CULTURE OF NINE HUMAN CELL LINES IN AN ESCO CELCULTURE® CO_2 INCUBATOR FOR NATURAL PRODUCT RESEARCH

Abstract

The enormous chemical diversity of natural products makes them an interesting subject for drug discovery. For instance, plant derivatives are tested on human cell lines to assess their therapeutic potential. In the study cited here, nine human cancer cell lines were cultivated in an Esco CelCulture® CO_2 incubator prior to use for the cytotoxic assay of 11 plant xanthone derivatives. Such study demonstrates the use of the CelCulture® for the successful culture of different cell lines in natural product research and cancer drug discovery.

Introduction

For the past decades, natural products have captured the interest of the scientific community as a rich source of potential therapeutic compounds. Natural products have such enormous chemical diversity that they continue to be of relevance in modern drug discovery.[1] One of the most active fields of natural product research is the screening of plant-derived compounds for cancer therapy.

This was the goal of a recent study that screened the chemotherapeutic potential of 11 compounds derived from *Mesua beccariana, Mesua ferrea,* and *Mesua congestiflora*. These compounds are xanthone derivatives, identified as mesuarianone, mesuasinone, mesuaferrin, mesuaferrin B, mesuaferrin C, 6-deoxyjacareubin, caloxanthone C, macluraxanthone, 1,5-dihydroxyxanthone, tovopyrifolin C, and α-mangostin. These compounds were tested on nine human cancer cell lines which include Raji, SNU-1, K562, LS-174T, SK-MEL-28, IMR-32, HeLa, Hep G2, and NCI-H23. These cell lines were maintained in an Esco CelCulture® CO_2 Incubator (CCL-170B-8).[2]

This application note shall review the cell culture requirements of these nine cell lines[3] and how the CelCulture® CO_2 Incubator was able to meet these needs, ultimately contributing to the success of screening the chemotherapeutic potential of the tested natural products.

Raji cell line

The Raji (ATCC® CCL-86™) cell line is a human lymphoblast cell line, specifically B lymphocyte cells exemplifying Burkitt's lymphoma. According to the ATCC website, the Raji cell line is a suitable transfection host. Raji cells must be maintained in RPMI-1640 medium with fetal bovine serum (final concentration of 10%). Cultures must be kept at 95% humidity, 5% CO_2, and 37°C.

SNU-1 cell line

The SNU-1 (ATCC® CRL-5971™) cell line is derived from human stomach epithelium exhibiting gastric carcinoma. These cells express surface glycoproteins carcinoembryonic antigen (CEA) and TAG72. The oncogenes myc+ and erb B2 + are also expressed. RPMI-1640 is the base medium and fetal bovine serum is added to a final concentration of 10% to complete the growth medium. Cells must be maintained at 95% humidity, 5% CO_2, and 37°C.

[1] Koehn and Carter 2005.
[2] Teh et al. 2013.
[3] ATCC website.

K-562 cell line

The K-562 (ATCC® CCL-243™) cell line is a human bone marrow lymphoblast cell line with chronic myelogenous leukemia (CML). These cells are to be maintained in Iscove's Modified Dulbecco's Medium (Catalog No. 30-2005). Fetal bovine serum must be added to a final concentration of 10% to complete the growth medium. Cells must be kept in an atmosphere with 95% humidity, 5% CO_2, and 37°C.

LS-174T cell line

LS-174T (ATCC® CL-188™) is a human colorectal adenocarcinoma (Dukes' type B disease) cell line. Culture requires Eagle's Minimum Essential Medium (Catalog No. 30-2003) supplemented with fetal bovine serum to a final concentration of 10%. LS-174T cells are to be maintained in an atmosphere with 95% humidity, 5% CO_2, and 37°C.

SK-MEL-28 cell line

The SK-MEL-28 (ATCC® HTB-72™) cell line is derived from human skin exhibiting malignant melanoma. This cell line could be used in transfection studies as this is a suitable host for transfection. Cells should be grown in Eagle's Minimum Essential Medium (Catalog No. 30-2003). Fetal bovine serum is to be added to complete the growth medium (final concentration of 10%). Cultures should be maintained at 95% humidity, 5% CO_2, and 37°C.

IMR-32 cell line

IMR-32 (ATCC® CCL-127™) is a human neuroblastoma cell line. This cell line could be used for transfection studies as host. The base medium for this line is the Eagle's Minimum Essential Medium (Catalog No. 30-2003) supplemented with fetal bovine serum to a final concentration of 10%. Cells must be maintained in a physical environment of 95% humidity, 5% CO_2, and 37°C.

HeLa cell line

The HeLa (ATCC® CRM-CCL-2™) cell line is a human cervical adenocarcinoma. Aside from being a suitable transfection host, this cell line is also useful for the screening of strains of *Escherichia coli* with invasive potential. Cultures should be maintained Eagle's Minimum Essential Medium (Catalog No. 30-2003) added with fetal bovine serum to a final concentration of 10%. Cells should be kept in 95% humidity, 5% CO_2, and 37°C.

Hep G2 cell line

Hep G2 (ATCC® HB-8065™) is a human hepatocellular carcinoma cell line. These cells are useful as transfection hosts. Cells are to be cultured in Eagle's Minimum Essential Medium (Catalog No. 30-2003). Fetal bovine serum must be added to a final concentration of 10% for a complete growth medium. Cells must be maintained in 95% humidity, 5% CO_2, and 37°C.

NCI-H23 cell line

The NCI-H23 (ATCC® CRL-5800™) cell line is derived from human lung epithelium exhibiting non-small cell lung cancer. This cell line thrives in RPMI-1640 Medium supplemented with fetal bovine serum to a 10% final concentration. Cells should be maintained at 37°C.

The abovementioned cell lines were used to screen for the cytotoxic activities of the 11 xanthone derivatives from *Mesua beccariana*, *Mesua ferrea* and *Mesua congestiflora*. MTT assay was employed to determine

the IC_{50} values of the compounds. Xanthone derivatives with diprenyl, dipyrano, and prenyl-pyrano substituent groups demonstrated cytotoxicity on almost all of the nine cell lines.[2]

CelCulture® CO_2 Incubator

Prior to the MTT assay, all nine cell lines used in the study were incubated in Esco CelCulture® CO_2 incubator (CCL-170B-8). The CelCulture® was able to maintain optimum growth conditions for all the cell lines: 37°C, 5% CO_2, and 95% humidity (Fig. 1).

The precise parameter control and uniformity of the CelCulture® CO_2 incubator ensures that all culture flasks are maintained at the same temperature, CO_2, and humidity conditions. Optimum temperature conditions are achieved via microcontroller PI control, direct heating, and air jacket insulation. The CO_2 value inside the chamber is precisely monitored by the IR CO_2 sensor. Humidified air is constantly delivered to all parts of the chamber via the forced convection method.

The robust contamination methods of the CelCulture® also contribute to the successful culture of all the nine cell lines. An ULPA filter, with 99.999% efficiency, maintains a sterile chamber. The chamber is maintained free of contamination with the 90°C moist heat decontamination cycle. This cycle runs overnight. The chamber is dry and clean at the end of the cycle. The outer body of the CelCulture® is protected from the growth of surface bacteria through the ISOCIDE™ antimicrobial powder coating that eliminates 99.9% of surface bacteria within 24 hours of exposure.

Fig. 1. The CelCulture® CO_2 Incubator (CCL-170B-8) for natural product research.

For studies with multiple cell lines, it would be beneficial to use a CO_2 incubator with multiple inner glass doors to avoid cross-contamination. Cross-contamination of cell lines has long been recognized as a problem in cell culture.[4] Multiple inner glass doors minimize the contact between flasks containing different cell lines and decrease the risk of mistaking one cell line for another.

[4] Nelson-Rees et al. 1981.

This multiple inner door design is available for Esco CelCulture® CO_2 incubators (Fig. 2). Four separate glass doors can be opened horizontally to allow access to defined sections of the incubator without affecting much the inner atmosphere of the chamber. This minimizes recovery time and contamination risks. The sealed inner door is also reversible as same as the outer door which can be installed to be opened either from right-to-left or from left-to-right.

Fig. 2. The CelCulture® CO_2 Incubator is available in the multiple inner door design, which minimizes cross-contamination of cell lines.

Conclusion

Natural product research contribute significant leads on potential chemotherapeutic substances. For instance, xanthone derivatives from *Mesua beccariana, Mesua ferrea,* and *Mesua congestiflora* showed cytotoxic activity on nine human cancer cell lines. These cell lines were cultivated in an Esco CelCulture® CO_2 incubator prior to MTT cytotoxic assay. This highlights the significance of the CelCulture® for the successful culture of different cell lines in natural product research and cancer drug discovery.

References

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