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Review article

Application of standard cell cultures and 3D *in vitro* tissue models as an effective tool in drug design and development

Aleksandra Amelian[a,b], Katarzyna Wasilewska[a], Diego Megias[c], Katarzyna Winnicka[a,*]

[a] Department of Pharmaceutical Technology, Medical University of Bialystok, Bialystok, Poland
[b] Department of Clinical Pharmacy, Medical University of Bialystok, Bialystok, Poland
[c] Confocal Microscopy Core Unit, Spanish National Cancer Research Centre, Madrid, Spain

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ABSTRACT

Cell culture systems are essential tools used in a wide range of biomedical and clinical studies. Two dimensional cell culture models (2D) provide basic information on cytotoxicity, penetration and accumulation of drugs in cells and they are of outmost importance when selecting new compounds of the desired biopharmaceutical properties as candidates for novel drugs. The improvement over 2D growing cells are three dimensional (3D) tissue models that mimic *in vivo* conditions and the functions of living tissue more accurately. These models reduce the cost of drug development, enable more efficient drug screening, minimise failure rate in medicine discovery and eliminate animal use during experiments. The article provides an overview of 2D cell cultures and 3D tissue models – their properties, basic procedures, conditions of culturing and applications.

Contents

Introduction

Two- (2D) and three-dimensional (3D) culture models constitute a basic tool in many fields of biomedical sciences. Cell cultures provide a fluent connection between biochemical (*in vitro*) and *in vivo* studies [1–3]. Properly selected and cultured cells allow one to answer a number of fundamental questions of pharmacological

* Corresponding author.
E-mail address: kwin@umb.edu.pl (K. Winnicka).

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and technological nature and are necessary to determine drug safety and the mechanism of its action. For this purpose, investigated substances and their precursors or metabolites are added to the culture medium and thus their action at the cellular or molecular level might be explained. The use of cellular models in toxicological studies exhibits many advantages, such as study simplicity of cellular and molecular processes, repeatability and the possibility of using small amounts of tested substances. Experiments conducted under controlled conditions enable the screening of newly synthesised groups of compounds, significantly reducing the cost incurred in expensive and time-consuming *in vivo* studies on animals [1,4,5], which require the Ethical Committee approval.

Rapid development of biotechnology has enabled the isolation and culturing of many types of cell lines. Cell lines can be purchased from cell line banks of which the best known are: The European Collection of Authenticated Cell Cultures (ECACC) established in Great Britain and the American Type Culture Collection (ATCC) [6,7], which is one of the largest animal cell line collection centres in the world. The choice of an appropriate cell line is primarily determined by its origin. In medicine and pharmacy, cells of human lines are most commonly used [3,4,8,9]. For example, to examine the penetration of a drug through the skin, mainly HaCaT cells are utilised due to their phenotypic similarity to keratinocytes [3,4]. The most frequently used cell lines are presented in Table 1.

Recently, the employment of stem cells for drug discovery and development has been discussed. Multipotent adult stem cells can be derived from organs or bone marrow and human embryonic stem cells (hES) – from foetal tissues or cord blood. As stem cells have a capacity of a potentially unlimited number of divisions and differentiate into cells of other types with more specialised function, they seem to be ideal *in vitro* models for drug efficacy and toxicity testing [10]. Moreover, stem cells can be genetically modified using reporter gene construct to improve efficiency of the tests and to provide specific disease models [11].

Types of cell culture models

Depending on the cultivation method, several culture models can be distinguished. Primary cultures are derived from cells isolated directly from small pieces of tissues or organs. These cells possess the ability to adhere to a surface and are known as adhesive cells. However, some cell lines can proliferate in suspension. Constant maintenance of medium movement provides the conditions for optimal growth and sustains an appropriate environment for suspension culture. Cells are incubated in special culture plates with embedded magnetic stirrers and oars in order to provide circulation of medium [12].

Another example of a cell culture model are cultures on microcarriers. These are cultures of adhesive cells on gelatin, porous or dextran carriers. Microcarriers provide an increased growth surface area in a small medium volume and therefore 20–50 times greater density of cell cultures can be obtained [13]. The application of cultures on microcarries was initiated by Wetzel who introduced dextran beads (Sephadex A-50) into cell suspension [14]. These carriers are suitable for cultivation in suspension with stirring, as well as in the flow system. In addition, this system protects cells from becoming damaged [12–15].

Spheroids, another example of a cell line culture, are characterised as cultures in which mutual spatial associations between cells are reproduced. Multicellular tumour spheroids exhibit features characteristic of tumour growth *in vivo* in the early stage of its development and therefore are regarded as a form between a monolayer cell culture and a spontaneously growing tumour. Morphologically, 3D-cell cultures are comprised of cells with different phenotypes which are in the quiescence phase of the cell cycle, proliferating and located in the centre of a spheroid cell with necrotic changes, due to a decrease in oxygen partial pressure towards the centre of the spheroid. Dividing cells occur mainly in 3–5 external layers [16,17].

Cell lines derived from normal diploid cells and tissues can be cultured during a limited period of time (finite cell lines). After 50 generations, diploid cells usually spontaneously die or undergo neoplastic transformation followed by the formation of a continuous culture. By subculturing of more than 70 times, a permanent cell line can be obtained. Continuous cell lines (immortal lines) are characterised by an indeterminate lifespan. Cells derived from continuous cell lines are usually polyploid, and of cancerous origin, providing the opportunity for indefinite divisions. Continuous cell lines can also be obtained from normal cells as a result of their transformation. The most popular continuous tumour cell line is HeLa isolated from an epithelial cervix tumour from a 31-year-old patient Henrietta Lacks, after whom the cells were named [5,18,19]. HeLa cell lines division is shown in Fig. 1.

Culture conditions

Air laiquid interface (ALI) is the most appropriate (both for 2D and 3D cell culture models) for stimulating conditions closely reflecting those *in vivo* [20,21] (Fig. 2).

A cell culture is preceded by the collection of an appropriate tissue or organ from the human organism. Next, isolated cells are cultured at $37\,^{\circ}C \pm 0.5$ in special incubators with controlled gas atmosphere providing 5% CO_2 which mimics the blood environment and replicates the *in vivo* conditions in which cells are able to survive, divide and differentiate.

Culture media developed for *in vitro* studies offer environmental conditions similar to those prevailing *in vivo*. The culture is a source of nutrients which contribute to the maintenance of physiological pH and osmolality [2,3]. The composition of culture

Table 1
Characteristic of selected human cell lines [3–5].

Cell Line	Origin	Application
HeLa, A431	Epithelial cervical cancer	Very rapid growth, cells commonly used in cancer research
Caco-2, HT29, HCT–116	Colon adenocarcinoma	Studies of absorption *via* intestinal epithelium, toxicity tests
U2OS	Osteosarcoma cells	Studies of transport and absorption of drugs
SkBr3, MCF-7, MDA-MB231, ZR-75-1	Breast cancer	Studies of transport and absorption of drugs, screening anticancer compounds
Calu-3	Serous cells of submucosal gland	Studies of absorption *via* bronchial epithelium, metabolic and transport model to study drug delivery to the respiratory epithelium
HaCaT	Adult human skin	Penetration of drug through the skin
16HBE140	Bronchial epithelium	Studies of absorption and excretion through the bronchial epithelium