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APPLICATION NOTE

stem cells

Stem Cell Differentiation Is Tuned by Oxygen Concentration and Pressure

There is tremendous enthusiasm around the use of stem cells in regenerative medicine. Since the discovery that adult cells could be treated with a specific gene cocktail to convert them into induced pluripotent stem cells (iPSCs), researchers around the world have been working to understand how this process could be implemented in the clinic for truly personalized medicine.

Realizing the potential for iPSCs, however, depends largely on the ability to culture and manipulate these delicate cells under very specific conditions — in some cases to maintain stemness markers, and in others to coax cells to differentiate into a desired cell type. This has been a significant technical challenge for the research community because conventional culturing systems are not precise enough to truly mimic the microenvironments in which stem cells would normally exist.

Stem cells have vast potential for use in regenerative medicine due to their ability to differentiate into multiple cell lineages. Harnessing this potential requires techniques to culture cells under conditions that allow for robust production and consistent function while avoiding unwanted genetic and epigenetic changes. Maintaining stemness and committing stem cells to specific lineages for effective generation of a target cell remain significant challenges when scaling production and culture sizes for therapeutic applications.

While extensive research has focused on soluble factors to optimize stem cell culture, conditions such as hypoxia, atmospheric pressure, and the composition and organization of the extracellular matrix are also important drivers of stem cell differentiation and cell function. However, no study to date has systematically analyzed the contribution of these factors in the maintenance and differentiation of stem cells, leading to uncertainty surrounding the extracellular factors that dictate stem cell state.

To address this, Xcell Biosciences has developed a novel stem cell culturing platform, the Avatar™ System, which allows for tunable control of the microenvironment and uniquely offers customizable settings for oxygen and hydrostatic pressure. In a recent study, Xcell scientists analyzed human pluripotent stem cells to characterize their underlying biology and to demonstrate the utility of the Avatar system. Cells iPSCs were cultured in minimally supportive media to allow stem cell state to differentiated

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into ectoderm, mesoderm, and endoderm cells. The Avatar system was used to tune environmental conditions and determine the impact of oxygen tension and pressure levels in guiding stem cell fate.

Study Results

For this project, scientists reprogrammed primary human dermal fibroblasts via episomal expression of key stemness factors (Sox2, Nanog, and Oct4) while cultured in altered a range of oxygen concentrations (1% – 5%) and atmospheric pressures (0 PSI – 5 PSI) using the Avatar system. Those results were compared with cells cultured using to conventional culture methods. Having generated iPSCs, scientists next then aimed to assess their pluripotency potential relative to the condition in which reprogramming and subsequent long- term culture was performed.

To determine, the pluripotency potential of iPSC colonies, scientists used three differentiation protocols to separately generate each germ layer: ectoderm, endoderm, and mesoderm. They and assessed relative efficiency through immunofluorescence staining for germ layer markers (Figure 1). Intriguingly Scientists saw significantly greater efficiency at commitment to ectoderm and mesoderm in iPSCs derived and cultured in normal physiological oxygen (5%) relative to standard incubator conditions. Interestingly, Moderate pressure
(2 PSI) and reduced oxygen had the most abundant effect, suggesting atmospheric pressure plays a critical role in regulating stem cell differentiation.

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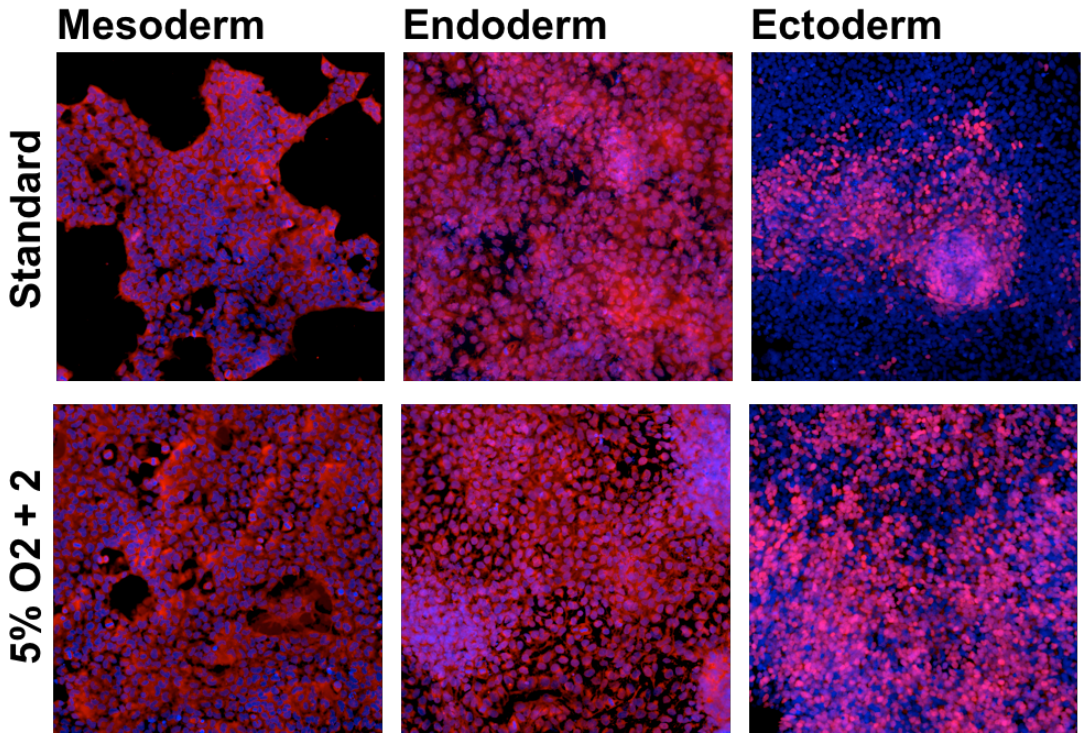

Figure 1: iPSCs generated and cultured in different environmental conditions were separately differentiated into the three germ layers. Efficiency of differentiation was assessed via immunofluorescence for critical respective germ layer markers.

These results are consistent with previous work implicating hypoxia signaling as an important regulator of pluripotency. They also provide strong evidence that environmental control could serve as a useful complement to current technologies for enhanced efficiency in stem cell growth, manipulation, and differentiation.

To learn more, scientists next wanted to used control of the cellular microenvironment to study the impact of oxygen and pressure on differentiation towards neuronal cells. Established iPSC cultures were exposed to different microenvironmental conditions and cultured in a neural induction media and then assessed for neural progenitor marker expression. Interestingly, iPSCs cultured under pressure led to significantly enhanced ectoderm commitment and eventually a neuronal morphology (Figure 2).

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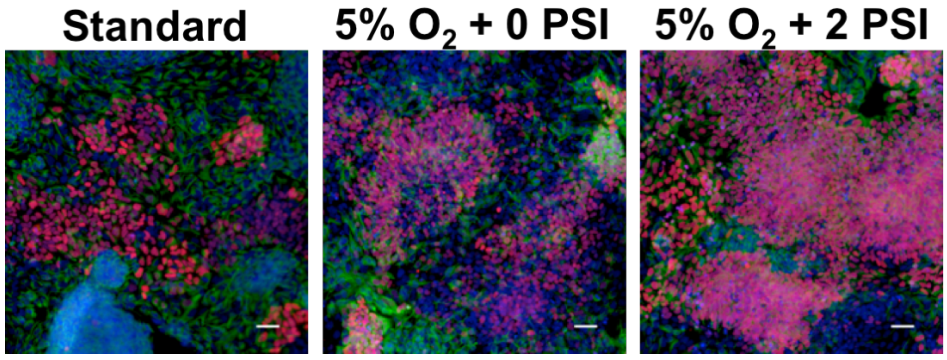

Figure 2: iPSCs cultured in different environmental conditions were differentiated into neural progenitor cells. Efficiency of differentiation was assessed via immunofluorescence for critical respective neural progenitor cell markers. (Nestin, Pax6)

These results provide exciting insight into new methods for enhancing neuron generation and expansion through microenvironmental control via the Avatar. This approach and might be key to therapeutic applications for iPSC-derived neurons in regenerative medicine.

Advancing Regenerative Medicine Through Next-Generation Culturing Tools

This project demonstrates the utility of the Avatar system for stem cell biology and indicates the potential for other regenerative medicine applications. With carefully cultured stem cells, it may ultimately be possible to generate highly functional, patient-specific adult cells for organ and tissue replacement. Stem cells grown in the Avatar system will yield important information about how the gene expression of these cells changes as they encounter new microenvironments. It might even be possible to adjust culture settings to mimic the disease environment of terminally differentiated cells, revealing an untapped source of data that could lead to the development of new, environment-specific stem cell treatments.

Visit us at www.xcellbio.com to learn more about the Avatar System.

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