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Rapid and effective modulation of dissolved oxygen levels in cell culture media for improved hypoxia studies



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Cell-based hypoxia studies are reliant on close control of O₂ concentration in the surrounding environment. Exposure to ambient O₂ (21%) concentrations for short or sustained periods of time can result in confounding results due to oxygen toxicity. Reduced O₂ cell culture incubators and workstations are examples of adopted measures designed to reduce this oxygen toxicity and to promote consistency of observation. We have identified a further layer of complexity to this scenario – culture media. With a starting O₂ concentration of approximately 10% O₂, a high salt content, and high operating temperature (37°C) it can take culture media approximately 3 hrs to reduce to 2% O₂ in the presence of cells and essentially never in the absence of cells and in large volumes.

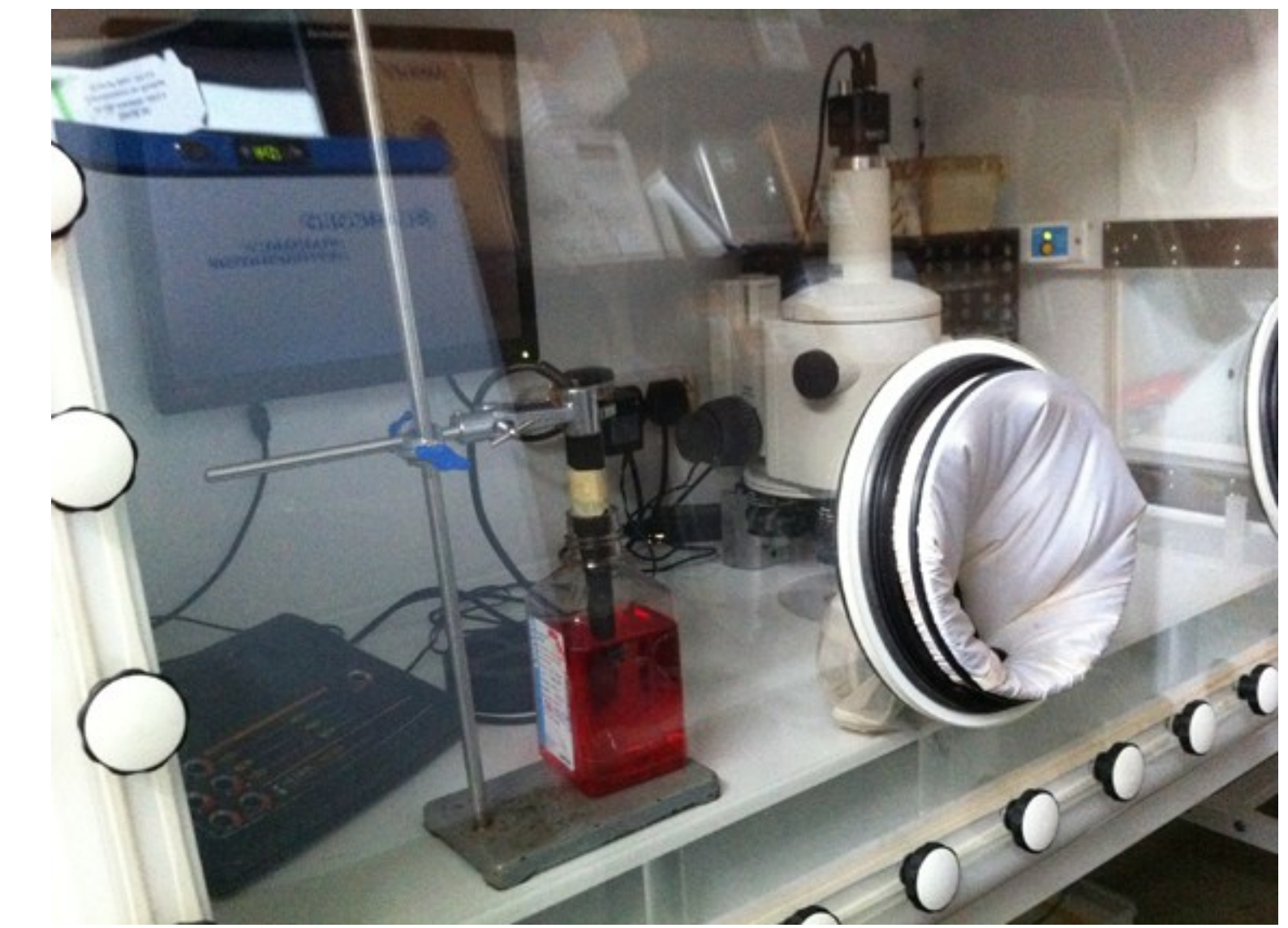
To overcome this deficiency we have developed a system for the rapid and effective modulation of dissolved oxygen levels in cell culture media. We have engineered a simple agitation device within a refrigerated unit with both O₂ and CO₂ gas level control. The system will reproducibly deoxygenate 500 ml bottled volumes of culture media with sterility retained through vented cap incorporation. Deoxygenation of large medium volumes to defined set-points (0.5%, 1%, 2%, etc) is readily achievable within 90 minutes.

Controlled experimentation to evaluate human mesenchymal stem cell (hMSC) recovery from bone marrow aspirate in a 2% O₂ workstation revealed a 50% improvement in both colony-forming unit-fibroblasts (CFU-F) and in absolute cell numbers when pre-deoxygenated (2% O₂) medium was used as compared to medium stored in 2% O₂ workstation prior to its use. The dramatic improvement in cell number was accompanied by widespread global transcriptional alterations at both transcript and exon levels as identified through Exon 1.0ST microarray analysis. This included 167 and 71 unique upregulated and down-regulated transcripts as compared to 2% O₂ workstation-alone recovered hMSC when compared to 21% O₂ recovered hMSC.

In summary we have developed a system for the rapid and effective modulation of dissolved oxygen levels in cell culture media. The application of this technology resulted in improved hMSC CFU-F and cell number recovery which was accompanied by widespread global transcriptional changes.

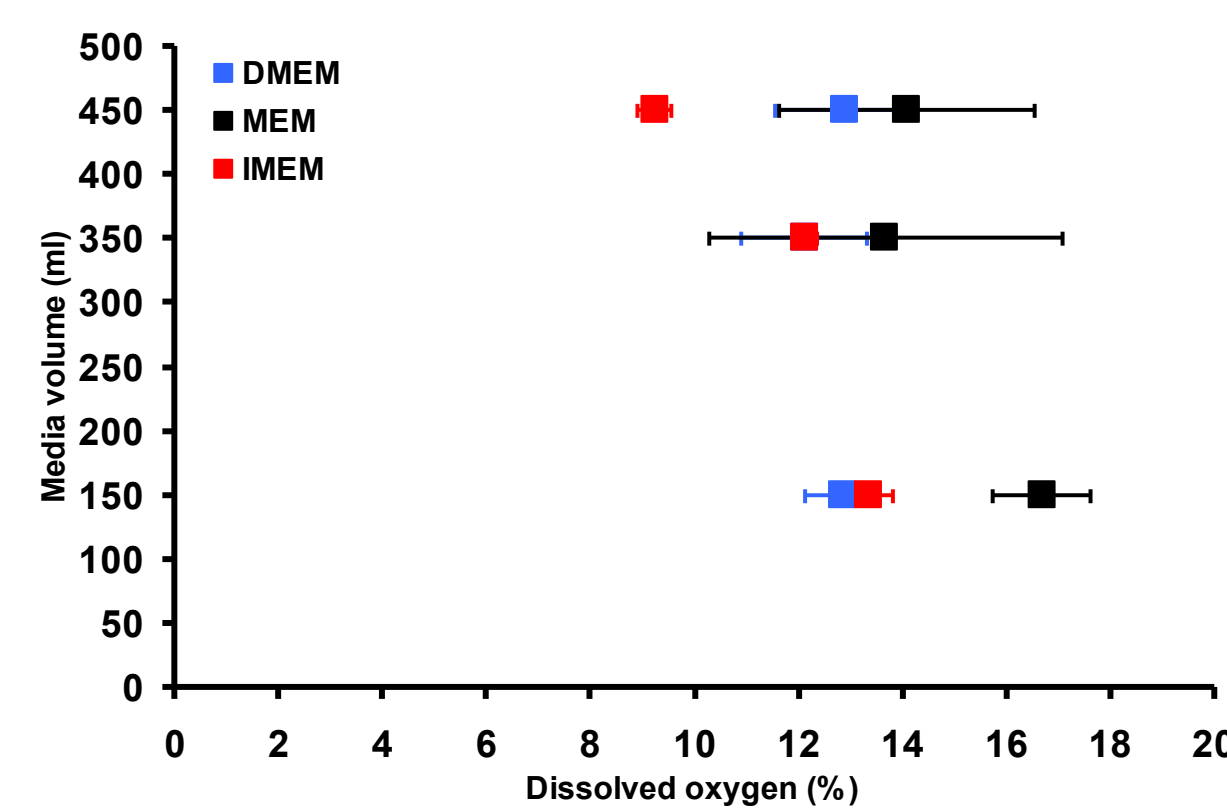


HypoxyCOOL prototype



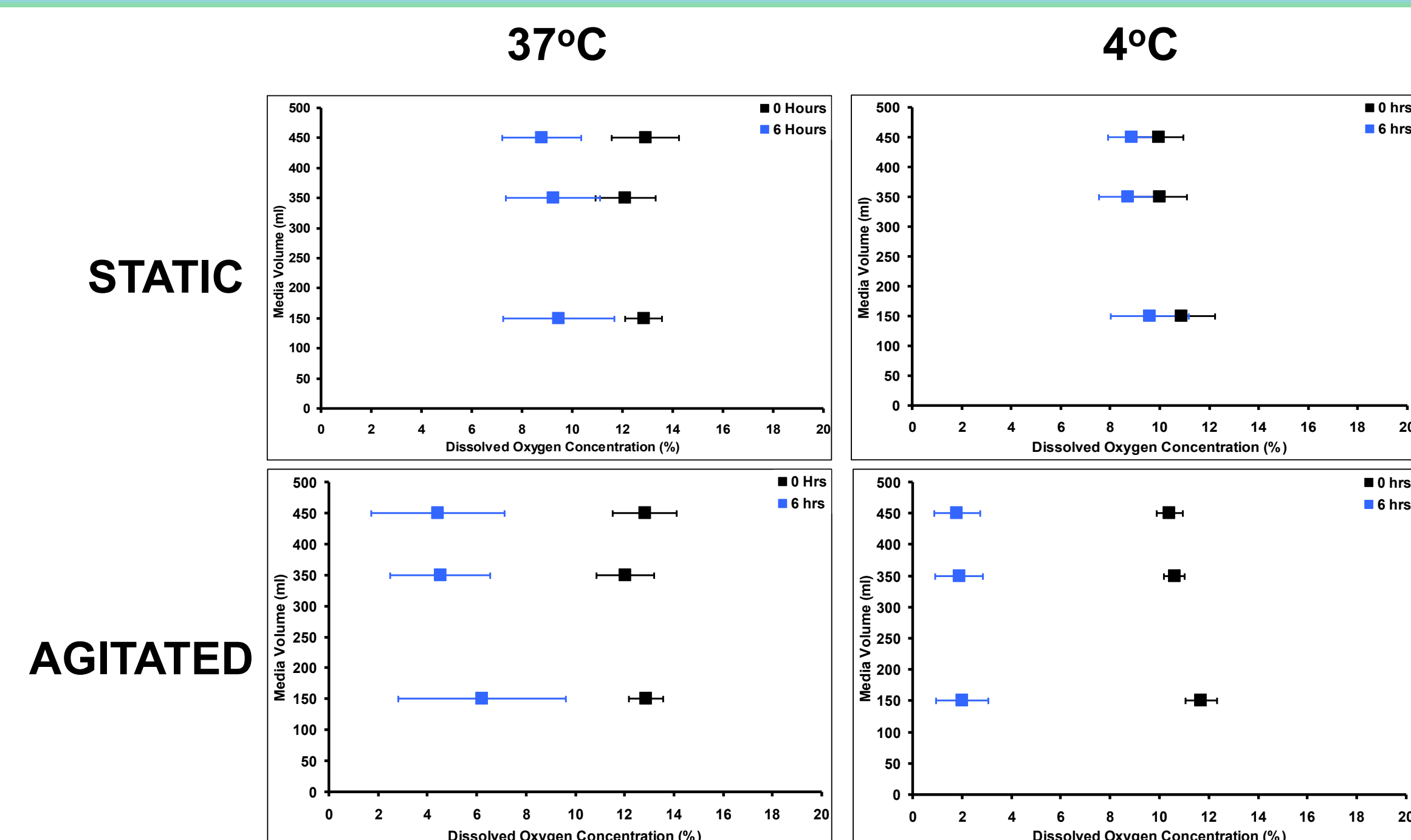
SCI-tive and dissolved oxygen sensor

Cell culture media has variable dissolved O₂ levels



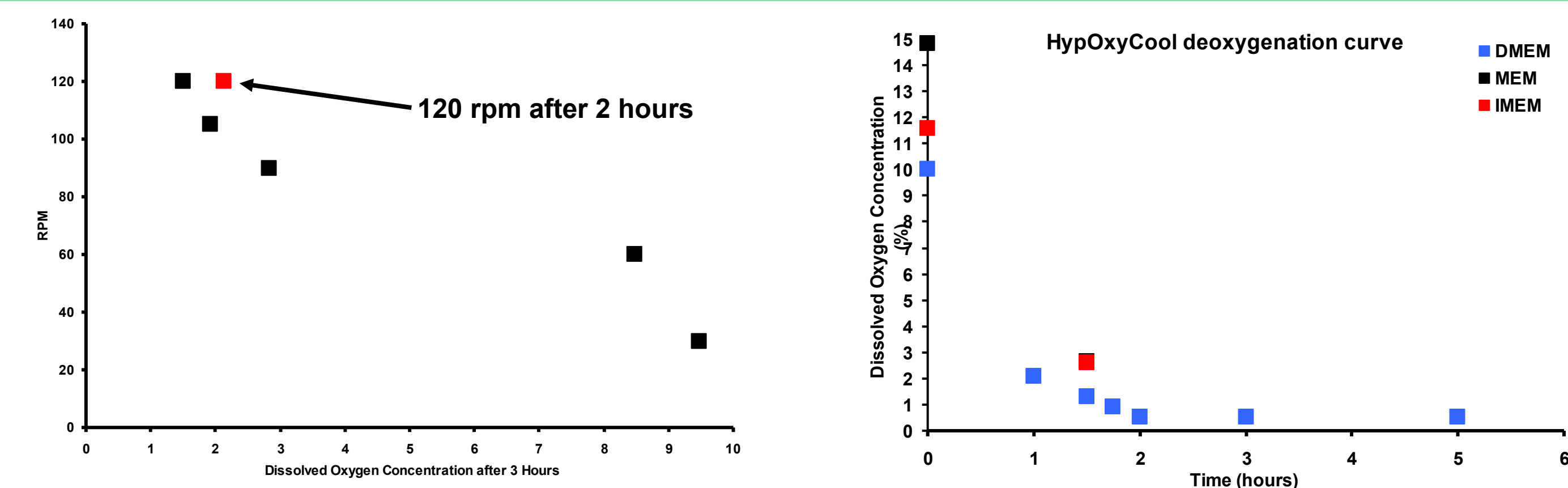
All dissolved O₂ measurements were performed with the HI 2400 Dissolved Oxygen Bench Meter with salinity and temperature correction.

Media will equilibrate to reduced O₂ after 6 hours at 4°C only



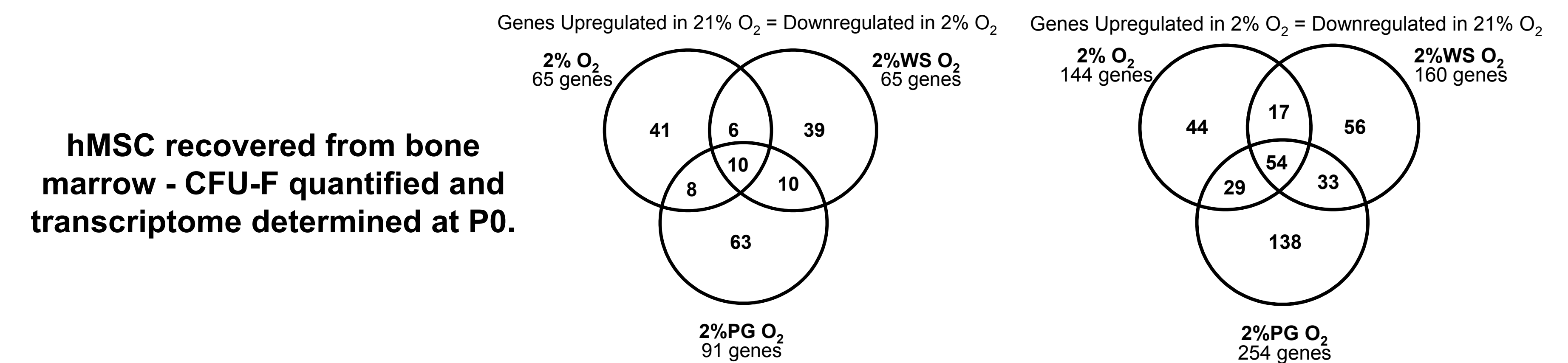
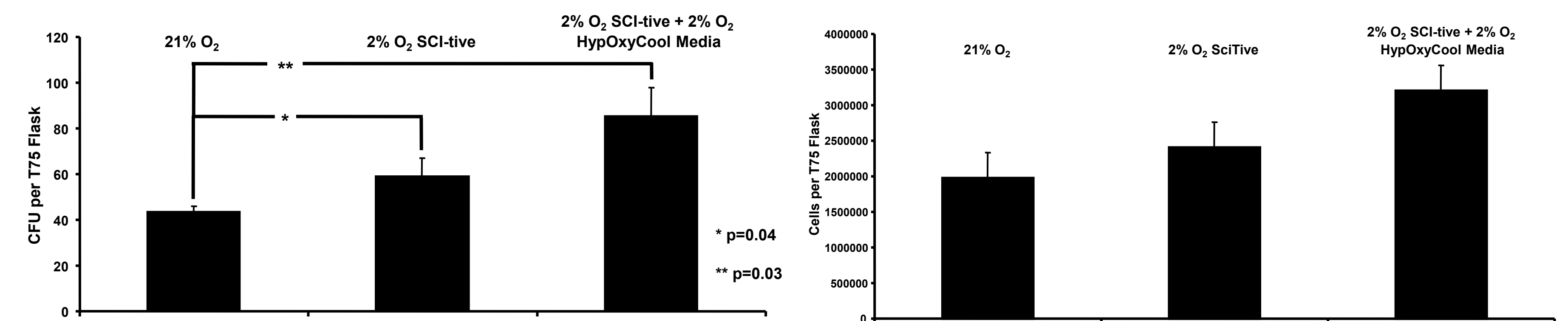
Media bottle placed into SCI-tive workstation operating at 2% O₂, opened, measured immediately and again after 6 hours.

Rapid deoxygenation to desired use point in HypoxyCOOL prototype



HypoxyCOOL unit set at 1% O₂, variable rpm, vented cap, 500ml media bottle. Measurements performed inside SCI-tive operating at 37°C, 2% O₂

Pre-deoxygenated media enhances hMSC recovery and stabilises transcriptome



hMSC recovered from bone marrow - CFU-F quantified and transcriptome determined at P0.

- Optimised frequency of CFU isolation
- Optimised cell numbers (per flask)
 - Greater CFU recovery with controlled rate of expansion
- Reduced transcriptional alteration
 - Maintenance of *in vivo* profile?

