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Review

Rationale employment of cell culture versus conventional techniques in pharmaceutical appraisal of nanocarriers

Manal A. Elsheikh [a,b], Yosra S.R. Elnaggar [a,*], Ossama Y. Abdallah [a]

[a] Department of Pharmaceutics, Faculty of Pharmacy, Alexandria University, Egypt
[b] Department of Pharmaceutics, Faculty of Pharmacy, Damanhour University, Egypt

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ABSTRACT

Nanomedicines are enjoying a widespread popularity realizing their intriguing potential to solve drug delivery obstacles. Assessment of major quality attributes of nanocarriers is a crucial process for approving their therapeutic outcomes. Disparate assessment methods that recently encompassed cell line technique were employed . Routinely, a cell line model was viewed as an excellent platform for gene and vaccine deliveries. However, its application in pharmaceutical assessment of nanocarriers was not so far overviewed. This review provides a meticulous look at cell culture implementations in evaluation of major quality attributes of nanocarriers, including oral permeability, cytotoxicity and efficiency of tumor targeting. Among others, cell culture technique strikes the right balance between predictability and throughput. It could circumvent drawbacks of in-vivo and in-vitro techniques while gathering privileges of both. Imperative pharmaceutical considerations demanded for proper application of this technique were emphasized. Furthermore, challenges encountered in assessment of versatile nanocarriers were highlighted with proposed solutions. Finally, future research perspectives in this theme issue were suggested.

Contents

Abbreviations: P-gp, P-glycoproteins; BEs, bioactive excipients; AP, apical; BL, basolateral; Papp, apparent permeability; CYP3A4, cytochrome P450 3A; HCC, hepatocellular carcinoma; BBB, blood–brain barrier; ER, Estrogen receptor.

* Corresponding author at: Department of Pharmaceutics, Faculty of Pharmacy, Alexandria University, 1 Khartoum Square, Azarita, Messalla Post Office, P.O. Box 21521, Alexandria, Egypt. Tel.: +20 1147591065; fax: +20 3 4873273.
E-mail address: yosra_pharm@yahoo.com (Y.S.R. Elnaggar).

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1. Introduction

Major restrains of new and old drug moieties are manifested in poor oral bioavailability, harmful side effects, and lack of tissue targeting. Nanomedicines have been recently employed to circumvent therapeutic obstacles of drug delivery. Unique characteristics of such nanoplatforms like nanometric range, content of bioactive excipients (BEs), and amenability for surface modification endow them to face numerous therapeutic challenges. Versatile classes of nanocarriers with desperate properties comprised the core of our recent research, including nanoemulsions, self-nanoemulsifying drug delivery system [1,2], liposomes and its modifications [3–5], solid lipid nanoparticles and nanostructured lipid carriers [6,7]. In addition, polymeric nanoparticles [8,9], metal-based nanocarriers [10], and inorganic nanocarriers (carbon nanotubes) are attracting intensified interest as well [11].

Proper assessment techniques should be adopted to guarantee therapeutic outcomes of nanocarriers. In the field of oral nanomedicine, assessment of intestinal permeability is the most indicative for bioavailability and efficacy. Accordingly, many attempts were implemented to circumvent permeability related hurdles. These could be achieved either via inclusion of bioactive excipients (BEs) in the nanosystem or by using the whole system for lymphatic targeting. On the other hand, in view of parenteral nanocarriers, appraisal of targeting and uptake efficiency is the most crucial parameter. In particular, high mortality rates were reported from side effects of anti-cancer drugs lacking targeting property. Thus, appraisal of targeting efficiency of parenteral nanocarriers via both passive and active mechanisms is essential as well. For both cases (oral and parenteral nanocarriers) cytotoxicity assessment would be badly required. When an already existing molecule or compound, is reintroduced in a nanomaterial form, it is considered as new chemical entity. Therefore, an update has to be performed to include nanosystem specific properties and toxicities [11,12] that was recognized as nanotoxicology. In a consequence, characterization of major quality attributes of nanocarriers, including permeability, cytotoxicity and targeting potential deemed crucial for fulfilling proposed therapeutic outcomes via different administration routes.

In the context of nanocarrier assessment, different in-vivo and in-vitro methods have been employed. In recent research investigations, cell culture technique could circumvent the drawbacks of in-vivo and in-vitro methods while gathering privileges of both. Cell culture technology was extensively employed in biochemical and immunological research disciplines. It is one of the major tools used in cellular and molecular biology, providing excellent model systems for studying the normal physiology and biochemistry of cells (e.g., metabolic studies, aging), mutagenesis and carcinogenesis. In addition, cell culture medium was used in Hybridoma technology for mass production of monoclonal antibodies. Besides, cultured cells are used in viral vaccine production and recombinant DNA technology either as a source of mRNA, gene sequence or as vector. Recently, such techniques gained an enormous interest in the field of drug development and pharmaceutical assessment of dosage forms with a recent highlight on nanocarriers [13].

Cell culture is the process by which cells are cultivated under controlled conditions outside the living animal (in vitro) for easier experimental manipulation and regulation of controls [14]. This technique offers privileges of reducing the animal's need, so it avoids the legal and ethical problems of animal experimentation and related variations. In addition, it avoids interference from biological molecules that occurs in vivo, so different secretions and elements will be feasibly monitored. Furthermore, homogeneous population of cells with similar growth requirements could be obtained. Therefore, cell characteristics will be maintained over several cell generations producing consistent and reproducible results [13–15].

Albeit cell culture technology was extensively utilized as a tool in biomedical field, their pharmaceutical applications were not so far overviewed. The rationale of the current review was to give an overview on implementations of cell culture models in pharmaceutical assessment of nanomedicines, with high emphasis on permeability assays, cytotoxicity studies and targeting efficiency as major quality attributes.

2. Terminology involved in cell culture methodologies

Before proceeding in cell culture applications in nanomedicine, it may be useful to summarize commonly used terminology of cell and tissue culture. The following definitions will help to explain different types of cultured cell, how to grow and expand cell population, in addition to cell distribution in monolayer and multilayer culture.

2.1. Primary culture

These are cells derived directly from intact or dissociated tissues or organ fragments taken directly from an animal. The cells have heterogeneous nature, and retain many of the differentiated characteristics of the original cells in vivo. However, most have a limited life span and ultimately die. Until subculture, a culture is considered a primary one, after that it is termed a cell line [13].

2.2. Cell line

These cells are subcultured from primary cells but undergo manipulation in the laboratory so that they can be propagated and passaged indefinitely with no apoptosis. The term cell line denotes that it consists of lineages of cells that originally exist in the primary culture, it also could be referred as Established or Continuous cell line. Unlike the unmanipulated primary cells, cell line may not mimic the original cells in in vivo cells–after several passages–due to their manipulation. Tumor cell lines are often derived from actual clinical tumors, for example Coca-2 cell line which is derived from human colonic adenocarcinoma, while in transformed cell lines, the transformation may be induced using viral oncogenes or by chemical treatment. For example Hela cell line (human epithelial cervical carcinoma) is transformed by human papillomavirus 18 (HPV18) to immortalized cell line. Most normal cells undergo limited number of subcultures, or passages before they stop growing due to senescence, so that they are referred to as finite cell lines, while tumor or transformed cell lines can divide more rapidly, indefinitely, and form tumors when reintroduced into animals. Another type of cell lines includes clonal cell line which is a cell population derived from a single cell by successive mitoses forming a genetically homogeneous population [13].

2.3. Sub-culture (passage, splitting)

Sub-culture is the dissociation of cells from each other and from substrate using proteolytic enzymes. Trypsin and/or EDTA are most commonly used in this process, so it is called trypsinization. Afterward, these cells are transfered or transplanted to a new culture vessel with nutrition media so as to propagate and expand the cell population for study. Reseeding this cell suspension generates a secondary culture, which can be grown up and subcultured again to give a tertiary culture, and so on. It is essential to determine, for each cell the type, source, and

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