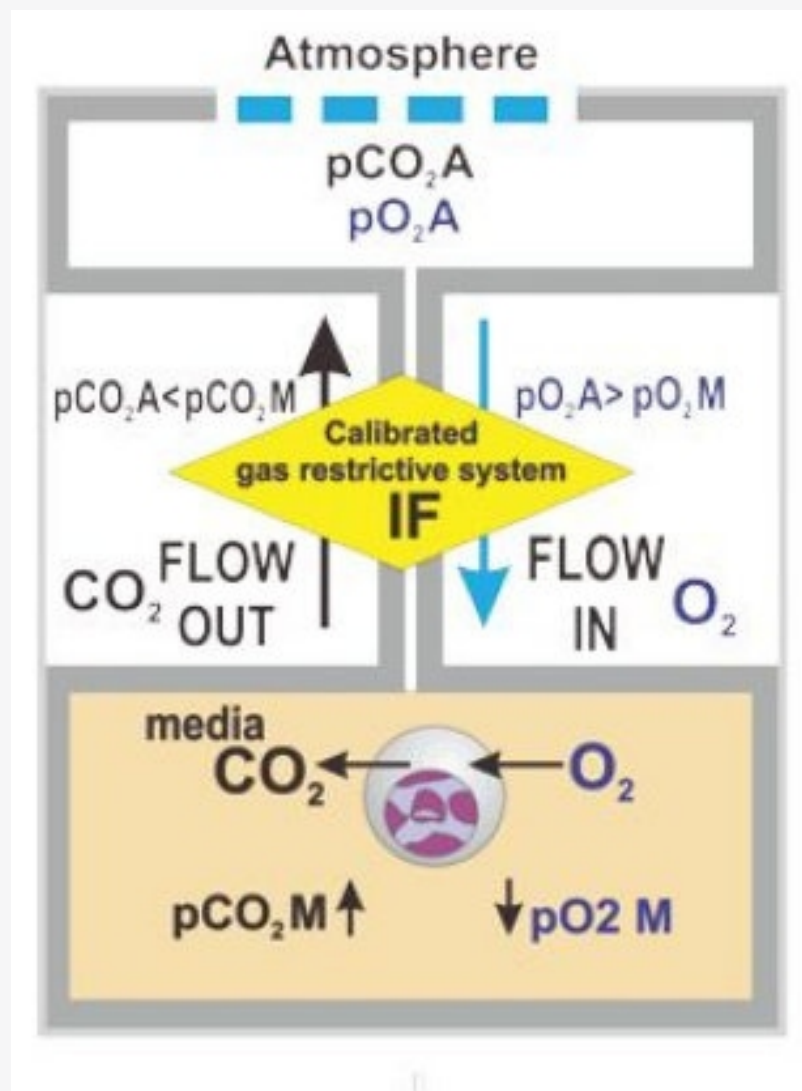


Cell culture inside chambers with limited oxygen transfer

Concepts and data

Petaka G3



Ducted Respiratory Chamber Bioreactors Cell Culture in Naturally Produced State Mimics True Physiologic Conditions

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Developments in stem [cell culture](#), regenerative medicine, and drug discovery are creating demand for cell culture under true physiologic conditions mimicking the atmosphere within living beings.

While former goals were just to keep cells alive and genetically stable, avoid contamination, and grow cells quickly in large numbers, investigators now know the subtle effects of unnatural cellular environments.

Atmospheric oxygen concentrations of in vitro cultures are far higher than that experienced by in vivo cells, and this difference has far-reaching consequences. Room air oxygen levels in cultures increase oxidation, mitochondrial activity, and forced cell proliferation, which inhibit other cell functions, causing misleading data as to tumor cell sensitivity to drugs and radiation.

Traditional labware, that is, flasks, bottles, and plates, are ambient atmosphere dependent—they take on the gas characteristics in which they are incubated. Without complex active interventions, cells are exposed to room air.

Cells in flasks or plates grow as a monolayer below a shallow layer of media directly exposed to air containing 21% oxygen, rapidly and constantly dissolving in the media, while true cellular oxygen levels are much lower. Room air contains almost no carbon dioxide, so

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supplemental tanked CO₂ is supplied, while low relative humidity in the lab requires a saturated water environment (water pans).

Excluding oxygen is particularly problematic, requiring sealed incubators, nitrogen flushing, and repeated attention to prevent the ingress of omnipresent atmospheric oxygen. A plethora of complex hypoxia chambers and glove boxes have been developed as active interventions to bring oxygen, carbon dioxide, and humidity levels in line with true physiologic conditions, but even so, wide fluctuations and failure of these active interventions is a constant danger.

Novel Bioreactor

Instead of trying to replicate natural conditions, a new strategy employs engineered devices, called Ducted Respiratory Chamber (DRC) [bioreactors](#). [Celartia's](#) Petaka® G3, instead of attempting to impose “normal” gas conditions on the cells through active incubator controls, passively allows cells to maintain their own oxygen, carbon dioxide, and humidity levels.

These levels are maintained automatically and independently from the laboratory or incubator conditions. Because the DRC devices act passively, they are inherently far simpler, more reliable, and less expensive than traditional methods of active gas control.

While the passively controlled gas environments within the DRC are far simpler than the active interventions required by incubators, they are actually far more dependable, as they are driven exclusively by natural laws.

The Petaka G3 DRC design is shown in *Figure 1*. The cell culture chamber is isolated on the injection side from the atmosphere by a self-sealing silicone injection port that allows the closed introduction of media and cells, including most eukaryotic cells types, small early-stage embryos, tissue fragments, and even needle biopsies.

On the venting side, the culture chamber is partially isolated from the atmosphere by an engineered, semi-closed respiratory duct having two

major portions. Closest to the reaction chamber, a series of small chambers act as water vapor condensers blocking evaporation, preventing alterations in media osmolarity, even at 37°C for 10 to 20 days at 10% RH.

The chambers also act as a capillary breaker to prevent media flow into the second portion, the respiratory duct that controls internal gas levels. The length and cross-section of the respiratory duct is engineered to partially restrict the diffusion of oxygen from the high levels of ambient air to create lower, physiologic levels of dissolved oxygen in the reaction chamber.

Observing Fick's Law, as oxygen is consumed inside the culture chamber, decreasing the partial pressure of oxygen in the media, oxygen diffuses from the outside atmospheric (higher) partial pressure, through the respiratory duct, to the lower partial pressure inside.

Diffusion is proportional to the concentration gradient, as regulated by the engineered design of the respiratory duct, and occurs entirely spontaneously and without outside intervention.

At the same time, the respiratory duct partially retains carbon dioxide from cellular metabolism to maintain a physiologically normal mild acidosis to balance pH. All gas exchange with the outside environment occurs through a 0.2 micron filtered vent, preserving the internal sterility of the device but allowing in and out gas movements to prevent pressurization issues when filling and emptying the bioreactors. These physical features of the Petaka G3 are labeled in *Figure 2*.

Cell culture in DRCs does not need supplemental oxygen-nitrogen balancing or CO₂ and humidity sources, eliminating the entire panoply of gas tanks, regulators, sensors, microprocessors, and water pans. This creates a doubled benefit: not only are cells cultured in more normal physiologic conditions, but the mechanics and costs of cell culture are greatly simplified and reduced.

Because the gas environment is automatically maintained, cultures can be incubated in virtually any kind of chamber providing warmth, and nothing else.

Naturally Produced Conditions

Many genes promoting cellular differentiation and expression are downregulated at atmospheric oxygen tensions. Naturally induced oxygen levels and cell growth in the DRC are depicted in *Figure 3*.

A series of DRC bioreactors were seeded with neonatally derived cardiomyocytes, 10^6 cells per DRC in RPNI 1640 media, and incubated in room air at 37.5°C. At time zero (left, *Figure 3*) seed cells and media at room air O_2 (~120 mm Hg) are injected into the DRC. Cells proliferate, consuming O_2 , causing the O_2 level within the DRC to begin to fall, but restricted diffusion through the respiratory duct partially replaces O_2 .

At about 5×10^6 cell count, internal O_2 levels enter the upper limits of physiologically normal tissues (~50 mm Hg). At about 8×10^6 cell count, O_2 diffusion through the respiratory duct approaches compensatory levels for O_2 consumption. Cells enter a differentiation phase, shown by a leveling of the doubling rate, expanding protein expression and normal gene expression, and are harvested while still within the band of normal tissue O_2 levels (5–50 mm Hg).

Because the cells control their own gas environment, there is little effect from outside gas conditions, and the DRC is effective in atmospheres from 500 meters below sea level up to elevations of 4,000 meters, and in relative humidity levels between 10% and 100%.

The DRC changes the paradigm of cell culture, replacing nearly a century of active attempts to humanly intervene to manipulate gas exchange with a self-regulating design driven by natural laws of diffusion to create dependable, and truly physiologic, gas environments.

Figure 1. Schematic of ducted respiratory bioreactor (DRC).

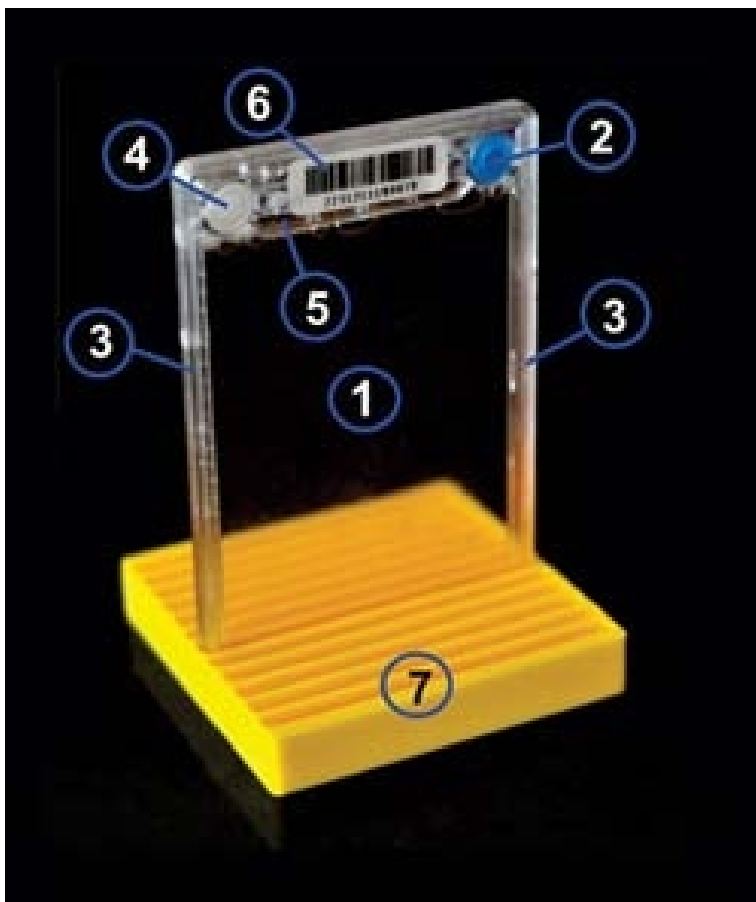
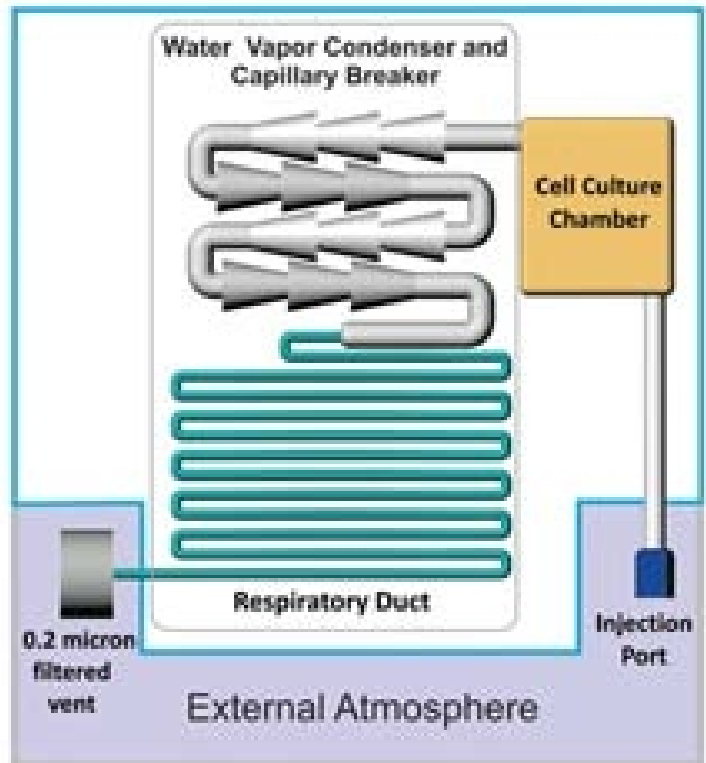


Figure 2. Petaka G3 DRC (1) cell culture chamber, (2) injection port, (3) respiratory duct, (4) 0.2 micron filter, (5) water vapor condensers and capillary breakers, (6) unique barcode. The DRC is shown upright (7) in a silicone stand.

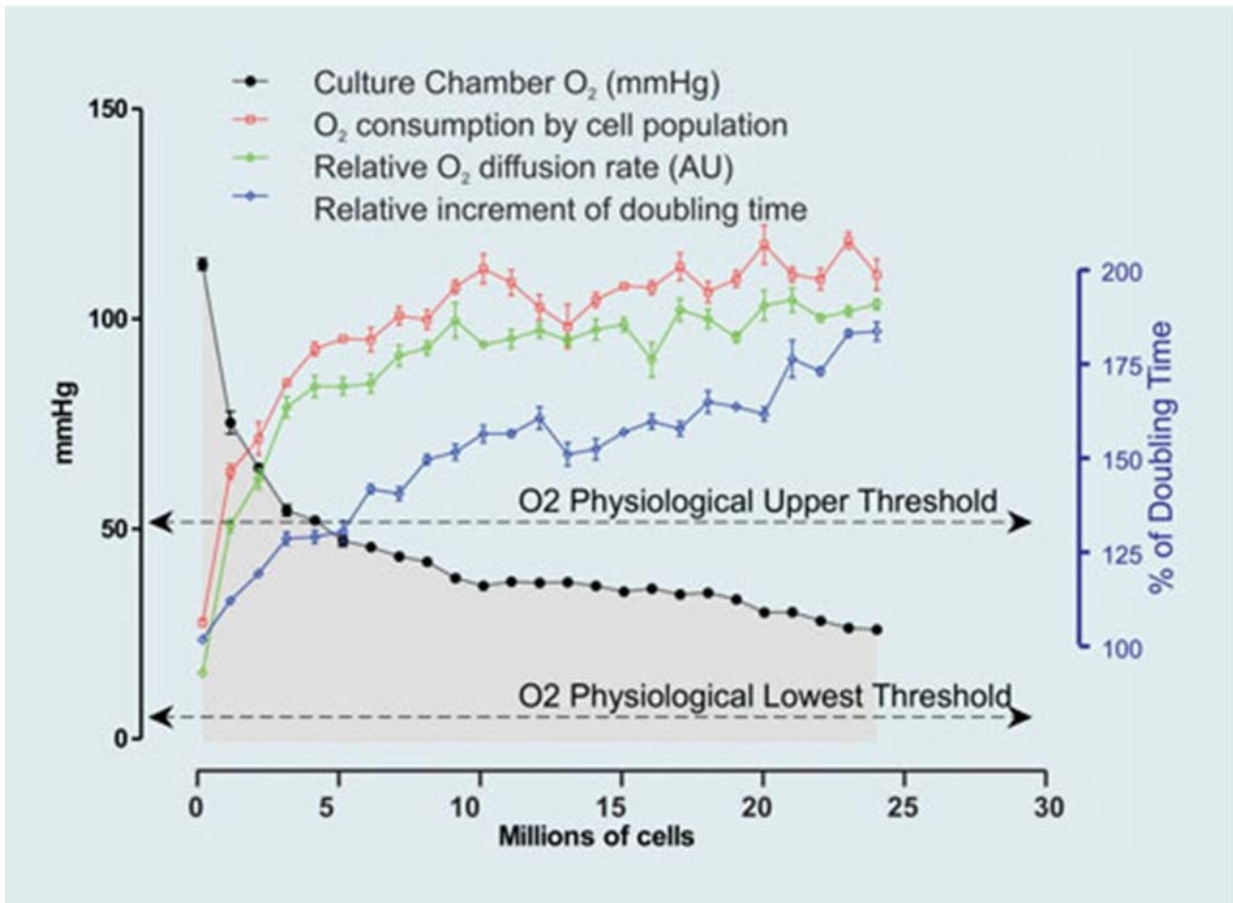


Figure 3. Self-regulating gas management in the DRC. Cells consume O₂, with restricted O₂ ingress, causing a first proliferative cell phase to evolve into a second differentiating cell phase for protein production and gene expression.