Genetic disease modelling: Leveraging advanced gene

editing technologies such as CRISPR-Cas9, mutations associated with hereditary cartilage diseases (e.g., osteogenesis imperfecta, chondrodysplasia) could be introduced into the cartilage organoids. This approach would allow for detailed observation of how these mutations affect cartilage formation and hypertrophy, providing unprecedented insights into disease mechanisms and potentially guiding the development of novel therapeutic strategies.

These proposed enhancements to the cartilage organoid model represent exciting frontiers in cartilage research. By expanding the capabilities and applications of this system, researchers can gain deeper understanding of cartilage biology, disease processes, and potential therapeutic interventions. This multifaceted approach holds the promise of accelerating drug discovery, improving our understanding of genetic cartilage disorders, and ultimately leading to more effective treatments for a wide range of cartilage-related conditions.

The groundbreaking strategy of constructing customised double-reporter organoid models, as demonstrated in the cartilage study, opens up a vast array of possibilities for tissue and organ system research. This approach has the potential to revolutionise developmental biology and regenerative medicine by providing powerful, real-time visualisation tools for complex biological processes. By extending this strategy to various organ systems, researchers can gain unprecedented insights, accelerate drug discovery, and enhance our understanding of human development and function.

In brain research, engineering hEPSCs with neuron-specific markers (e.g., MAP2-mCherry) and glial cell markers (e.g., GFAP-eGFP) can establish sophisticated brain organoid models. These models would offer invaluable insights into neurodevelopmental processes, mechanisms underlying neurodegenerative disorders, and the pathophysiology of psychiatric conditions. For liver studies, constructing hEPSCs with hepatocyte markers (e.g., ALB-mCherry) and bile duct cell markers (e.g., CK19-eGFP) would enable the creation of advanced liver organoid models. These would be crucial for studying liver development and regeneration, investigating mechanisms of metabolic diseases, and conducting high-throughput drug toxicity testing.

Developing hEPSCs with pancreatic β-cell markers (e.g., INS-mCherry) and α-cell markers (e.g., GCG-eGFP) would facilitate the establishment of sophisticated pancreatic islet organoids. These models would be instrumental in elucidating islet development processes, simulating type 1 and type 2 diabetes pathogenesis, and screening drugs that promote β-cell regeneration or functional recovery. In the field of respiratory research, engineering hEPSCs with alveolar epithelial cell markers (e.g., SPC-mCherry) and tracheal epithelial cell markers (e.g., FOXJ1-eGFP) would enable the creation of advanced lung organoid models, crucial for studying lung development and regeneration, investigating mechanisms of chronic obstructive pulmonary disease, and exploring the pathogenesis of pulmonary fibrosis.

The integration of multiple organoid models presents an exciting opportunity to create "human-on-a-chip" systems. These complex models would allow researchers to study interactions between different organ systems, investigate regulatory mechanisms of the neuro-endocrine-immune axis, and evaluate systemic effects of drugs and environmental factors. This approach could revolutionise our understanding of human physiology and drug development processes.

The potential of these dual- or multi-colour reporter systems extends far beyond the examples provided. They offer a platform for numerous technological developments and pathogenesis research opportunities, including studying cellular plasticity in development and disease, investigating cell-cell interactions in complex tissue environments, real-time monitoring of cellular responses to various stimuli or interventions, and high-throughput screening of compounds affecting specific cell populations. In conclusion, the extension of this double-reporter organoid strategy to various organ systems represents a paradigm shift in biomedical research, promising to accelerate our understanding of human development, disease mechanisms, and drug responses.

Despite the remarkable potential of organoid technology in elucidating disease mechanisms and screening therapeutic drugs, several aspects require further refinement to achieve effective simulation of physiological and pathological environments. A primary challenge lies in establishing high-throughput protocols for generating organoids with consistent batch quality to ensure experimental reproducibility, particularly when modelling complex structures such as brain tissue. Insufficient vascularisation in organoids directly constrains their size and complexity. Although Wei et al.[11] ingeniously circumvented this limitation in their cartilage organoid model, as cartilage is naturally avascular, the majority of organoid systems face significant challenges due to limited nutrient and oxygen diffusion, potentially compromising their functionality. Furthermore, organoids typically lack immune components and inter-organ interactions, limiting their utility in modelling systemic diseases or immune responses. The maintenance of phenotypic stability during passaging and long-term culture presents additional challenges.

Recent advances in precision cancer therapy have yielded promising developments. Yin et al.[12, 13] developed a matrix-free patient-derived tumour-like cell cluster (PTC) based on primary dissociated tumour tissues, capable of recapitulating the tumour microenvironment. This is a special type of patient-derived organoid that retains epithelial, fibroblast, and immune cells from the original tumour, capable of self-assembly under optimised culture conditions. The PTC technology enables rapid generation of micro-tumour models for various cancers, including gastric, intestinal, breast and lung malignancies, eliminating the need for matrigel and immunodeficient animals. Clinical trials demonstrated predictive efficacy rates of 93%[12] and 89%[13] for drug responses. However, despite incorporating immune cells, PTCs exhibit limited immune cell diversity, notably lacking T cells. Similarly, these PTCs also

lack endothelial cells. These limitations constrain their utility in predicting responses to immunotherapy or angiogenesis-targeted interventions.

In comparison to two-dimensional cultures, organoids recapitulate complex three-dimensional tissue architecture and cell-cell interactions more effectively, yet they incur significantly higher cultivation costs. Compared to animal models, human-derived organoids offer reduced temporal and financial investments. However, organoids lack the systemic context provided by animal models, including immune responses and inter-organ interactions. Instead of serving as a replacement for existing models, organoids should be regarded as a complementary tool in the research process, bridging the gap between simple *in vitro* systems and complex *in vivo* models. The specific choice of model for research should fully consider the purpose and objectives of the study to balance research accuracy and costs, including time and financial expenses, rather than blindly employing organoid technology.

Regarding the use of human stem cells, particularly embryos, in organoid research, ethical considerations should receive sufficient attention. As organoid technology advances, enabling the construction of increasingly biomimetic structures and functions, particularly in cerebral organoids, questions regarding consciousness and sentience become pertinent. These emerging challenges necessitate the development of more precise ethical guidelines through collaborative efforts between scientists, ethicists and regulatory authorities across different nations.

Author contributions

Both authors contributed to conceptualising, writing, reviewing, editing and proofing the manuscript, and approved the final version of the manuscript.

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Conflicts of interest statement

The authors declare no conflicts of interest.

1. Li, M.; Izpisua Belmonte, J. C. Organoids - preclinical models of human disease. *N Engl J Med.* **2019,** *380*, 569-579.
2. Han, X.; Cai, C.; Deng, W.; Shi, Y.; Li, L.; Wang, C.; Zhang, J.; Rong, M.; Liu, J.; Fang, B.; He, H.; Liu, X.; Deng, C.; He, X.; Cao, X. Landscape of human organoids: ideal model in clinics and research. *Innovation (Camb).* **2024,** *5*, 100620.
3. Sato, T.; Vries, R. G.; Snippert, H. J.; van de Wetering, M.; Barker, N.; Stange, D. E.; van Es, J. H.; Abo, A.; Kujala, P.; Peters, P. J.; Clevers, H. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature.* **2009,** *459*, 262-265.
4. Zhang, F. L.; Hu, Z.; Wang, Y. F.; Zhang, W. J.; Zhou, B. W.; Sun, Q. S.; Lin, Z. B.; Liu, K. X. Organoids transplantation attenuates intestinal ischemia/reperfusion injury in mice through L-Malic acid-mediated M2 macrophage polarization. *Nat Commun.* **2023,** *14*, 6779.
5. Qu, M.; Xiong, L.; Lyu, Y.; Zhang, X.; Shen, J.; Guan, J.; Chai, P.; Lin, Z.; Nie, B.; Li, C.; Xu, J.; Deng, H. Establishment of intestinal organoid cultures modeling injury-associated epithelial regeneration. *Cell Res.* **2021,** *31*, 259-271.
6. Atamian, A.; Birtele, M.; Hosseini, N.; Nguyen, T.; Seth, A.; Del Dosso, A.; Paul, S.; Tedeschi, N.; Taylor, R.; Coba, M. P.; Samarasinghe, R.; Lois, C.; Quadrato, G. Human cerebellar organoids with functional Purkinje cells. *Cell Stem Cell.* **2024,** *31*, 39-51.e6.
7. Hendriks, D.; Pagliaro, A.; Andreatta, F.; Ma, Z.; van Giessen, J.; Massalini, S.; López-Iglesias, C.; van Son, G. J. F.; DeMartino, J.; Damen, J. M. A.; Zoutendijk, I.; Staliarova, N.; Bredenoord, A. L.; Holstege, F. C. P.; Peters, P. J.; Margaritis, T.; Chuva de Sousa Lopes, S.; Wu, W.; Clevers, H.; Artegiani, B. Human fetal brain self-organizes into long-term expanding organoids. *Cell.* **2024,** *187*, 712-732.e38.
8. Wang, S.; Wang, X.; Tan, Z.; Su, Y.; Liu, J.; Chang, M.; Yan, F.; Chen, J.; Chen, T.; Li, C.; Hu, J.; Wang, Y. Human ESC-derived expandable hepatic organoids enable therapeutic liver repopulation and pathophysiological modeling of alcoholic liver injury. *Cell Res.* **2019,** *29*, 1009-1026.
9. Yuan, X.; Wu, J.; Sun, Z.; Cen, J.; Shu, Y.; Wang, C.; Li, H.; Lin, D.; Zhang, K.; Wu, B.; Dhawan, A.; Zhang, L.; Hui, L. Preclinical efficacy and safety of encapsulated proliferating human hepatocyte organoids in treating liver failure. *Cell Stem Cell.* **2024,** *31*, 484-498.e5.
10. Xie, C.; Liang, R.; Ye, J.; Peng, Z.; Sun, H.; Zhu, Q.; Shen, X.; Hong, Y.; Wu, H.; Sun, W.; Yao, X.; Li, J.; Zhang, S.; Zhang, X.; Ouyang, H. High-efficient engineering of osteo-callus organoids for rapid bone regeneration within one month. *Biomaterials.* **2022,** *288*, 121741.
11. Wei, X.; Qiu, J.; Lai, R.; Wei, T.; Lin, Z.; Huang, S.; Jiang, Y.; Kuang, Z.; Zeng, H.; Gong, Y.; Xie, X.; Yang, J.; Zhang, Y.; Zhang, S.; Zou, Z.; Gao, X.; Bai, X. A human organoid drug screen identifies α2-adrenergic receptor signaling as a therapeutic target for cartilage regeneration. *Cell Stem Cell.* **2024.** doi: 10.1016/j.stem.2024.09.001.
12. Yin, S.; Xi, R.; Wu, A.; Wang, S.; Li, Y.; Wang, C.; Tang, L.; Xia, Y.; Yang, D.; Li, J.; Ye, B.; Yu, Y.; Wang, J.; Zhang, H.; Ren, F.; Zhang, Y.; Shen, D.; Wang, L.; Ying, X.; Li, Z.; Bu, Z.; Ji, X.; Gao, X.; Jia, Y.; Jia, Z.; Li, N.; Li, Z.; Ji, J. F.; Xi, J. J. Patient-derived tumor-like cell clusters for drug testing in cancer therapy. *Sci Transl Med.* **2020,** *12*, eaaz1723.
13. Yin, S.; Yu, Y.; Wu, N.; Zhuo, M.; Wang, Y.; Niu, Y.; Ni, Y.; Hu, F.; Ding, C.; Liu, H.; Cheng, X.; Peng, J.; Li, J.; He, Y.; Li, J.; Wang, J.; Zhang, H.; Zhai, X.; Liu, B.; Wang, Y.; Yan, S.; Chen, M.; Li, W.; Peng, J.; Peng, F.; Xi, R.; Ye, B.; Jiang, L.; Xi, J. J. Patient-derived tumor-like cell clusters for personalized chemo- and immunotherapies in non-small cell lung cancer. *Cell Stem Cell.* **2024,** *31*, 717-733.e8.

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