Development and Validation of a Closed Chamber for Cell Culture Experiments in Space

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Abstract

Here, we report the features of a novel closed cell culture chamber (DCC) for use in experimental settings in space, and the results of subsequent validation tests. The DCC consists of a basal plate, a film and two medium ports. The basal plate and film are made from clear polystyrene. Oxygen and carbon dioxide gas exchange is performed through the thin film. The two medium ports are made from silicone in which syringe needles are inserted, and the culture medium is exchanged. The corona discharge treatment is applied on the basal plate to change the nature of the surface depending on the requirements for cell adhesion. Here, cell culture was performed using a human hepatoma cell line (HepG2), a hamster pancreatic β-cell line (HIT-T15) and a mouse fibroblast cell line (L929) in both DCCs and regular culture dishes. We confirmed that there were no differences in cell shape, growth rate, and dissolved oxygen concentration. Although the DCC was developed for use in space, it can also be used for cell transportation on the ground. ©2014 Jpn. Soc. Biol. Sci. Space; doi: 10.2187/bss.28.1

Introduction

Many experiments have been performed while in orbit. However, only a few experiments using mammalian cells have been performed (Lorenzi et al., 1993) because of the following difficulties:

In general, mammalian cells are cultured in a tissue culture dish or a flask on the ground, but these cannot be used in orbit, because the liquid is lost under microgravity conditions. In addition, as fresh medium is continuously required for cell cultivation, careful consideration must be given as to how the medium exchange is performed in microgravity in order to avoid leakage. Furthermore, the cells require oxygen supplementation for their respiration, but must be able to discharge CO₂ in order to avoid medium acidification. Although the O₂ and CO₂ gases are exchanged directly between air and medium on the ground, gas exchange parameters are altered in orbit.

To culture mammalian cells, the temperature, humidity, and carbon dioxide concentration should be constantly maintained. The Cell Biology Experiment Facility (CBEF) was installed in the Japanese Experiment Module (JEM) of the International Space Station (ISS) in 2002 (Ishioka et al., 2004; Yano et al., 2012). The CBEF can provide a constant environment (temperature, humidity and carbon dioxide concentration) for life science experiment. The environment for cultivation of mammalian cells is now completed.

Here, we developed a completely closed cell culture chamber (DCC) to culture mammalian adherent cells while in orbit. The DCC consists of a clear film and base plate for cell observation and two medium ports for medium exchange. The film also enables the exchange of oxygen and carbon dioxide. In this study, we validated the performance of the DCC on the growth rate, cell morphology and dissolved oxygen concentration in several commonly used mammalian cell lines.

Materials and Methods

Cells and culture media

L929 mouse fibroblast cells (Riken BioResource Center, Tsukuba, Japan), HepG2 human hepatoma cells (Riken BioResource Center) and HIT-T15 hamster pancreatic β-cells (DS Pharma Biomedical Co., Osaka, Japan) were grown in Dulbecco’s Minimal Essential Medium (DMEM) containing 4,500 mg/L D-glucose and 25 mM HEPES (Gibco BRL, Invitrogen Co., Carlsbad, CA) supplemented with 10% (v/v) fetal bovine serum (Equitech-Bio, Inc., Ingram, TX) and 1% penicillin/streptomycin (10,000 U/mL penicillin and 10,000 μg/mL streptomycin; Sigma Chemical Co., St. Louis, MO). Cells were subcultured twice a week in 25 cm² tissue culture flasks (Asahi Glass Co., Tokyo, Japan) in a 37°C humidified incubator in an atmosphere of 5% CO₂ in air.

Cell Culture in DCC

Previously cultured L929, HepG2 and HIT-T15 cells were washed twice with phosphate buffered saline (PBS; GIBCO), and detached with 1 mL Trypsin-EDTA (0.25% trypsin, 1 mM EDTA; GIBCO) at 37°C. After 10 min trypsinization, the cells were transferred to 15-mL centrifuge tubes (Asahi Glass Co.) with 5 mL medium, and centrifuged for 3 min at 1,000 rpm. The supernatant was removed, the pellet was resuspended with 1 mL medium, and the cell concentration was adjusted to 3.2 x 10⁶ cells/mL for L929, 12.8 x 10⁴ cells/mL for HepG2 and 12.8 x 10⁴ cells/mL for HIT-T15 with medium. The cells were inoculated into DCCs (4.64 mL) and into 60-mm tissue culture dishes (6.32 mL, Asahi Glass Co.) or 35-
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Fig. 1. Photograph of Cell Experiment Culture Chambers (CECC). The CECC, which was developed for space experiment consists of an upper plate, gas permeable membrane, gasket, middle plate, culture plate and lower plate. The CECC equips 2 ports to exchange the old medium for the fresh medium or the buffer. The specifications are follows: (a) dimensions of small type CECC: 55 x 70 x 12 mm, (b) dimensions of large type CECC: 55 x 122 x 12 mm.

mm tissue culture dishes (2.82 mL, Asahi Glass Co.), as the depth of medium was 3 mm. The L929, HepG2 and HIT-T15 cells were cultured for 3 d in a 37˚C incubator at 5% CO₂ in air, and the cell number was counted.

In long term culture of HepG2, the cell concentration was adjusted to 2.6 x 10⁴ cells/mL, and the cells were inoculated into DCCs (4.64 mL) and into 35-mm tissue culture dishes (2.82 mL) and counted at 4, 6 and 8 d after inoculation.

Oxygen concentration

The concentration of L929 cells were adjusted to 3.2 x 10⁴ cells/mL and inoculated into DCCs (4.64 mL) and into 35-mm tissue culture dishes (2.82 mL). The concentration of dissolved oxygen was measured using MicroX TX3 (PreSens, Germany) at 1, 3, and 5 d after inoculation. The sensor needle of MicroX TX3 was inserted through the septum port of DCCs and the silicone port of tissue culture dishes in which a hole had been previously made and filled with silicone gum (RTV). The distance of the tip of sensor needle to the cell layer was set to 0.5 mm.

Results

Development of DCC

Before developing the DCC, we first developed a closed culture chamber (Cell Experiment Culture Chamber, CECC) for cell culture (Fig. 1). The CECC is a reusable culture vessel and has already been used for cell culture experiments in the International Space Station (ISS) in March 2009 using A-6 cells and A-8 cells isolated from Xenopus (Dome Gean Project) and in May 2010 using gold fish scales (Fish Scales Project) (Suzuki et al., 2009). The medium in the CECC was exchanged to buffer by using a Pre-Fixation Apparatus (PFK), and the buffer was exchanged to the fixative by using a Cell Fixation Kit (CFK) in orbit (Yano, 2011).

The DCC is a disposable type culture vessel that takes advantage of CECC (Fig. 2). This chamber is previously sterilized by gamma radiation and is ready-to-use without the need for complicated setup. The DCC consists of a baseplate and gas-permeable film made from polystyrene and 2 septum ports made from silicone. The corona discharge treatment is applied to the baseplate to allow cell attachment as the CECC. The culture area is 15.5 cm² and the depth of medium is 3 mm. The cell inoculation or medium exchange is performed through the 2 septum ports with the syringes and needles. Commercially available 18-22 gauge needles are normally used and there is no leakage from the septum ports, even following repeated insertion and removal of needles.

Validation of DCC

The L929, HIT-T15 and HepG2 cells were cultured in DCCs for 3 d in a 37˚C humidified incubator in an atmosphere of 5% CO₂ in air. The morphology of cells cultured in the DCCs was not significantly different from that of cells cultured in 35-mm tissue culture dishes (Fig. 3). The growth of the cells in the DCCs was also not significantly different from that in the dishes (Fig. 4). In long term cultures of HepG2 in the DCCs, the growth rate is similar to that observed in culture dishes (Fig. 5). The cell morphology and growth were also tested in a Chinese hamster ovary cell line, CHO-K1, and the Madin-Darby canine kidney cell line, MDCK, and no differences were observed between DCCs and dishes in cell culture (data not shown). In addition, morphology and growth rate of L929 cells was not affected by culture in a DCC manufactured 2 years ago (data not shown). The concentration of dissolved oxygen (DO) in the culture medium was measured in L929 cells in DCCs and tissue culture dishes (Fig. 6). When the needle type DO sensor MicroX TX3 was used, the DO concentration in DCC was easily measured through the septum port. The DO concentration in the DCCs was similar to that in the dishes at 1, 3 and 5 d after inoculation. The cells
Fig. 3. Cells cultured in DCC and tissue culture dishes. The mouse fibroblast cell line (L929) cells were cultured 3 d in the DCC (A) or a culture dish (B). The hamster pancreatic β-cell line (HIT-T15) was cultured 3 d in the DCC (C) or a culture dish (D). The human hepatoma cell line (HepG2) was cultured 3 d in the DCC (E) or a culture dish (F). The cell density was adjusted to 0.96 x 10^4 cells/cm^2 for L929 and 3.83 x 10^4 cells/cm^2 for HIT-T15 and HepG2. Bar: 250 μm.

Fig. 4. Cell growth in DCC and tissue culture dish. (A) mouse fibroblast cell line (L929), (B) hamster pancreatic β-cell line (HIT-T15) and (C) human hepatoma cell line (HepG2) were cultured in DCCs and tissue culture dishes. The cell density was adjusted to 0.96 x 10^4 cells/cm^2 for L929 and to 3.83 x 10^4 cells/cm^2 for HIT-T15 and HepG2, and the cells were counted 3 d after inoculation. (n = 3)
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reached confluence at 5 d post-inoculation, and the DO concentration was 2.16 ± 0.2 ppm in DCCs. In general, the range of the optimal DO concentration required for cell growth was between 30% and 60% (Ozturk and Palsson, 1990). This is equivalent to a range between 2.06 and 4.12 ppm at 37 ºC.

The DCC complies with all of the requirements for space experiments that require mammalian cells. For example, the morphology and growth rate of the various cells cultured in DCC are not different compared with those in normal tissue culture dishes. Furthermore, the use of a completely closed chamber prevents any contamination and leakage. A transparent film and base plate enables simple microscopic observation. Finally, inclusion of two septum ports allows aseptic medium exchange or buffer addition. The DCC has already been used for the space experiments. The human neuroblastoma cell line, SK-N-SH was cultured in DCCs to investigate the biological effects of space radiation in March 2010 (Neuro Rad Project). The rat skeletal muscle cell line, L6G8C5 (L6) was also cultured in DCCs to investigate the effect of the Cbl-b ubiquitin ligase on microgravity-mediated muscle atrophy in March 2010 (Myo Lab Project, Harada-Sukeno et al., 2009).

The DCC will be useful not only for cell culture experiments in orbit, but also for the transportation of cells. In general, cells are frozen and kept on dry ice during transportation to prevent cell deterioration, but considerable time and effort are spent in order to prepare and ship these packages. To circumvent the freezing step and use cells immediately after arrival, it would be advantageous if cells could be incubated in normal culture conditions during the transportation. The DCC may be suitable for this purpose, because it prevents leakage of medium during transportation, and oxygen and carbon dioxide gas can be also be exchanged through the gas permeable film.

After standardizing these DCC properties, we now use the chambers in an automatic cell culture system (Koike et al., 2012). The DCC can keep itself aseptic, and the system can automatically transfer the chamber with no leakage.

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