eISSN (Online): 2598-0580

Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

The Potential of CO_2 Incubator "Sriwijaya CO_2 Incubator" Against Cell Culture Proliferation in Invitro Study as Smart Controlling-Based CO_2 Incubator for Cell Culture

Wardiansah[1*], Msy Rulan Adnindya[1], Rachmat Hidayat[2]

[1]Department of Anatomy, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia
[2]Department of Biology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

ARTICLE INFO

Keywords:
Incubator
In Vitro Study
Fibroblast Cell
Culture Cell
Cell Lines.

Corresponding author:
Wardiansah

E-mail address:
wardiansah@gmail.com

All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.32539/bsm.v5i3.207

ABSTRACT

Introduction. A CO_2 incubator is an essential tool for the initiation of the proliferation of primary culture cells or cell lines. In principle, this tool works by keeping the sample cell line at an optimum temperature of 37°C and 5% carbon dioxide supply. The ability of the CO_2 incubator to maintain temperature and supply of 5% carbon dioxide are essential points in the development of the CO_2 incubator. This study is an attempt to convince the potential of Sriwijaya CO_2 Incubator in maintaining the proliferation ability of cultured cells in an in vitro study. **Methods.** This study is an experimental pre-post test that explores the percentage of viability of primary culture cells (fibroblasts) before and after incubation in CO_2 incubators. The object of this study was fibroblast cells obtained from the prepuce of patients who performed circumcision. **Results.** Fibroblast cell proliferation in CO_2 incubators shows an increase in the number of fibroblast proliferation which can be seen with the increasing number of cells visualized by inverted microscopy. **Conclusion.** Sriwijaya CO_2 incubator has the potential to be used in in vitro research to trigger the growth and proliferation of fibroblast cells.
.

1. Introduction

A CO_2 incubator is an essential tool in the success of in vitro research using cell culture, either primary cell culture or cell line culture.[1] A CO_2 incubator is a tool used in the proliferation of primary cell culture or cell line. Utilization of primary and line cells is essential in biomedical studies or drug or vaccine development.[2] The study of cells is a starting point in conducting biomedical studies or in vaccine and drug development. The existence of a CO_2 incubator sometimes becomes a new problem in carrying out in-vitro studies with cultured cells. Quite a few laboratory facilities have difficulty procuring CO_2 incubators, due to limited funds for procuring the equipment. Sometimes researchers have to spend further effort and money to find a laboratory with CO_2 incubator facilities.

A CO_2 incubator is an essential tool for the initiation of the proliferation of primary culture cells or cell lines. In principle, this tool works by keeping the sample cell line at an optimum temperature of 37°C and 5% carbon dioxide supply.[3,4] The ability of the CO_2 incubator to maintain temperature and supply of 5% carbon dioxide are essential points in the development of the CO_2 incubator. Sriwijaya CO_2 Incubator is an incubator designed to work using a smart controlling system

controlled by an Arduino microcontroller. Arduino works automatically, controlling the temperature to be stable at 37°C and supply CO_2 at 5% position. This tool is a breakthrough for all researchers who want to have a CO_2 incubator at an economical price but still have good precision. This study is an attempt to convince the potential of Sriwijaya CO_2 Incubator in maintaining the proliferation ability of cultured cells in an in vitro study.

2. Research Methods

Study design

This study is an experimental pre-post test that explores the percentage of viability of primary culture cells (fibroblasts) before and after incubation in CO_2 incubators. The object of this study was fibroblast cells obtained from the prepuce of patients who performed circumcision, obtained from Dr Moh Hoesin General Hospital Palembang. This research has received approval from the Medical and Health Research Ethics Committee (No. 143 / KEPKK-FKUNSRI / X / 2020).

Fibroblast cell culture

Fibroblast cells were obtained from the prepuce of an 11-year-old boy patient. During the trip, the skins were stored in a transportation medium consisting of Dulbecco Minimal Essential Medium (DMEM Gibco USA®) 1 time, Penstrep 400 units-400µg / ml and Amphotericin B 10 µg / ml and carried in a box filled with ice. In the laminar flow cabinet, the peels were soaked in 10% povidone-iodine for 3 minutes, washed with phosphate buffer salt solution (PBS) 3 times for 10 minutes while being cleaned of heavy tissue debris. In the last washing, the epidermis is cut and removed; the dermis is taken and cut into pieces about 20 mm^3 in size, then cultured by explant technique (Viale, 2000). The pieces of the dermis are spread in a 25 cm^2 flask which has been filled with complete medium. The complete medium consisted of DMEM 1 times 87.950 ml, fetal bovine serum (Gibco USA®) 10 ml, amphotericin B (Fungizone-Gibco USA) 1 ml (250 µg/ml) and Pens-Strep (Gibco-USA) 1 ml (10,000 units / 10,000 µg / ml The cuttings of the dermis were incubated in an incubator at 370C and 5% CO_2 for 24 hours After 24 hours when the cut was attached to the base of the flask, the medium at the base of the flask was sucked off and replaced with 2 ml of complete medium until all the pieces submerged dermis.

Sriwijaya CO_2 incubator development

Sriwijaya CO_2 Incubator was developed as an environmentally friendly and very economical CO_2 incubator. Whereas an incubator box used styrofoam, which is then given a layer of thermal insulation on the part and we use the heatsink and DC fan 12 V from the old desktop computer Heatsink and DC fan mounted on the inner wall of the styrofoam. Furthermore, the DS18B20 temperature sensor is used, which is placed on the heatsink, which is located in the Styrofoam box. On the inside of the Heatsink in Styrofoam is placed a CO_2 sensor GC-0017.

To control temperature and CO_2 concentration in the Styrofoam box, use an Arduino microcontroller device. The Arduino microcontroller is coding the DC fan speed regulation as well as coding the CO_2 supply regulation into the Styrofoam box. Thus, Arduino plays a role in controlling fan speed for temperature regulation and controlling CO_2 supply.

Assessment of fibroblast viability

The procedure for measuring fibroblast proliferation in single-layer cultures refers to the 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide or abbreviated MTT protocol (catalogue no: CAS # 298-9391; Bio-Basic). In summary, the procedure is: the medium from each well is sucked and discarded, washed with sterile PBS 3 times (use a blue pipette tip connected to a sterile 23 G needle). The tip of the needle should not touch the bottom of the well. µl of the complete medium for fibroblast growth was added 50 µl of MTT solution with a content of 50 mg/ml in sterile PBS. The microplates were then wrapped in aluminium foil and incubated at 37 ºC 5% CO2 for 4 hours. Then, add 200 µl of Dimethyl Sulfoxide (DMSO) 99% solution and add 50 µl of glycine buffer on top. The blue colour in each well is read the optical density by spectrophotometer 570 nm. The principle of this

method is the redox reaction that takes place inside the cell. MTT (3- (4,5-Dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) is reduced to formazan salt by the enzyme succinate dehydrogenase present in the mitochondria of living cells. The reaction was allowed to occur for 4 hours then a stopper reagent was added. The stopper reagent will lyse the cell membrane so that the formazan salt can leave the cell and dissolve the formazan salt. The formazan salt formed is quantified by a spectrophotometer and measured in the form of absorbance. The higher the absorbance, the more cells are alive (high cell viability). The amount of fibroblast proliferation was measured by the proliferation index by comparing live cells in treated fibroblasts divided by living cells in normal fibroblasts, multiplied by 100%. The unit is a percentage.

Data analysis

Data analysis was performed with the help of SPSS Version 25 for Windows software. Percentage of each fibroblast cell culture was assessed for the percentage of viability then compared with the percentage of culture on days 1 and 5 after being inserted into the CO_2 incubator with the culture inserted into the CO_2 incubator using dependent analysis T-test, $p < 0.05$.

3. Results

Figure 1 shows a microscopic visualization of fibroblast cell proliferation in CO_2 incubators. Increasingly, it shows an increase in the number of fibroblast proliferation which can be seen with the increasing number of cells visualized by inverted microscopy.

Figure 2 shows the cell viability percentage of fibroblast cells. The graph shows that day by day, fibroblast cell proliferation increases. The statistically very significant increase was shown on the proliferation of day 5, which increased significantly compared to day 1, $p < 0.00$.

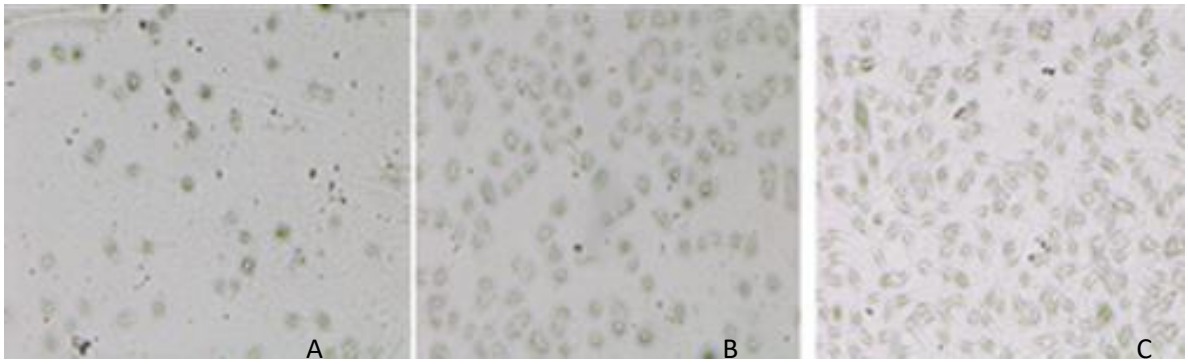

Figure 1. Cell viability in MTT Assay. A. 1 days. B. 3 days. C. 5 days

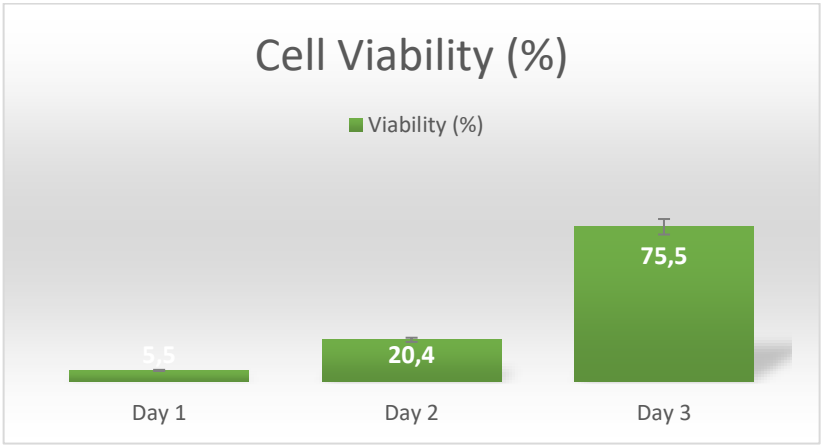

Figure 2. Percentage Cell Viability in MTT Assay

4. Discussion

Sriwijaya CO_2 Incubator is a self-develop incubator by utilizing an Arduino microcontroller to maintain consistency of CO_2 supply and maintain a stable temperature at the incubator. The use of thermal insulators is quite effective in keeping the temperature and CO_2 supply stable. The use of Arduino is quite effective in controlling CO_2 supply and temperature from the incubator box, proven by the effectiveness of Sriwijaya CO_2 incubator in triggering the proliferation of fibroblast cell cultures. The success of temperature sensors and CO_2 sensors in maintaining and controlling temperature and CO_2 concentration in the box dramatically affects the success of this incubator to function correctly and optimally. The optimum temperature of 37°C is the optimal temperature in triggering various cellular metabolic reactions, where the uncontrolled decrease or increase in temperature causes cell failure in metabolism which leads to failure of cells to grow and proliferate. Likewise, CO_2 is an important gas that plays a role in various metabolic reactions of cells. Excess or lack of CO_2 concentration causes the failure of cells to grow and proliferate.[5-10]

5. Conclusion

Sriwijaya CO_2 incubator has the potential to be used in in vitro research to trigger the growth and proliferation of fibroblast cells.

6. References

1. Mullis KB. The unusual origin of Incubator CO2. Scientific American. 1990;262(4):56–61. 64–5. [PubMed] [Google Scholar]
2. Weier HU, Gray JW.Incubator CO2. DNA. 1988;7(6):441–7. [PubMed] [Google Scholar]
3. Dworkin AM, et al. Merkel cell polyomavirus in cutaneous squamous cell carcinoma of immunocompetent individuals. J Invest Derm. 2009;129(12):2868–74.[PubMed] [Google Scholar]
4. VanGuilder HD, Vrana KE, Freeman WM.Cell Cultures. BioTechniques. 2008;44(5):619–26. [PubMed] [Google Scholar]
5. Valasek MA, Repa JJ. Incubator CO2. Advances in physiology education. 2005;29(3):151–9. [PubMed] [Google Scholar]
6. Stahlberg A, Thomsen C, Ruff D, et al. Incubator CO2 analysis of DNA, RNAs, and Proteins in the same single cell. Clin Chem. 2012;58:1682–1691. [PubMed] [Google Scholar]
7. Botes M, de Kwaadsteniet M, Cloete TE. Application of Incubator CO2 for the detection of microorganisms in water. Food Microbiol. 2011 Aug;28(5):848–61.[Google Scholar]
8. AUTHOR/S. TITLE OF CHAPTER. In: Bolognia J, Jorizzo J, Rapini R, editors. Dermatology. Vol. 2008. Elsevier; Spain: 2008. PAGE RANGE. [Google Scholar]
9. Postollec F, Falentin H, Pavan D, et al. Recent advances in quantitative PCR (qPCR) applications in food microbiology. Food Microbiol. 2011 Aug;28(5):848–61.[PubMed] [Google Scholar]
10. Smith C, Osborn M. Advantages and limitations of quantitative PCR (qPCR)-based approaches in microbial ecology. F
11. EMS Micro