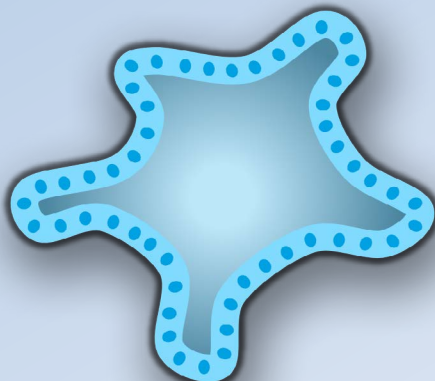




Technical Note

Optimization of Physioxic Organoid Cell Culture for Research and Drug Discovery



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Introduction

Low oxygen conditions, or hypoxia, play a significant role in the culture of organoids, which are three-dimensional, self-organizing structures derived from stem cells that mimic the architecture and function of real organs. Organoids have become invaluable tools in biomedical research and drug discovery, offering a more physiologically relevant model compared to traditional two-dimensional cell cultures. Furthermore, the NIH mandate to transition away from animal models adds further reliance on the quality of organoid and other cellular models. The significance of low oxygen in organoid culture lies in its ability to better replicate the *in vivo* microenvironment, enhance stem cell maintenance, and improve the functionality and maturation of organoids.

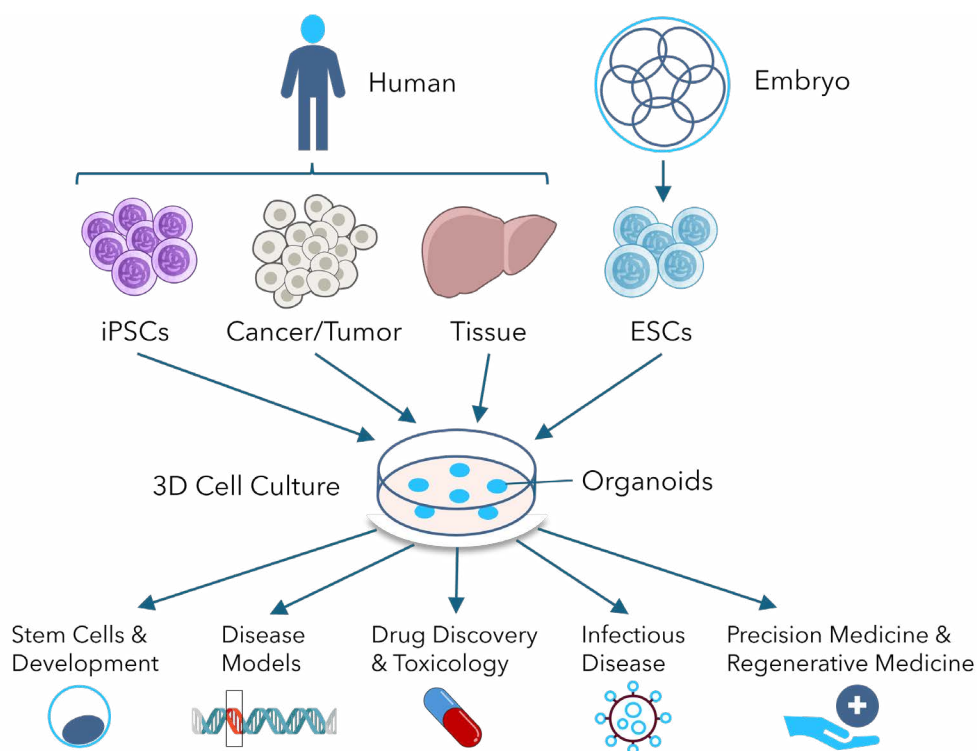


Fig. 1. A summary of organoid cell sources and applications.

Replicating the *In Vivo* Microenvironment

In the human body, oxygen levels vary significantly across different tissues and organs. For instance, the physiological oxygen (physioxia) concentration in most tissues ranges from 1% to 11%, which is much lower than the 21% oxygen typically found in ambient air. Traditional cell cultures are often maintained at atmospheric oxygen levels, which can lead to oxidative stress and altered cellular behavior. In contrast, culturing organoids under low oxygen conditions that reflect physioxia (typically 1-5% O₂) more accurately mimics the biological environment of many tissues, particularly those with high metabolic activity or limited vascularization, such as the brain, liver, and intestines. This improved mimicry enhances the relevance of organoids as models for studying human biology and disease.

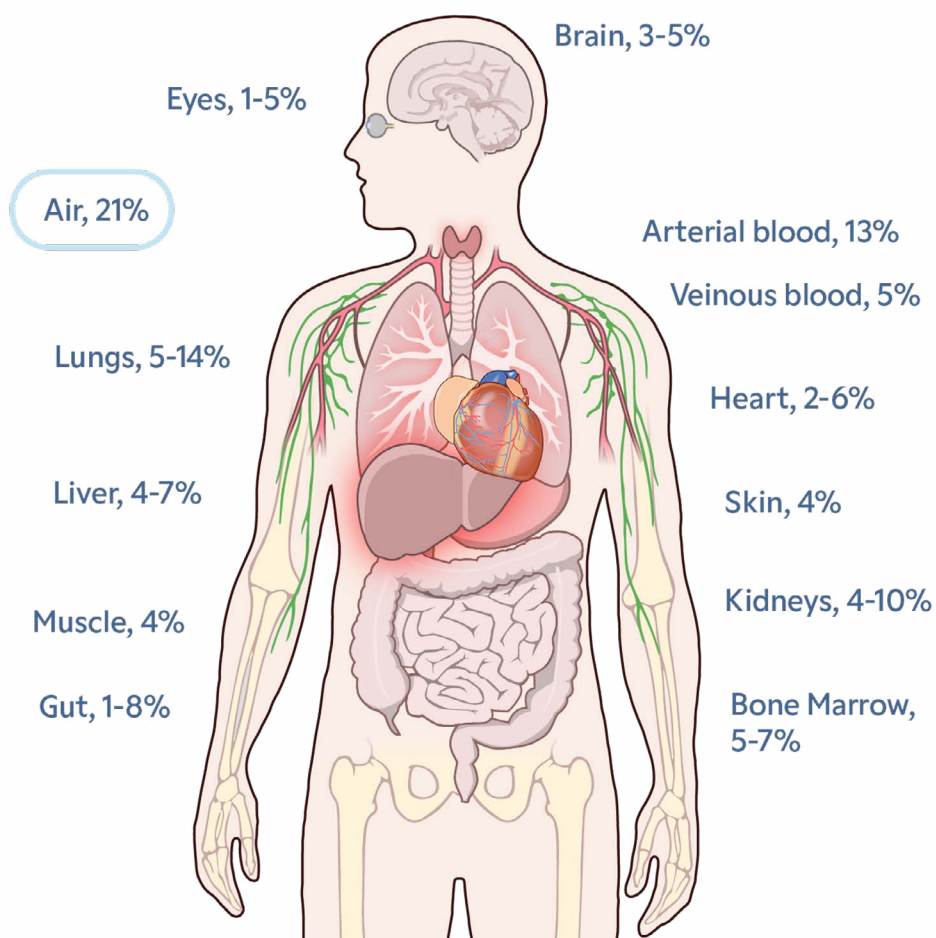


Fig. 2. Physiological oxygen levels in human organs and tissues.

Enhancing Stem Cell Maintenance and Proliferation

Low oxygen conditions are crucial for maintaining the stemness of progenitor cells within organoids. Stem cells, including pluripotent stem cells (PSCs) and adult stem cells, naturally reside in hypoxic niches *in vivo*, such as the bone marrow or intestinal crypts. Hypoxia promotes the self-renewal and proliferation of these cells by activating Hypoxia-Inducible Factors (HIFs), which regulate genes involved in cell survival, metabolism, and differentiation. By culturing organoids under low oxygen, researchers can better preserve the stem cell population, ensuring the long-term expansion and stability of organoid cultures. This is particularly important for applications requiring large-scale organoid production, such as high-throughput drug screening.

Improving Organoid Functionality and Maturation

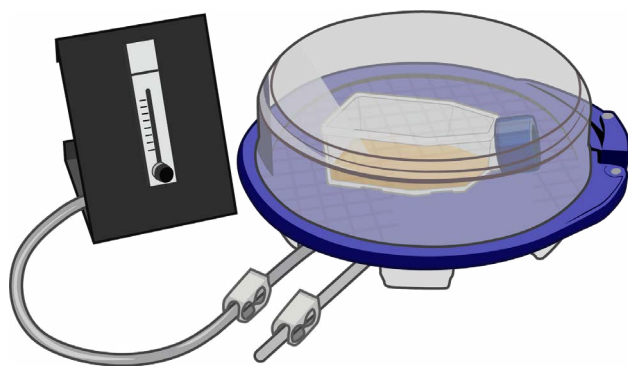
Low oxygen conditions also influence the differentiation and functionality of organoids. For example, in brain organoids, hypoxia has been shown to promote the generation of specific neuronal subtypes and improve the maturation of neural networks. Similarly, in intestinal organoids, low oxygen enhances the formation of crypt-villus structures and the expression of functional markers. These effects are mediated by HIF signaling, which modulates key developmental pathways and metabolic processes. The activity of HIFs influences hundreds of genes which would otherwise be altered by atmospheric oxygen. By optimizing oxygen levels, researchers can generate organoids that more closely resemble their *in vivo* counterparts, thereby improving their utility for modeling diseases and testing therapeutic interventions.

Applications for Disease Modeling and Drug Discovery

The ability to culture organoids under low oxygen conditions has significant implications for disease modeling and drug discovery. Many diseases, including cancer, ischemic conditions, and neurodegenerative disorders, are associated with hypoxia. By replicating these conditions *in vitro*, researchers can study disease mechanisms and identify potential therapeutic targets more effectively. For instance, cancer organoids cultured under hypoxia exhibit enhanced tumorigenic properties, such as increased invasiveness and drug resistance, making them valuable tools for studying tumor biology and screening anti-cancer drugs. Additionally, low oxygen conditions can improve the predictive accuracy of organoid-based drug assays, as they better reflect the physiological context in which drugs will ultimately act.

Challenges and Future Directions

Despite its benefits, culturing organoids under low oxygen conditions presents technical challenges, such as the need for specialized equipment and precise control of oxygen levels. Moreover, the optimal oxygen concentration may vary depending on the organoid type and research application, requiring careful optimization. Future research should focus on standardizing hypoxia protocols and exploring the interplay between oxygen levels and other culture parameters, such as nutrient availability and mechanical cues. Advances in microfluidic and bioreactor technologies may further enhance the ability to control oxygen gradients and create more complex organoid models.



Learn how Embrient's [Modular Incubator Chamber](#) provides a convenient solution for controlled hypoxia environments.

In conclusion, low oxygen conditions are critical for the successful culture of organoids, enabling researchers to better replicate the *in vivo* microenvironment, maintain stem cell populations, and improve organoid functionality. These advancements have profound implications for biomedical research and drug discovery, offering more physiologically relevant models for studying human biology and disease. As the field of organoid research continues to evolve, optimizing oxygen levels will remain a key factor in unlocking the full potential of these innovative tools.

References

These references below highlight the role of hypoxia in mimicking physiological conditions, enhancing stem cell maintenance, and improving organoid functionality:

1. Hypoxia and Stem Cell Maintenance

Ito, K., & Suda, T. (2014). Metabolic requirements for the maintenance of self-renewing stem cells. *Nature Reviews Molecular Cell Biology*, 15(4), 243–256.

Relevance: This review discusses how low oxygen levels (hypoxia) are critical for maintaining stem cell self-renewal and pluripotency, which is essential for organoid culture. It highlights the role of hypoxia-inducible factors (HIFs) in regulating stem cell metabolism and survival.

2. Hypoxia in Intestinal Organoids

Yin, X., et al. (2020). Hypoxia-induced HIF-1 α promotes intestinal epithelial cell proliferation and enteroid formation. *Cellular and Molecular Gastroenterology and Hepatology*, 10(3), 567–580.

Relevance: This research highlights how hypoxia enhances the proliferation and differentiation of intestinal stem cells, leading to improved crypt-villus structure formation in intestinal organoids. HIF-1 α signaling is identified as a key mediator.

3. Hypoxia in Cancer Organoids

Drost, J., & Clevers, H. (2018). Organoids in cancer research. *Nature Reviews Cancer*, 18(7), 407–418.

Relevance: This review discusses the use of cancer organoids under low oxygen conditions to model tumor microenvironments. Hypoxia is shown to enhance tumorigenic properties, such as drug resistance and invasiveness, making cancer organoids more representative of in vivo tumors.

4. Hypoxia and Drug Discovery

Walaas, G.A., et al. (2023). Physiological hypoxia improves growth and functional differentiation of human intestinal epithelial organoids. *Frontiers in Immunology*, 14:1095812.

Relevance: This study emphasizes the importance of hypoxia in improving the physiological relevance of organoids for drug screening. Low oxygen conditions are shown to enhance the predictive accuracy of organoid-based drug assays.

5. Hypoxia in Disease Modeling

Ziółkowska-Suchanek I. Mimicking Tumor Hypoxia in Non-Small Cell Lung Cancer Employing Three-Dimensional In Vitro Models. *Cells*. 2021 Jan 12;10(1):141.

Relevance: This review highlights the use of hypoxia in lung cancer organoids to better replicate the tumor microenvironment and improve drug screening outcomes.

These references collectively underscore the importance of low oxygen conditions in organoid culture, demonstrating its role in enhancing physiological relevance, stem cell maintenance, and functionality. They also highlight the potential of hypoxia in advancing disease modeling and drug discovery.