Pluripotent stem cells (PSC), including embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC), hold promise for cell replacement therapies and embryonic developmental studies. Efficient differentiation to desired cell types remains a major obstacle, and residual PSC within differentiated populations are problematic because of their potential to form teratomas. Most PSC research is performed in high, non-physiological partial pressures of O₂ (pO₂). However, cells during embryonic development are exposed to much lower pO₂ and are affected by the expression of O₂-sensitive developmentally-important transcription factors, including hypoxia inducible factor-1 (HIF-1). This doctoral work demonstrates that physiological pO₂ markedly influences differentiation to mesodermal, ectodermal, and endodermal derivatives, with enhanced differentiation to cardiomyocytes in particular, and reduces tumorigenic residual PSC from the resulting differentiated cellular populations.

Mouse and human ESC (mESC and hESC) and mouse iPSC (miPSC) were cultured at defined gas pO₂ (pO₂gas) on silicone rubber membranes having high oxygen permeability to control cellular pO₂ (pO₂cell). By using silicone rubber membranes, pO₂cell at the cell-membrane interface was maintained virtually equal to pO₂gas, and rapid equilibration of pO₂cell occurred after a change in pO₂gas. Cellular attachment was achieved by coating with extracellular matrix protein before seeding.

Low pO₂ affected differentiation to mesoderm, ectoderm, and endodermal derivatives, with the most dramatic results being increased cardiomyocyte (mesoderm) differentiation. The best results were acquired by differentiation of mESC for 6 days at 36 mmHg pO₂gas followed by 142 mmHg for 15 days, resulting in up to 57% cardiomyocytes and 304 cardiomyocytes generated per initial mESC without purification, factors of 5 and 9 higher, respectively, than differentiation entirely at 142 mmHg. This low-pO₂ increase in cardiomyocytes required HIF-1. Low pO₂ culture for the first 6 days increased the expression of Mesp1/2 by 3x, which helps restrict mesodermal cells to the cardiovascular lineage, but did not increase Brachyury T expression, an early mesodermal marker, compared to culture at 142 mmHg pO₂gas, thereby suggesting that low pO₂ acted by restricting the fate of early mesoderm towards cardiomyocytes. Low pO₂ decreased the fraction and number of Nestin+ cells (ectoderm) by 3x for mESC, but enhanced expression of the endodermal genes Sox17, Foxa2, Hnf4a, and Pdx1.

In addition to affecting cell fate, low pO₂ for extended periods after completing differentiation drastically reduced the amount of residual PSC within, and the tumorigenic potential of, differentiated cell populations. Pluripotency marker (Oct4 and
Nanog) expression and the fraction and number of Oct4+ cells was reduced by up to four orders of magnitude at low compared to high O₂. This low-pO₂ decrease in residual PSC required HIF-1. Upon implantation into immunocompromised mice, low pO₂-differentiated cells did not form tumors or did so slower than high pO₂-differentiated cells, consistent with reduced residual PSC in the low pO₂-differentiated populations. Cell sorting to remove cells expressing PSC surface markers from differentiated populations after extended low pO₂ culture further reduced residual PSC and tumor formation rate.

These findings establish that culture pO₂ is important for almost every aspect of PSC differentiation and were reproducible among mESC, hESC, and miPSC. Using highly-O₂ permeable silicone rubber culture dishes allowed for accurate control of cellular O₂ exposure. One of the major hurdles in regenerative medicine is producing a sufficient amount and purity of the desired cell type from stem cells without contaminating PSC. By modulating pO₂ during different stages of differentiation, substantial increases in cardiomyocyte cell yields was achieved. Extended culture at low pO₂ further decreased residual PSC by several orders of magnitude. pO₂ control, alone or combined with other methods, could be applied to future cell therapy protocols to generate and increase the safety of differentiated cells.

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