

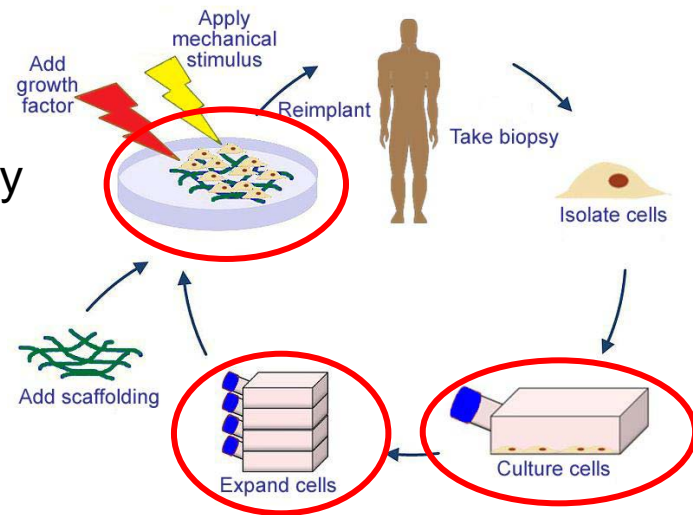


Culture Your Cells at Physiological Oxygen for Relevant Results

Mary Kay Bates, M.S.
Global Cell Culture Specialist

Why Culture Cells in Lower Oxygen?

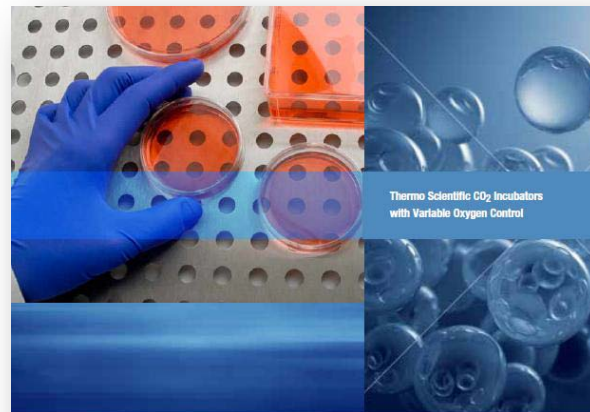
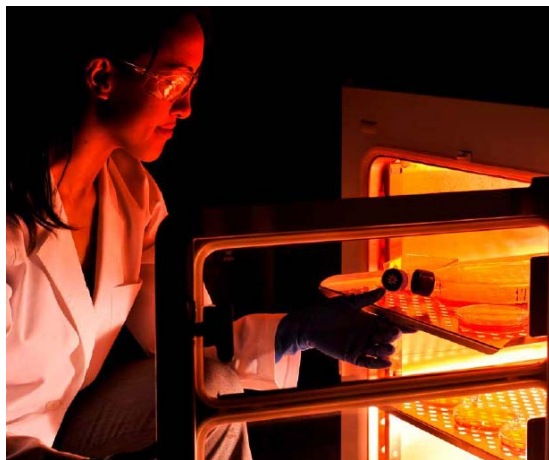
- Cell culture is slowly moving away from cell “lines”
- Use of cells that better mimic conditions in the body
 - Primary cells
 - Stem cells
 - 3D culture scaffolds
- Cells or cell products for use inside the human body
 - Vaccines
 - Biotherapeutics
 - Cell therapy
- Requires careful maintenance of conditions
- Consider how to mimic conditions in the body
 - Biological functional studies
 - Drug, cosmetic studies, diagnostic tests



Why Culture Cells in Low Oxygen?

“We use the tri-gas incubator because the integrity of the cells is better, they develop better, and they are healthier.”

--Neurobiology researcher, clinical research institute



5 reasons

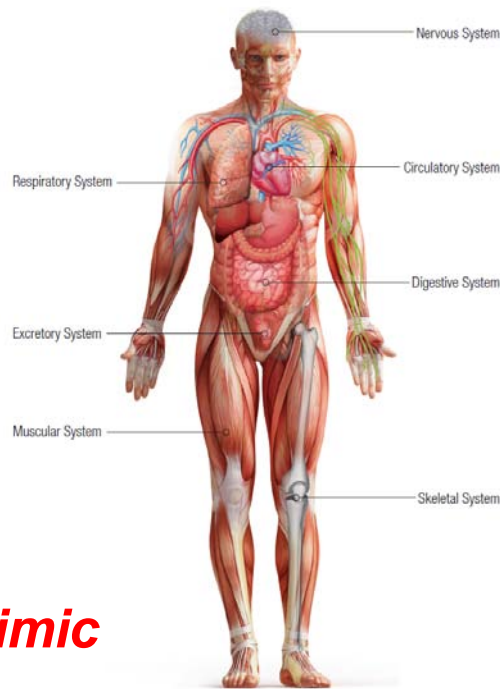
to enhance your cell culture using a Thermo Scientific CO₂ incubator with variable oxygen control

Thermo
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Oxygen in the Body are Lower Than in Air

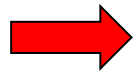
- Earth's Atmosphere: 20 – 21%
- Human Tissues:
 - Lungs: 14%
 - Arteries: 12%
 - Liver, heart, kidneys: 4% - 12%
 - Brain: 0.5% - 7%
 - Eyes: 1 – 5%
 - Bone marrow: 0% - 4%

➔ *Culturing cells in lower oxygen will better mimic conditions inside the human body*



Oxygen: A Delicate Balance

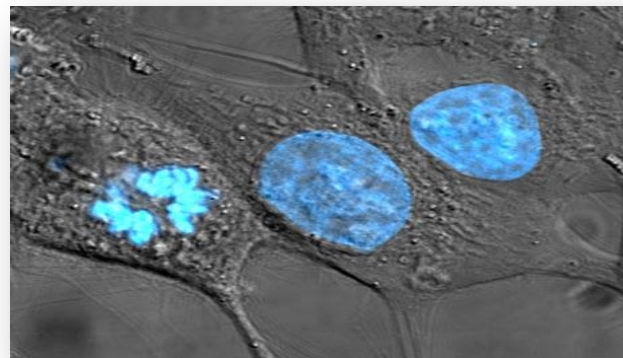
- Oxygen concentration inside cells is carefully balanced (HIF-1)
 - Cells can sense oxygen and react to conditions
 - Balance supply and demand
- Most activities and functions of cells are regulated by oxygen concentration in the cell
- Clearly, the air we breathe is way too high for most cells
- It is time to re-think our traditional cell culture at atmospheric O₂



To best simulate conditions in the body, cells should be cultured at hypoxia, or low oxygen conditions

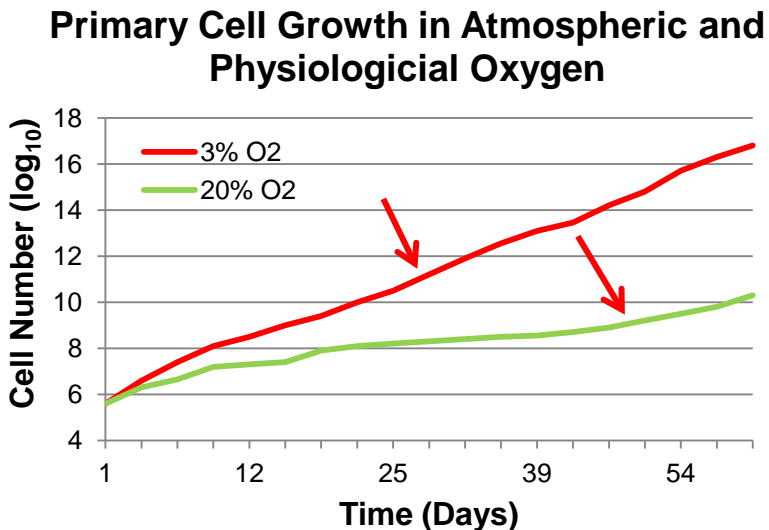
Oxygen Concentration Affects Cultured Cells

- Atmospheric oxygen:
 - Increases cellular differentiation
 - Increases oxidative damage to DNA
 - Increases expression of stress proteins
- Lower Oxygen
 - Cells grow faster
 - Cells live longer
 - No oxidative damage
 - Normal gene expression



Less Oxygen = Faster Cell Growth and Longer Life

- Experiments using primary cells better mimic cells in the body
- Many primary cell types grow faster and live longer when cultured under hypoxic (low oxygen) conditions



Adapted from Parrinello et al. *Nature Cell Biology* 2003

Stem Cells Require Low Oxygen for Proper Growth

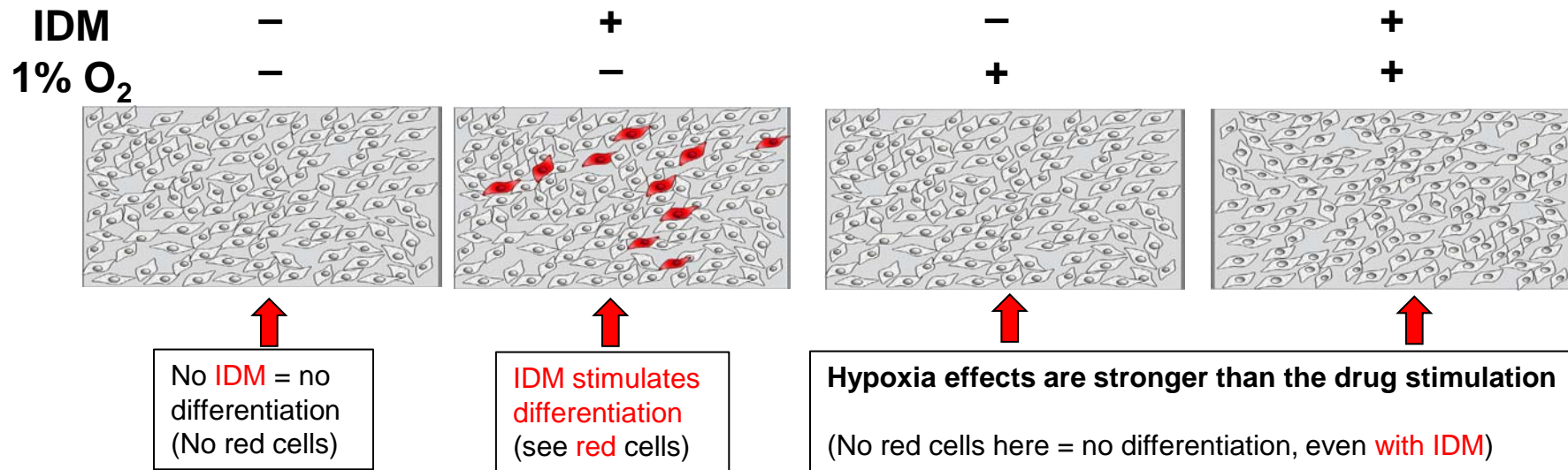
“Our lab mandates this (5% oxygen in the Thermo Scientific™ Heracell™ tri-gas incubator) in order to mimic conditions in the body, so that cells are as close to those conditions as possible and nothing is different. All of the signals for proper epigenetics are there.”

--Stem cell researcher at biomedical research institute



Hypoxia Can Regulate Cellular Differentiation

- Under hypoxic conditions, ADVM stem cells do not differentiate into more “developed” cells, even when stimulated with drugs (IDM)



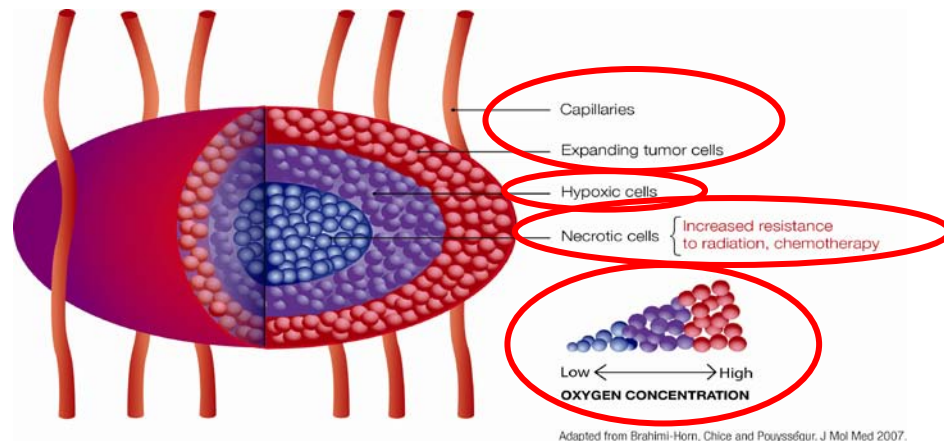
Modified from Lin, Lee and Yun, *J Biol Chem* 2006



Thus, stem cells and primary cells should be cultured in low O₂ to maintain their non-differentiated state

Hypoxia Also Important in Tumor Growth

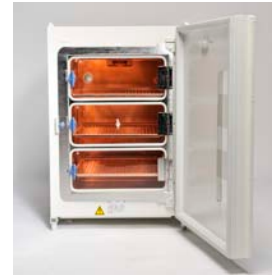
- Tumor microenvironments have an oxygen gradient
- Center of tumors with very low oxygen are necrotic (dying)
- These areas are linked to resistance to drugs and radiation, and to continued tumor progression



Oxygen control is increasingly used in cancer research

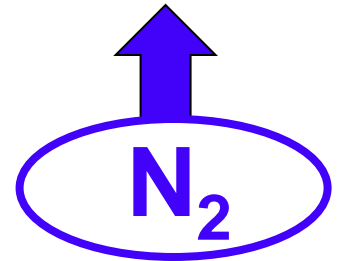
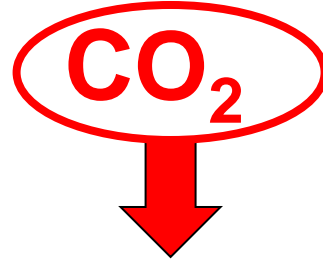
Tri-Gas Incubators: How They Work

- Tri-Gas incubators reduce oxygen concentration by pumping in nitrogen gas
- Sensor measure oxygen concentration and trigger N₂
 - Fuel cell
 - Zirconium oxide
- Oxygen concentration is set via control panel, like for CO₂
 - Use with CO₂ only for standard incubator
- Options for hypoxic or hyperoxic culturing



Thermo Fisher Scientific Leads with Tri-Gas Incubators

- Introduced the tri-gas incubator in 1979
 - Only two years after Packer and Fuehr recommended lower oxygen for cultured cells in 1977
 - FDA 510(k) registered for use with human patient samples
- Adding in nitrogen gas is technically tricky
 - CO₂ gas sinks
 - N₂ gas rises
 - Humidity, temperature and CO₂ affected



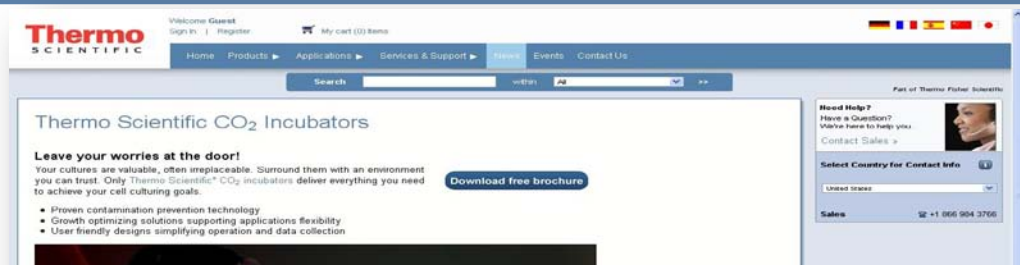
Active air circulation is the only way to keep conditions uniform throughout the chamber!

Summary and Conclusions

- New cell culture applications require conditions that better mimic those inside the human body, including low oxygen (hypoxia)
- Low oxygen conditions provide faster cell growth, longer life for primary cells, fewer stress responses, reduced DNA damage and less differentiation of stem cells
- Hypoxia is connected to tumor growth and aggressiveness, and to tumor resistance to treatment
- Thermo Scientific tri-gas incubators are offered with variable oxygen control and many choices for optimum cell growth
 - Uniform environment
 - Fast recovery

 ***Nearly everyone who cultures cells should consider hypoxic conditions***

Technical Resources



thermoscientific.com/co2incubators

