eVitroKhem. 2025; 4:241
doi: 10.56294/evk2025241

REVIEW

Medical Applications and Challenges of Cell Culture Systems

Aplicaciones médicas y desafíos de los sistemas de cultivo celular

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Cite as: Gonzalez-Argote J. Medical Applications and Challenges of Cell Culture Systems. eVitroKhem. 2025; 4:241. https://doi.org/10.56294/evk2025241

Submitted: 01-10-2024 **Revised:** 07-02-2025 **Accepted:** 23-07-2025 **Published:** 24-07-2025

Editor: Ahmed M. Abu-Dief

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ABSTRACT

Introduction: cell culture systems allow investigation of physiological, pathological, and pharmacological processes under controlled conditions, tracing back to techniques developed since the early 20th century and enhanced by the discovery of induced pluripotent stem cells in 2006.
Method: a total of 16 articles in Spanish and English were retrieved from Scopus, Science, and Springer using the keywords "2D cell culture," "3D cell culture," "regenerative medicine," and "drug screening," with more than 60 % published in the last five years.
Results: two-dimensional cultures are characterized by simplicity, low cost, and scalability in cytotoxicity assays and drug screening, although they exhibit low biological fidelity. Three-dimensional systems better reproduce tissue architecture, generate nutrient and oxygen gradients, and promote more physiological gene expression profiles, making them suitable for tissue engineering, organoid development, and regenerative medicine. Identified applications include monoclonal antibody production, vaccines, cell therapies, and viral diagnostics. However, technical complexity, matrix standardization, and reproducibility remain limiting factors.
Conclusions: cell culture techniques enable in vitro study of cellular physiology and pathology. Two-dimensional monolayers and three-dimensional constructs offer distinct advantages and limitations. Their applications include drug screening, regenerative medicine, vaccine production, and diagnostics.

Keywords: 2D Cell Culture; 3D Cell Culture; Regenerative Medicine; Drug Screening.

RESUMEN

Introducción: los sistemas de cultivo celular permiten investigar procesos fisiológicos, patológicos y farmacológicos en entornos controlados, remitiendo a técnicas desarrolladas desde principios del siglo XX y potenciado por el descubrimiento de las células iPSCs en 2006.
Método: un total de 16 artículos en español e inglés fueron extraídos de Scopus, Science y Springer; utilizando como palabras clave: cultivo celular 2D, cultivo celular 3D, medicina regenerativa, cribado de fármacos; siendo más del 50 % de los últimos cinco años.
Resultados: los cultivos 2D destacan por su simplicidad, bajo coste y escalabilidad en ensayos de citotoxicidad y cribado de fármacos, aunque presentan baja fidelidad biológica. Los sistemas 3D reproducen mejor la arquitectura tisular, generan gradientes de nutrientes y oxígeno y favorecen perfiles de expresión génica más fisiológicos, siendo idóneos en ingeniería tisular, desarrollo de organoides y medicina regenerativa. Se identificaron aplicaciones en producción de anticuerpos monoclonales, vacunas, terapias celulares y diagnóstico virológico. Sin embargo, la complejidad técnica, la estandarización de matrices y la reproducción de resultados siguen siendo limitantes.
Conclusiones: las técnicas de cultivo celular permiten el estudio in vitro de la fisiología y la patología

celular. Las monocapas bidimensionales y los constructos tridimensionales ofrecen ventajas y limitaciones distintas. sus aplicaciones incluyen el cribado de fármacos, la medicina regenerativa, la producción de vacunas y el diagnóstico.

Palabras clave: Cultivo Celular 2D; Cultivo Celular 3D; Medicina Regenerativa; Cribado de Fármacos.

INTRODUCTION

This novel technology includes culturing cells outside of their natural biological context, giving researchers a controlled environment in which to investigate cellular responses under diverse conditions.[1]

Cell culture technology has altered how we see and study cellular behavior, disease processes, and therapeutic interventions.[1] It refers to laboratory methods that enable the growth of eukaryotic or prokaryotic cells in physiological conditions.[2] Others consider it as the removal of animal cells and its propagation and cultivation in vitro in an artificial environment that is suitable for its growth.[3]

The roots of cell culture can be traced back to the late 19th century when Wilhelm Roux and Ross Harrison cultured nerve tissue and frog embryo cells, respectively. This technique was first developed in the early 20th century as a method of studying animal cell behavior in vitro.[1,3,4] In 2006, the work of Takahashi and Yamanaka introduced induced pluripotent stem cells (iPSCs), a transformative concept that facilitated the reprogramming of somatic cells into a pluripotent state, bypassing ethical concerns associated with embryonic stem cells.[5]

This technique involves the growth and maintenance of cells in a controlled environment outside of their natural context, offering insights into cellular physiology, replicate disease mechanisms, novel drug testing, and regenerative medicine.[1,2] One of the goals of regenerative medicine is to produce differentiated cells in the laboratory that can be used to heal tissues when they have lost the ability to regenerate for themselves.[6,7]

Culture systems are an important research tool because of enabling supervision of the conditions that the cells are in. Especially the examination of normal cellular processes' cell culture systems serves too many fields such as the toxicology studies, development of new drugs, and treatment methods.[8]

Cell culture systems are indispensable tools in modern biological and biomedical sciences, offering controlled platforms for the in vitro study of cellular processes, pharmacodynamics, and disease mechanisms. A bibliographic review was carry out, the objective was to describe the medical applications and technical challenges of culture systems.

METHOD

A bibliographic investigation was carried out. To this end, a search was conducted in databases such as PubMed, Scopus, and Springer using the following keywords: 2D cell culture; 3D cell culture; Regenerative medicine; Drug screening. These terms were used in combination with the connector "and." A total of 16 articles were selected, more than 60 % of which were published in the last five years, in both Spanish and English. Relevant information was extracted, and after organizing and synthesizing it, a well-structured development was achieved.

RESULTS

When tissues degenerate or become damaged, the affected cells have to be replaced so the tissues can keep performing their roles. This regenerative potential exists thanks to populations of stem cells in each tissue, which can divide to produce more stem cells maintaining a constant pool of stem cells for repair or differentiate into specialized cells to replace damaged cells.[6]

Cell differentiation is a common situation that is provided by internal cellular program and environmental conditions. Knowing the characteristics and factors of cellular differentiation enables the cells that are studied in the cell culture to be easily manipulated.[8] Cell structure changes can influence nuclear morphology, which may alter the transcription and translation of genes.[4]

The requirements that cells and cell- derived products need to meet to be used in the clinic are changing rapidly, and the tests used to assess these requirements can also propagate variability.[6]

Cells cultured in the lab can be classified into three different types: primary cells, transformed cells, and self-renewing cells. Primary cells, such as fibroblasts obtained from skin biopsies and hepatocytes isolated from liver explants, are directly isolated from human tissue. Transformed cells can be generated either naturally or by genetic manipulation. Self-renewing cells include, for example, embryonic stem cells, induced pluripotent stem cells, neural and intestinal stem cells. These cells carry the capacity to differentiate into a diversity of other cells types, while their self-renewing property allows for long-term maintenance in vitro.[2] Segregated according to their differentiative potential, stem cells delineate into totipotent stem cells, pluripotent stem cells, multipotent stem cells, and unipotent stem cells.[5]

System characteristics

Factors such as extracellular matrix (ECM) composition, stiffness, and mechanical forces are key in determining the differentiation pathways of stem cells. For instance, variations in ECM stiffness can direct stem cells toward specific lineages; softer matrices tend to promote neurogenic differentiation, while stiffer matrices support osteogenic differentiation. Mechanical forces like wall shear stress and circumferential strain also significantly influence stem cell fate by modulating cellular signaling pathways and gene expression. These mechanical cues, in combination with the biochemical environment, create a complex microenvironment that ultimately governs stem cell behavior and differentiation.[5]

The goal to create an environment that allows for maximum cell propagation is achieved primarily through the incubator (i.e., temperature, humidity, O2, and CO2 tensions) and the basal cell culture medium and its supplements. This includes not only the supply of nutrients such as carbohydrates, vitamins, amino acids, minerals, growth factors, hormones, but also components that control physicochemical properties such as the culture's pH and cellular osmotic pressure.[2]

The choice of a cell line for cell culture depends heavily on the functional properties and specific readouts required of the cell model.[2] To culture most of the cell types outside of a living body, artificial devices are usually required to allow the cells to adhere and grow. Glass devices such as coverslips were most commonly used in the first few decades of cell culture history. Later, plasma treated polystyrene was invented by the Falcon Plastics Company and showed excellent properties for cell adhesion and growth.[4]

In terms of cell culture experiments, digital microfluidics has various advantages over typical enclosed microfluidic chips. One key advantage is the capacity to pinpoint and manipulate specific chemicals and cells. The use of sub-microliter volume droplets reduce reagent use while matching the scale of an experiment to the size of cells, allowing for precise and efficient cell handling. This property makes DMF an invaluable tool for studying smaller cell populations, and for examining cell heterogeneity within populations.[9,10]

Types

There are several ways to culture stem cells, depending on the source of the cells themselves.[5,11]

Primary cell culture: Primary cells are directly isolated from tissues and have a finite lifespan in culture. They closely resemble them in vivo counterparts and are ideal for studying cellular behavior and physiology. Continuous cell lines: immortalized cell lines are derived from cancerous or transformed cells and can replicate indefinitely. These lines are commonly used for large-scale experiments, drug screening, and vaccine production.[1]

The evolution of these methodologies has led to the development of two primary systems: two-dimensional (2D) and three-dimensional (3D) cultures. Each presents distinct structural and functional characteristics that influence cellular behavior and experimental outcomes.

2D cell culture

Two-dimensional cell culture is a commonly used technique to grow and maintain cells in the laboratory.[12] These cell culture devices allow adherent cells to grow in a monolayer on a two-dimensional planar surface under static conditions. With the established techniques in cell seeding, subculture, cryopreservation, and harvesting, it is convenient to perform in vitro experiments, typically using a single cell type, in a 2D and static environment at a relatively low cost.[4] It provides straightforward access to nutrients, gases, and reagents, making it particularly suitable for high-throughput screening, cytotoxicity assays, and fundamental mechanistic studies.

Despite the convenience and the extensive use, these traditional 2D and static culture devices have been questioned recently regarding them in vivo relevance because in a living body, cells are grown in a three-dimensional and dynamic, rather than a 2D and static, environment.[4]

The main differences between 2D and 3D cell culture systems can be seen in cell shapes, nutrient distribution, formation of cellular junctions, cell proliferation rates, responsiveness to stimuli, and gene or protein expression profiles. These differences can affect the cellular functions as well.[13] In a traditional 2D cell culture system, the lack of a suitable 3D background environment and structural framing will influence cell behavior. For instance, normal epithelial cells always lose their differentiation ability and perform like cancer cells when they grow as 2D monolayer cells.[4,12] As a result, their predictive value in translational and pathophysiological contexts is limited.

3D cell culture

Traditional monolayer cultures lack the complexity of native tissues. 3D cell culture techniques, like spheroids and organoids, aim to replicate tissue-like structures, allowing researchers to study cellular interactions and responses more accurately.[1]

Various biomimetic materials including extracellular matrix (ECM) as well as synthetic materials are currently used as scaffolds in 3D culture systems.[14] The 3D culture of cells in hydrogels is the most widely used and relevant

culture technique to resemble in vivo like interactions between cells and the native extracellular matrix (ECM). Although numerous encapsulation techniques and hydrogel compositions are available, methacrylated collagen or gelatin hydrogels are among the most widely used hydrogels and represent an emerging versatile matrix for 3D cell culture.[15]

The research has emphasized the importance of three-dimensional (3D) culture systems, demonstrating their superiority in maintaining stem cell pluripotency and enhancing differentiation potential compared to traditional two-dimensional (2D) approaches.[5] The shift to 3D cell culture is a significant advancement in laboratory research, as it provides a more physiologically relevant model for studying cellular processes and disease. While some challenges remain to be addressed, the advantages of 3D culture outweigh the limitations of 2D culture.[12] However, they often require sophisticated equipment and protocols for setup and maintenance.

These models foster improved cellular morphology, differentiation, and gene expression profiles compared to their 2D counterparts. They also generate nutrient and oxygen gradients, simulating in vivo microenvironments and enhancing the physiological relevance of the system. Accordingly, 3D cultures are increasingly utilized in tissue engineering, oncology research, regenerative medicine, and organoid development.

The advances in stem cell culture technologies, responsive 3D biomaterials, and automation and monitoring of 3D culture processes have enabled the creation of human 3D in vitro models that more closely mimic the in vivo situation, thus being relevant for drug discovery, disease modeling, and personalized medicine approaches.[15] It is also an innovative approach in cancer research to bridge the gap between conventional 2D culture and in vivo tumors.[14]

Co-culture systems, mimic interactions between different cell types within an organ, this system, provide insights into cellular cross-talk and how cells influence each other's behavior. Stem cell culture consists in the cultivation of pluripotent and multipotent stem cells, this practice holds immense potential for regenerative medicine, disease modeling, and drug testing due to their ability to differentiate into various cell types.[1]

Applications

The areas in which the cell culture is used can be counted as monoclonal antibody, viral and insect vaccine, enzyme, hormone, interleukin, and growth factor productions.[8] Authors consider that cell culture systems play a pivotal role in biomedical research and clinical development, providing essential in vitro platforms for studying cellular physiology, drug responses, tissue regeneration, and disease mechanisms.

Many research works are conducted using rodent stem cells, (especially mouse ones), and the outcomes of these works suggest that cultured stem cells can help in the treatment of joints diseases (e.g., rheumatoid arthritis), peritonitis, colitis, and many more.[5] Cell lines are an attractive option for producing trophoblastic EVs on a large scale due to their ease of manipulation and ready availability. One of the most commonly utilized immortalized cell lines serving as an analogue of trophoblast cells is the human choriocarcinoma JAr cell line.[13] In medical research, cell culture systems are extensively used for toxicology screening, pharmacokinetics, and drug efficacy evaluations.

Cardiovascular diseases remain one of the most common deaths causes all around the world, and the possibility of heart regeneration is very promising. The researchers are also examining the usage of cell lines, which are induced into male germ-like cells, in the treatment of male infertility.[5]

Cell culture is a fundamental technique that can be accomplished in hospital diagnostics and microbiology laboratories if infectious viral agent is suspected. This technique was used in discovering Ebola virus in a suspected yellow fever patient and vice versa in several studies.[3]

The growing understanding of the role of extracellular vesicles (EVs) in embryo maternal communication has sparked considerable interest in their therapeutic potential within assisted reproductive technology, particularly in enhancing implantation success. The inherent properties of EVs in cellular communication hold great potential for diagnostic and therapeutic applications across a spectrum of diseases, including cancer, regenerative disorders, cardio vascular, and infectious diseases, etc.[13]

Recent advances in metagenomics with deep sequencing techniques have made it possible to analyze the genome of microorganism without isolating the virus via cell culture. This is done via high-throughput sequencing using random amplified DNA product and comparison of sequences with available extensive bank of sequences for the final identification of the detected agent.[3]

Difficulties

Despite their numerous applications, cell culture systems face considerable difficulties that limit their translational potential.

The abundance of erroneous and irreproducible results in the scientific literature is specifically caused by common problems in cell culture, such as cross contamination between different species or within the same species, misidentification of cells, genetic drift, bacterial, fungal, yeast, viral, or chemical contamination, and the lack of strict quality control testing.[5]

Indeed, microbiological infections represent the main problem for the maintenance of cells in vitro. Infectious agents such as bacteria are toxic for eukaryotic cells and ultimately lead to cell death.[2] In cell cultures, contamination can come from chemical and biological sources. Indicators of this contamination typically include sluggish cell growth, morphological abnormalities, abrupt pH changes in the media, and higher concentrations of dead or floating cells in the culture.[5]

Various studies have been conducted on the development of implant materials and treatment procedures for implant placement. However, the occurrence of peri-implantitis has become an increasingly prominent concern, affecting the stability of surrounding tissues and, consequently, the longevity of implants.[16]

These limitations underscore the need for continuous optimization of culture conditions, matrix design, and analytical methodologies to ensure reliable data generation and clinical relevance.

CONCLUSIONS

Cell culture techniques enable in vitro study of cellular physiology and pathology. Two-dimensional monolayers and three-dimensional constructs offer distinct advantages and limitations. Applications include drug screening, regenerative medicine, vaccine production, and diagnostics.

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FINANCING

The authors did not receive financing for the development of this research.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORSHIP CONTRIBUTION

Conceptualization: Javier Gonzalez-Argote.
Data curation: Javier Gonzalez-Argote.
Formal analysis: Javier Gonzalez-Argote.
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Validation: Javier Gonzalez-Argote.
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Drafting - original draft: Javier Gonzalez-Argote.
Writing - proofreading and editing: Javier Gonzalez-Argote.