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The only device for cell preservation in suspended animation at room temperature

Cells are normally cryopreserved for storage and shipping, however cryopreservation is a strenuous process for cells. Cryopreservation media contains toxic chemicals, such as DMSO and glycerol, to avoid ice crystal formation that kills cells.

Very low temperatures induce suspended animation in cells, characterised by cell cycle arrest, membrane changes, body shrinkage, spindle disorganisation resulting in some scattering of chromosomes (1), and metabolic interruption.

These are identical changes that also occur in living animals in hibernation, where tissues and cells exposed to a specific environmental event such as deeply cold temperatures, hypoxia, environmental low pH, or excess of CO₂ in the atmosphere.

Oxygen supply and cell suspended animation

Exposure to oxygen is essential for nearly all living vertebrates; however some of them can survive for hours in absence of oxygen. In anoxia, those vertebrate tissues enter a state of suspended animation where all apparent visible cell movement ceases, including cell division and motility.

In this "dormant state" cell cycle is halted during the S and G2 phases (1), and are more resistant to radiation and drugs commonly used as chemotherapy agents (2).

Some invertebrate cells have the ability to survive in the absence of molecular oxygen (anoxia) for months (3). The survival of anoxia depends on the organism's ability to conserve energy usage by shutting down non-essential cellular functions, maintaining stable and low permeability of membranes, and synthesising ATP by glycolytic processes (4).

As a reaction to the decrease in the capacity for aerobic energy production, cells increase anaerobic energy production and decrease energy demand. In mild hypoxia, oxidative phosphorylation is low but active, therefore maintaining some aerobic energy production, up regulating hypoxia-inducible transcription factor 1 (HIF-1), and genes involved

in anaerobic energy production, such as glycolytic enzymes, glucose transporters, and antioxidants (catalase, superoxide dismutase, etc) which protect free radical-induced damage.

In a deep anoxia of 0,01 mmHg of O₂, oxidative phosphorylation ceases, reducing the capacity to generate energy. In this environment, the cell reduces energy demand and declines cellular activity.

In certain embryos, exposure to anoxia leads to a complete arrest of cell cycle progression, and consequent embryo developmental suspension. Invertebrate embryonic cells can remain suspended for 24 h or more, and upon return to normoxia, will recover with a high viability. These embryonic cells survive the hypoxic stress by reducing the rate of energy consumption, preventing the occurrence of irreversible damages.

Hypoxia triggers sophisticated defense mechanisms, such as HIF-1-mediated response and carbon monoxide formation, which competes with oxygen for binding to iron-containing proteins, and can protect against hypoxia-induced lethality by inducing suspended animation.

Similar effects produce certain chemical compounds, such as hydrogen sulphide, that can produce reversible inhibition of cytochrome C

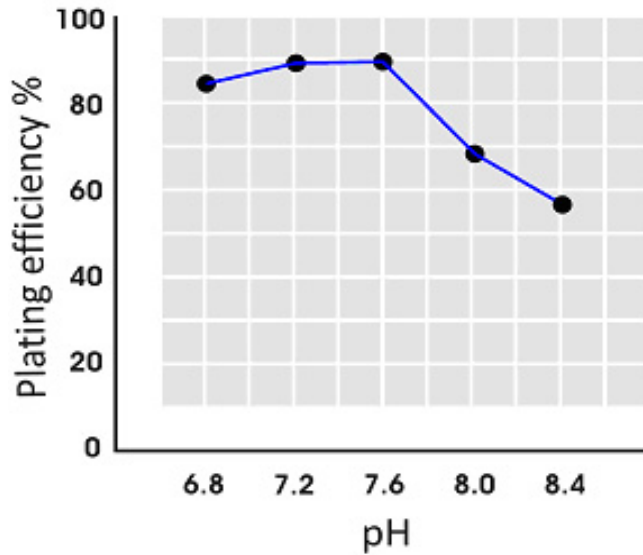


Figure 1. pH effect on the HeLa cells plating efficiency. (Ceccarini, C. I Effect of pH on plating efficiency, serum requirements, and incorporation of radioactive precursors into human cells. *In vitro*, 1975, Vnl. II, No. 2, 78-86.)

G2 that is selectively implicated within the specific temperature band. However, short exposures to more severe hypothermia (4 - 10 °C) induces cell cycle arrest in G1/S transition.

During animal hibernation the mitotic index is greatly reduced in tissue cells which are accumulated within the G2 phase. This allows complete reversibility after arousing. It is assumed that the block in G2 prevents the cells from possible damage and allows a safe reversible return to cell cycle (8).

Media pH and cell plating efficiency

Different cultured cells lines have different optimum pH for growth in culture. Cell cultures not at optimal pH fail to proliferate at lower saturation density. There are studies almost half a century old that demonstrate that a pH below the optimum level reduces cell motility as observed with time-lapse movies (9). However, cells that are density-inhibited at a sub optimal pH can be induced to proliferate to a higher density by increasing the medium pH to an optimum level (10).

The effect of pH on cell culture plating efficiency is considerable. Tumor cell lines and transformed cell lines have slightly higher plating efficiencies when the pH changes from 6,8 to 7,6. However, at more alkaline pH, the plating efficiency drops rapidly (11) (Figure 1).

oxidase (5). Suspended animation in organisms reversibly reduces the metabolic rate and, consequently carbon dioxide production and oxygen consumption.

Heat supply and cell suspended animation

Metabolism and oxygen needs to diminish logarithmically with respect to temperature (6). Cell cycle progression is temperature sensitive, in culture; many types of mammalian cells do not pass the G2/M transition in moderate hypothermia (16 - 20 °C). However, progression through G1, S and mitosis is not blocked at these temperatures (7). As a result, at least one check point is present during

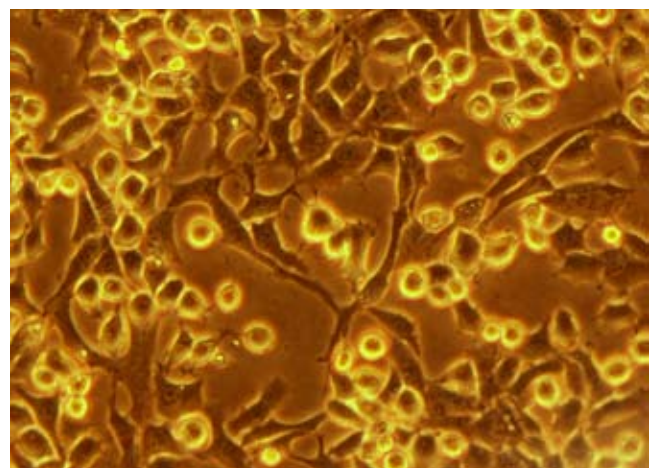


Figure 2. Petaka, the cell culture device with auto regulated gas exchange system. CHO cells cultured inside Petaka at 37,5 °C, 10% RH, 0,002% atmospheric CO₂, 20% atmospheric O₂, at sea level.

Days of Culture	PetakaG3 37 °C	PetakaG3 20 °C	T flask 37 °C	T flask 20 °C
7	1.5%	0.3%	28%	17%
14	3%	0.6%	55%	35%
28	6%	1.2%	92%	90%

Table 1.- Level of dehydration in Petaka G3 incubated in 10% RH atmosphere, compared to other open cell culture devices such as T flask and others, incubated in identical conditions.

In vitro cell dormancy and cell preservation at room temperature

Petaka is Celartia's proprietary cell culture device designed for all vertebrate derived cells (Figure 2). Petaka has an embedded gas transfer regulation system (US patent 7,514,256) which provides the cells with a calibrated combination of oxygen supply and a restrained release of CO₂, combined they both permit cell growth that auto regulates an optimal amount of dissolved oxygen in the media, and consequently a suitable pH as well.

Moreover, this system reduces the water evaporation of the media to negligible levels avoiding cell culture dehydration for extended periods up to 8 - 12 weeks (Table 1).

When cells reach a level of growth that initiate the contact inhibition, dissolved O₂ concentration in the media remains at a level of borderline hypoxia (see above), and the pH is maintained slightly below the optimum level for cell proliferation (see above), this environment supports a completely reversible arrest of cell growth as explained above (Figure 3).

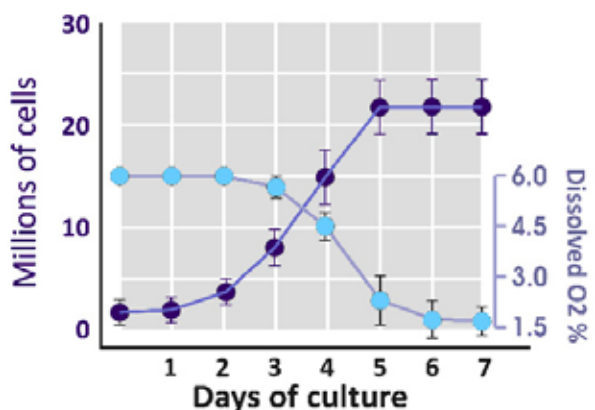
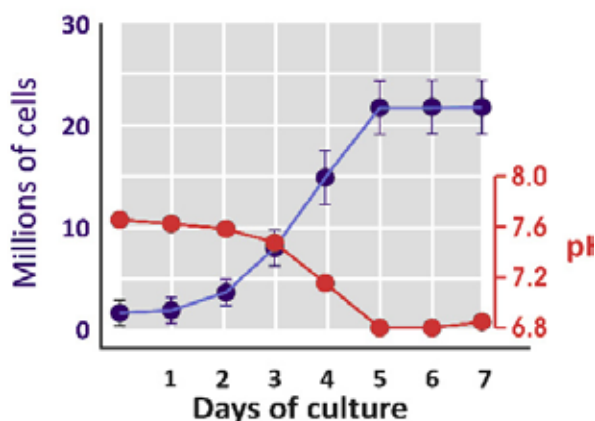


Figure 3. pH auto control in Petaka G3. CHO cells, results of 20 cultures. The number of cells determines the pH level within the optimal band, and the pH regulates the cell growth. Dissolved O₂ partial pressure auto control in Petaka G3. CHO cells, results of 20 cultures. The number of cells determines the DO concentration within the optimal band, and the DO concentration regulates the cell activity.

In this state, the exposure of the culture devices to moderate hypothermia (16 - 20 °C) induces a cellular variation of suspended animation in culture (*In vitro* cell dormancy) which concentrates the cells in G2 phase of the cycle (9,12), showing a very low metabolism, indicated through the Lactic acid and CO₂ production (pH fall) (Figure 4).

The result of this special dormant state is that cells remain for long periods of time (weeks or months, depending on the cell type) unchanged, with only the required necessity of maintaining the temperature in moderate hypothermia.

This together with the fact that Petaka doesn't allow dehydration allows maintenance of the cells in this dormant state for long periods of time, minimising risks of cell damage.

Petaka is a perfect device to investigate the molecular basis of suspended animation of vertebrate beings in cultivated cells, under different experimental settings, including complete anoxia.

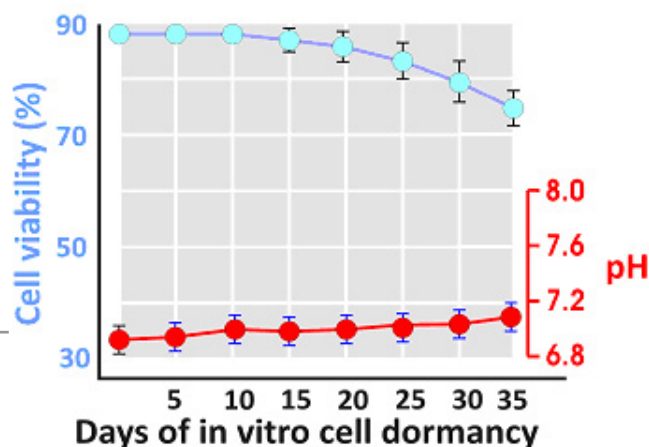


Figure 4. Confluent CHO cells maintained at 20 °C for 35 days. *In vitro* cell dormancy maintains high cell viability at 20 °C with a low metabolic activity as indirectly shown by the invariability of the media pH.

***In vitro* cell dormancy applications**

In vitro cell dormancy has been used with clear advantageous results for intercontinental cell shipping, long lasting drug effect on cells and offering infinite flexibility for laboratory scheduling.

Intercontinental cell shipping in Petaka renders the cryopreservation process and equipment unnecessary reducing logistics costs significantly and increasing safety of the culture (never exposed to unscheduled thawing). It has been proved that transits as much as 30 - 40 days are possible without significant loss of viability.

Moreover, upon reception of dormant cells in Petaka, it is not immediately necessary to transfer them to new devices or even effect a media change. The cell recipient will normally have the

flexibility of a number of days to arouse the cells depending on the duration the transit.

For drug discovery *in vitro* cell dormancy has a special value and benefit because small doses and long lasting drug effects can be studied.

Many researches have used Petaka to mimic the tumor cells resistance to chemotherapy whilst in a dormant state, as well as inflammatory responses from macrophages to dormant tumor cells.

Laboratory timing schedules benefit from the cell dormancy feature of Petaka as it offers infinite flexibility to sub culture at their convenience and not when confluence dictates it to be done.

References:

1. Sathanathan, A. H., Ng, S. C., Trounson, A. O., Bongso, A., Ratnam, S. S., Ho, J., Mok, H. and Lee, M. N. The effects of ultrarapid freezing on meiotic and mitotic spindles of mouse oocytes and embryos. *Gamete Research* 1988, 21: 385-401.
2. Pamela A. Padilla and Mark B. Roth. Oxygen deprivation causes suspended animation in the zebrafish embryo. *Proc Natl Acad Sci U S A*. 2001 June 19; 98(13): 7331-7335.
3. Brown, J. M. *Cancer Res.* 1999, 59, 5863-5870.
4. Anderson GL Responses of dauerlarvae of *Caenorhabditis elegans* (Nematoda: Rhabditidae) to thermal stress and oxygen deprivation. *Can. J. Zool.* 1978, 56, 1786-1791.
5. PW Hochachka. Defense strategies against hypoxia and hypothermia. *Science* 1986, 17 Vol. 231 no. 4735 pp. 234-241.
6. Reiffenstein, R. J.; Hulbert, W. C.; Roth, S. H. Toxicology of hydrogen sulfide. *Annu. Rev. Pharmacol. Toxicol.* 1992, 32, 109-134.
7. Horvath, S. M., B. K. Hutt, G. B. Spurr and G. E. Stevens: Some Metabolic Responses of Dogs Having Low Body Temperature. *Science*. 1953, 118:100.
8. Rieder CL, Cole RW. Cold-shock and the Mammalian cell cycle. *Cell Cycle*. 2002, 1(3):169-75.
9. Kruman II, Ilyasova EN, Rudchenko SA, Khurkhulu ZS. The intestinal epithelial cells of ground squirrel (*Citellus undulatus*) accumulate at G2 phase of the cell cycle throughout a bout of hibernation. *Comp Biochem Physiol A*. 1988, 90(2):233-236.
10. Taylor, A. C. Responses of cells to pH changes in the medium. *J. Cell Biol.* 1962, 15: 201-209.
11. Ceccarini, C. & Eagle, H. Induction and Reversal of Contact Inhibition of Growth by pH Modification. *Nature (London) New Bowl*, 1971, 233, 271-273.
12. Ceccarini, C. I Effect of pH on plating efficiency, serum requirements, and incorporation of radioactive precursors into human cells. *In vitro*, 1975, Vnl. II, No. 2, 78-86.
13. Todd G. Nystul, J.P. Goldmark, Pamela A. Padilla, Mark B. Roth. Suspended Animation in *C. elegans* Requires the Spindle Checkpoint. *Science*, 2003: 7, Vol. 302. no. 5647, pp. 1038 - 1041.

Benefits of Cell Dormancy in Petaka

- Cell Storage without cryopreservation
- Cell Storage without DMSO
- No media alteration
- Sterile preservation
- Safe handling
- Flexibility to lab schedules
- Safe cell transit at room temperature
- High cell viability after transit

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