UDC 608:576 https://doi.org/10.15407/biotech17.01.020

DIRECTIONS OF DEVELOPING NEW TECHNOLOGIES BASED ON CULTIVATION OF ANIMAL CELLS AND TISSUES

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Received 2024/01/03
Revised 2024/01/16
Accepted 2024/02/25

Animal cell cultures have found applications in various fields, from basic to applied research. This includes studying the fundamentals of cell biology, mechanisms of the cell cycle, specialized cell functions, cell-cell and cell-matrix interactions, toxicity testing for the study of new drugs, gene therapy for replacing non-functional genes with functional cells, characterization of cancer cells, understanding the role of various chemical substances, viruses, and radiation in cancer cells, vaccine production, monoclonal antibodies, and pharmaceuticals. Additionally, the cultivation of viruses for using in vaccine production, such as for diseases like rabies, hepatitis B, and measles, is another important application of animal cell cultures.

Aim. The purpose of this study was to analyze the literature data on the use of animal cell lines in genetic engineering, therapy, xenotransplantation, biopharmaceuticals, the food industry, and research.

Methods. An analytical review of literature data was conducted using the information analysis of Medline (PubMed), Web of Science and Scopus databases, Google Scholar, the Cochrane Central Register of Controlled Trials (CENTRAL), and other sources up to the current year 2023 using the keywords: “animal cell lines”, “immunobiological preparations”, “xenotransplantation”, “biopharmaceuticals”, “genetic engineering”.

Results. An analysis of research related to the use of animal cells in the biopharmaceutical industry was carried out, and considerations regarding the prospects for their use in various research and production technologies were outlined.

Conclusion. The technology of cultivating animal cells has become a fundamental tool in the development of research in the field of biotechnological sciences. The ability to culture animal cells *in vitro* has allowed the development of innovative methods, such as induced pluripotent stem cell (iPSC) and organ-on-a-chip models, which have provided valuable information about disease mechanisms and potential therapeutic targets. Although there are some challenges with the use of animal cells related to variability in differentiation efficiency and concerns about safety and efficacy, further studies are needed to optimize protocols and overcome these limitations. Overall, animal cell culture technology remains an important component of modern biomedical research and has the potential to revolutionize the field of regenerative medicine.

Key words: animal cell lines, immunobiological preparations, xenotransplantation, biopharmaceuticals, genetic engineering.

Cultivation of animal cells is a fundamental method in biotechnology that allows for the propagation and study of cells obtained from various animal species. The process of cultivation animal cells encompasses several fundamental aspects: the selection of cell type (depending on the experiment’s purpose). Primary cells are isolated from tissues and have a limited lifespan in culture, whereas immortalized cell lines can be cultivated indefinitely. The optimization of culture medium medium containing nutrients, growth factors, and additives necessary for cell growth, the composition of which varies depending on the cell type and desired outcome. The supporting of aseptic conditions

is very importent aspects when working with cell cultures [1].

The cultivation of animal cells is utilized in many fields of modern science, such as biomedical research, where they serve as valuable models for studying cellular physiology, metabolism, cell signaling, and cellular responses to various stimuli. They play a crucial role in deepening our understanding of disease mechanisms, drug development, and toxicological screening. They can be used for the production of recombinant proteins, monoclonal antibodies, vaccines, and viral vectors. Moreover, these products have important therapeutic and diagnostic applications in treating diseases such as cancer, autoimmune disorders, and genetic disorders [2].

Methods of cell isolation, reproduction and cultivation

Methods of isolation are constantly improving to enhance the efficiency and yield of specific cell types, as this stage is crucial in the subsequent cultivation process. The primary methods used for cell isolation include enzymatic digestion and mechanical disruption. Proteolytic enzymes such as trypsin or collagenase are utilized to separate cells from the extracellular matrix. Mechanical disruption involves physical methods such as grinding or cutting as well as the use of tissue homogenizers to release the desired cells. After the initial isolation stage, animal cells need to be propagated *in vitro*. Traditional methods involve culturing in flasks or dishes containing appropriate nutrient media supplemented with nutrients, growth factors, and serum. Techniques such as subculturing and passaging are used to maintain cell viability and prevent overgrowth. Additionally, specialized culture conditions have been developed, such as the use of extracellular matrix coatings and three-dimensional (3D) culture systems to enhance cell attachment, proliferation, and functionality.

The development and optimization of nutrient media are critical aspects of animal cell culture technology. Nutrient media contain essential nutrients, growth factors, and additives necessary for cell growth and proliferation. The composition of the medium must be carefully controlled to ensure it supports cell growth and meets the experiment's goals. The first step in formulating and optimizing nutrient media is selecting a basal medium. Basal media are commercially available formulations containing essential components required for cell growth, such as amino acids, vitamins, and minerals. The next step is adding supplements that can enhance cell growth, differentiation, and function. To ensure optimal cell growth, the concentration and timing of supplement additions need to be carefully controlled.

Bioreactors for cultivating animal cells

Bioreactors are complex technical devices that provide optimal conditions for the development or action of biological agents, resulting in the biosynthesis of useful compounds (target products), or the biotransformation or biodegradation of any organic substances (substrates), predominantly of natural origin [1]. Various microorganisms, cells and tissues of plants and animals, as well as enzymes, are often used as biological agents for the production of biologically active substances, pharmaceuticals, biopolymers, food products, and cosmetics. However, when planning such technological processes, it should be taken into account that animal cell cultures are sensitive to shear stress due to their rigid cell wall. This requires the application of different approaches to bioreactor design for their cultivation, significantly different from bioreactors for microbial fermentation [2]. However, this has not reduced the demand for their use, as the need for testing and developing medicinal products only grows over the years, and animal cultures are the most convenient tool for this activity [3].

The bioreactor system allows for the control of in vitro cultivation conditions. To achieve spatial uniformity in cell distribution within the environment, agitation must be provided. Mass transfer also plays an important role in maintaining cell integrity and their adequate consumption of nutrients and oxygen [4]. Therefore, it is advantageous to use bioreactors with agitation, but at the same time, they can damage cells. However, a non-agitated reservoir can also damage cells due to the formation of strong vortex flow, inducing low-frequency layering [5]. At present, disposable bioreactors with aerated agitation are the most favorable for the culture of animal cells. Among their advantages are higher production capacity, greater flexibility, prevention of cross-contamination, reduction in cleaning costs, and downtime reduction. The productivity of these bioreactors depends on many design parameters, which combined with the physicochemical and rheological properties of the environment, constitute a unique variable, the so-called volumetric gas-liquid mass transfer coefficient [6].

The use of animal cells in cell engineering and biopharmaceuticals

The most vigorous development is occurring in the production of protein-based biopharmaceutical products. Proteins can be specific even to a particular species, and synthesizing large proteins without the use of living organisms is very difficult, often simply impossible. Additionally, the three-dimensional structure of the protein, which forms in the human body with the help of chaperone proteins regulating protein folding, plays a crucial role in the protein's construction.

For biopharmaceuticals, one of the main directions is the cultivation of animal cells, especially mammalian cells, including those of humans. In 2021, 34% of new drugs approved by the FDA in the United States were biological products: vaccines, monoclonal antibodies, recombinant proteins, and so forth. From mammalian cells, we have long been able to obtain various products such as hormones, enzymes, cytokines, antibodies, bone morphogenetic proteins, blood-clotting factors, and more. Insect cells are also used, for example, to obtain a vaccine against human papillomavirus. Insect cells infected with a viral vector (baculovirus) can also produce recombinant proteins, mainly fragments of the virus, which are then used to manufacture vaccines [7].

It is worth noting that immortal cell lines (tumor or immortalized) are most commonly used, as they have the ability of indefinite proliferation. The most widespread cell line for the production of biopharmaceuticals is the Chinese Hamster Ovary (CHO) cell line. As of 2014, this line was utilized in 75 percent of studies involving mammalian cells, and currently, in 2023, there is a wealth of research on this cell line in scientific databases regarding the production of biopharmaceuticals. This cell line offers several advantages: cells can grow in suspension culture, in serum-free defined media, which greatly facilitates standardization and enhances the safety of the final product.

Additionally, these cells are quite resistant to changes in culturing conditions and have a higher yield of recombinant proteins. In addition to protein synthesis, post-translational modifications of proteins are possible in these cells, resulting in the production of biologically active forms of proteins similar to those produced in the human body. Furthermore, over the years of usage, researchers and manufacturers have thoroughly studied the genetic structure of this cell line [8].

Also common is the use of the following cell lines of animal origin: NS0 mouse myeloma cells, BHK-21 baby hamster kidney cells, Sp2/0-Ag14 (a non-immunoglobulin-producing line formed by the fusion of BALB/c mouse spleen cells and P3X63Ag8 mouse myeloma cells), and so on [8].

One of the most widespread biopharmaceutical products obtained industrially using mammalian cells is monoclonal antibodies. The challenge in producing monoclonal antibodies on a large scale lies in the fact that B-lymphocytes, like most healthy primary cells, divide slowly and have a short lifespan outside the body. The idea arose to combine B-lymphocytes with an immortal cell line to obtain monoclonal antibodies. Thus, in 1975, hybridoma technology was invented. Essentially, this technology combines the properties of healthy B-lymphocytes with the ability of tumor cells to infinitely divide. The general principle of hybridoma technology is to induce the fusion of B-lymphocytes and cells, such as myelomas, forming hybrids that produce the necessary antibodies while also having the ability to divide infinitely and rapidly.

Monoclonal antibodies have countless applications in both research and clinical practice. In research and diagnostics, these include immunostaining, enzyme-linked immunosorbent assay (ELISA), chemiluminescent immunoassay, blood group determination, among others. In clinical settings, monoclonal antibodies are widely used for the treatment of malignant neoplasms, infectious diseases, increasing tolerance to transplants, and more [9].

Monoclonal antibodies can function in the body on their own, targeting cells that need to be destroyed. However, there are therapies where antibodies are used as a method of targeted drug delivery. One drawback of chemotherapy and radiotherapy is that the drugs negatively impact not only cancerous but also healthy cells. Therefore, it is possible to attach a chemotherapeutic drug or a radioactive particle to an antibody specific to cancer cells, and the release of the drug or particle will occur only after binding to the antigen (cancer cell), thus reducing the negative impact on healthy tissues. Antibodies conjugated with fluorescent proteins or various enzymes are used for immunofluorescence assays (IFA) in research.

There are also bispecific antibodies, i.e., antibodies that bind to two different targets simultaneously, such as a malignant and an immune cell, literally attracting the immune cell to the malignant one, which will consequently be destroyed by the immune cell [10].

Animal cells in organ-on-chip development

For a long time, 2D and 3D animal cell cultures have been utilized in research, but in recent years, organ-on-chip (OOC) technology has emerged at the forefront and rapidly entered research practices worldwide.

The obvious advantages of microfluidic technologies include:

– reduction in sample volume and reagent consumption, thus lowering costs;

– complete control over fluid mixing, wide possibilities for adjusting temperature conditions (heating, cooling) quickly and with minimal expenses;

– ability to work with small objects;

– excellent reproducibility, stable results;

– possibility of creating inexpensive disposable chips;

– rapid mass transfer due to high surface-to-volume ratio;

– increased reaction efficiency, especially when combined with other methods;

– ability to integrate chips with microelectronic systems: most modern chips are microelectromechanical and allow control of fluid movement and temperatures.

The combination of organ-on-chip with cellular biology methods introduces its own adjustments to research techniques and enables the modeling of physiological processes in the human body using in vitro multicellular human organ models [11].

Organ-on-chip is a multi-channel (channels ranging in size from tens to hundreds of microns) three-dimensional microfluidic in vitro platform, which, with a certain degree of scaling, allows the modeling of tissues, organs, and their systems, replacing a living organism with a model and reducing the time and cost of research. The reaction chambers of the device are filled with living cells (a single cell layer for a simple system or cells of different types for complex systems), which are connected with membranes and placed on opposite sides for accurate organ modeling.

Thus, microfluidic devices of this type have a multicellular architecture associated with tissues and physicochemical microsystems, hich are an analogy to the human body.

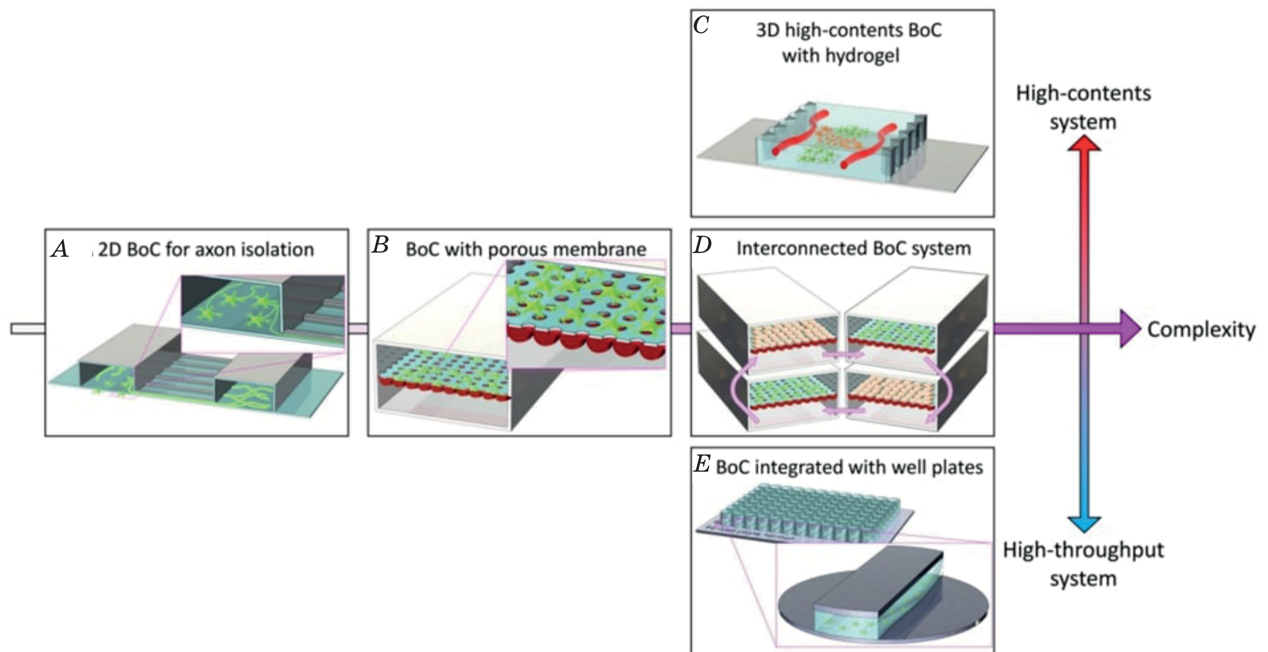
Organ-on-chip can be useful in studying chronic pathophysiological reactions, modeling cell interactions with metabolites, gases with circulating cells, designing the effects of various drugs on organs and tissues, and determining their effectiveness or harm. Since the technology is relatively young, there is no single classification of chips; however, they can still be categorically divided based on their design and functional purpose (Figure).

Microfluidic devices in combination with 3D culture techniques are advancing further, enabling the modeling of fluid flow (such as blood or its substitute) as well as mimicking physical actions (such as heart contractions). Multiple layers of different cell types can be created on chips, and signal transmission between them can be facilitated using a porous structure [13].

Placing multiple organs on a single chip helps understand how different organs can work together and, for example, metabolize certain substances. An example of such a combination is 3D cultures of human lung and liver cells on a chip connected by a fluid channel. The speed and direction of the nutrient medium circulating between cultures can be controlled using a smartphone. The channel system allows two different cultures to share the surrounding environment and "communicate" by emitting different signaling molecules without directly interacting [14].

Selecting the right cells for 3D culture on a chip is a separate complex task, taking into account that primary cells vary from donor to donor and have a short lifespan, reducing experiment controllability. Immortalized and tumor cell lines proliferate too actively, making the model inadequate. At the moment, the best solution is to use cells derived from embryonic or induced pluripotent stem cells.

A kidney-on-a-chip model, which would be useful for high-throughput screening for drug toxicity testing and studying pharmacokinetics, is a combination of 3D cultures of various kidney cells with a platform that replicates the microenvironment and structure of the kidney *in vivo*. Such a model should enable the co-cultivation of different kidney cells and maintain functional interactions between them (such as between glomerular endothelial cells and podocytes), provide the synthesis of functional transporters, as well as metabolic and endocrine functions. Today, models of the glomerulus and proximal and distal tubules have already been developed, but a fully integrated nephron-on-a-chip, which integrates all of this together, has not yet been created. The direct combination of existing models does not result in a "kidney-on-a-chip" platform.



Development process of brain-on-chips (BoCs):
A — Cells are cultured on 2D glass, which allows cell culture separation. *B* — Nerve and endothelial cells are attached on both sides of the membrane. *C* — Hydrogel modeling in BoC that more closely mimics neural tissue. *D* — Culture media are circulated by a pump through the connection of several BoCs. *E* — BoC is placed in a regular 96-well plate to miniaturize.

Other structural components of the nephron, such as the thick ascending limb, cortical collecting duct, are still not well characterized enough to create a microfluidic chip [12].

However, despite active research and progress in this field of science, there are still certain shortcomings of these systems that deserve more attention in program improvement. One of the current issues is the selection of materials for creating such chips, which is complicated by the need for high biocompatibility, complexity of creation, manufacturing (especially to meet wide use in laboratory research), and providing adequate prices or reusability for commercial distribution. Moreover, in creating organ-on-chip systems, it is important to correctly scale organoids according to their human counterparts, which is achieved through mathematical modeling of microfluidic elements (channels/capillaries, vessels, circulatory systems), which also requires improvement. Furthermore, attractive systems that integrate multiple organs also have their drawbacks; for example, the more organs they integrate the less sensitive the design becomes, and the higher the risk of obtaining unreadable and inaccurate results.

The development of microprocessing technologies (such as photolithography, microfabrication) has enabled rapid and relatively inexpensive production of microfluidic chips, as well as their standardization. However, there are several challenges still impeding the creation of a fully functional organ-on-chip model: from difficulties in obtaining and maintaining required cells to properties of the materials used. Some chip materials, commonly used ones like polydimethylsiloxane, leach solvents and nutrients. For instance, for the aforementioned kidney-on-chip to become a standard, it must undergo repeated testing for sensitivity and specificity, meaning standard cell responses to known nephrotoxicants.

The application of organ-on-chip technology in high-throughput drug screening is feasible if experiments are conducted concurrently on dozens of chips, and if microfluidics is combined with other methods, such as mass spectrometry: this would allow for investigating candidate compounds, tracking genetic, proteomic, and metabolomic changes in cells from different tissues within one system. Interestingly, remote control of conditions such as flow rates of nutrient mediums, temperature, and so forth, essentially allows housing dozens or even hundreds of organoids on chips in a single small room, and to operate such a «preclinical research factory» would require only a few personnel [14].

It is important to note that the application of organ-on-chip technology for fully replacing preclinical research is not only associated with advances in biology but also with regulatory requirements. For example, regulatory bodies for the cosmetic industry already permit conducting research only in vitro; hence, the abandonment of animals is becoming almost ubiquitous in this field. However, in more critical industries-namely, pharmaceuticals-years of research may pass before the use of microfluidic chips is officially permitted as an alternative to classical preclinical studies. Nevertheless, most experts agree that in the foreseeable future, around 10–15 years, this will become a reality.

Gene engineering and xenotransplantation

Cell lines of animals play an important role in gene therapy. Gene therapy involves the introduction, removal, or alteration of a therapeutic or working copy of a gene to treat a disease or defect. Gene therapy holds great promise; however, the challenge of transferring genetic material into cells remains a significant technical problem and requires cell cultivation *in vivo*. The first step in gene therapy is identifying the «faulty» gene. After this, the gene is isolated, generated, and constructed for proper expression. Gene integration with subsequent delivery of genetic material *in vivo* or *ex vivo* is crucial for the success of gene therapy. In in vivo therapy, genetic material is directly introduced into the body at a specific location, while in *ex vivo* processing, target cells are treated outside the patient's body. *Ex vivo* technique involves gene therapy in cultivated cells that multiply and are subsequently transferred to the target tissue. Several clinical trials of gene therapy have already been approved and conducted worldwide [15].

Gene engineering revolves around the idea of reprogramming genes to produce new proteins. Animal cell cultures can be used for transfection to produce new proteins in large quantities for clinical research or therapeutic purposes. Gene engineering modifies DNA sequences in genomes using molecular biology technologies. Gene engineering allows for the production of cells with stable expression, thereby minimally affecting the properties of host cells; moreover, in combination with induced pluripotent stem cell technology, genetic errors can be corrected, which is also utilized in medicine [16].

One of the most widespread technologies is Clustered Regularly Interspaced Palindromic Repeats associated protein 9 (CRISPR-Cas9) technology, which is used for genome editing in various organisms. The CRISPR/Cas system consists of the Cas nuclease and two separate RNA components: programmable crRNA (CRISPR RNA) and fixed tracrRNA (trans-activating crRNA). The Cas protein can cleave invading phage DNA into small fragments, which are then integrated into the CRISPR array as spacers. Later, the CRISPR array transcribes to generate crRNA and complementary tracrRNA, forming a double-stranded RNA structure that recruits Cas proteins for cleavage. Alongside the crRNA-targeted sequence on the invading DNA, a short sequence called the protospacer adjacent motif (PAM) plays a crucial role in adaptation and interference stages, which the CRISPR/Cas complex recognizes during binding to the target DNA. The absence of the PAM sequence can alter the affinity between Cas and the target DNA, as specific recognition of the PAM sequence serves to distinguish non-self-target sequences from non-target sequences. However, current knowledge of the CRISPR/Cas9 system at biochemical and crystallographic levels is insufficient and requires further research, including a deep analysis of the Cas9 protein, one of the main components of the CRISPR/Cas9 system. Additionally, in many eukaryotic species such as mice, fruit flies, zebrafish, worms, and humans, specific delivery methods of Cas9, gRNA, and donor oligonucleotides to cells and tissues have been developed. For example, the CRISPR/Cas9 system used for creating transgenic mice can be fused with other proteins or effectors for control or stimulation of expression or initiation of the CRISPR/Cas9 system *in vivo*. Nevertheless, the frequency of off-target mutations in various CRISPR/Cas9 systems remains a problem [12]. For further improvement of CRISPR-Cas gene editing technology, it is necessary to enhance gene delivery efficiency and suppress off-target effects.

Recombinases are a proven technology for site-specific recombination of cellular genomes in many organisms. Currently, Cre (causes recombination) recombinase demonstrates high efficiency in transferring foreign genes into site-specific integration as an additional method of reprogramming. Some regions exist in the cellular chromosome where the inserted foreign gene is less susceptible to suppression. The recognition sequence of the recombinase is integrated

into the target genomic site, such as Genomic safe harbors (GSH), with the preemptive use of genome editing technologies, resulting in the generation of master cell lines that can be reprogrammed. Additional exogenous genes are efficiently integrated into the target locus of the cell genome, indicating expected transgene expression. Numerous studies have been conducted, and new technologies are being developed to promote efficient and reliable targeted transgene transfer for the creation of host-producer cell lines in industrial settings [17].

Gene engineering has made a significant contribution by providing the ability to selectively modify genome segments used in xenotransplantation. Pig organs are quite similar in size and physiology to human organs, making them excellent candidates for xenotransplantation. Unfortunately, most pig cells' surfaces contain a specific Gal epitope (galactose alpha-1,3-galactose linkage), including cells from kidney and heart tissues. Humans have antibodies to the Gal epitope.

Thus, when pig cells or organs are introduced into humans or primates, the combination of the Gal epitope and existing antibodies leads to acute rejection. Nevertheless, for organs such as the heart and kidneys, it has been concluded that overcoming the barrier of hyperacute rejection requires overcoming the Gal epitope. Now that this barrier of hyperacute rejection has been overcome, it is possible to investigate other genetic modifications necessary to overcome acquired immunity. It is quite likely that before successful pig organ transplantation to humans, additional changes will be required. An important point is that there is already technology for precise addition or deletion of genes, and it is believed that many obstacles in the way of xenotransplantation can be overcome through additional genetic modifications.

Conclusion

Animal and human cells play a significant role in biopharmaceuticals. The most crucial direction of cultivating these cells is to obtain therapeutic proteins, including monoclonal antibodies. Monoclonal antibodies not only play a role in treating many diseases but also in diagnostics and research. Bioreactors are a key element when we aim to produce valuable materials in biological processes, and the case of animal cell cultures is no exception. For the production of soluble metabolites released by animal cells, bioreactors based on animal cells have been constructed based on configurations developed for microbiological processes. From a practical point of view, the main considerations to be taken into account are the mechanical fragility and slow growth rate of animal cells.

Therefore, bioreactors used for cultivating these cells must be operated under strictly controlled conditions and take these characteristics into account. Genetic engineering and xenotransplantation are important research directions that open up possibilities for using animal cell lines to obtain vital organs and tissues for people in need of transplantation. Creating animal cell lines with genetic modifications that ensure compatibility with the human body opens up prospects for avoiding the problem of organ donor shortages and reducing the risks of rejection after transplantation. In addition, genetic engineering allows for additional modifications of cell lines to reduce the risks of viral infections, increase the viability of transplanted organs, and improve their functional characteristics.

Furthermore, the use of «organ-on-a-chip» technology, combined with methods of cell biology, is gaining momentum, allowing for the design of physiological processes of the human body on models of multicellular human organs *in vitro* [11].

Funding

The study was carried out as part of the research work of the Department of Translational Medical Bioengineering, Faculty of Biomedical Engineering of the National Technical University of Ukraine "Ihor Sikorsky Kyiv Polytechnic Institute" "Development of innovative biomedical technologies and products for the diagnosis and treatment of human pathologies" (2020–2024, State registration number 0119U103789).

REFERENCES

1. *Chandra, V., Tiwari, A., Pant, K.K., Bhatt, R.* (2022). Animal Cell Culture: Basics and Applications. In: Verma, P. (eds) I*ndustrial Microbiology and Biotechnology*. Springer, Singapore. https://doi.org/10.1007/978-981-16-5214-1_24
2. *Richter M, Piwocka O, Musielak M, Piotrowski I, Suchorska WM, Trzeciak T.* From Donor to the Lab: A Fascinating Journey of Primary Cell Lines. *Front Cell Dev Biol*. 2021, V.9:711381. https://doi.org/10.5772/66905
3. *Yunus Celik, Kadir Alptekin, S. Furkan Demirden, Suphi S. Oncel*, A novel low shear horizontal bioreactor design for the production of animal cells: Effect of bioreactor dynamics on the 3D spheroid formation of HepG2, *Biochemical Engineering Journal*. 2023, V.196:108952. https://doi.org/10.1016/j.bej.2023.108952
4. *Verma A, Verma M, Singh A.* Animal tissue culture principles and applications. *Animal Biotechnology*. 2020, P. 269–293. https://doi.org/10.1016/B978-0-12-811710-1.00012-4
5. *Brucato A., Busciglio A., Scargiali F. Unbaffled,* Stirred Bioreactors for Animal Cell Cultivation. *Current Developments in Biotechnology and Bioengineering*. 2017. P. 97— 142. https://doi.org/10.1016/b978-0-444-63663-8.00005-7
6. *Argang Kazemzadeh, Cynthia Elias, Melih Tamer, Ali Lohi, Farhad Ein-Mozaffari,* Mass transfer in a single-use angled-shaft aerated stirred bioreactor applicable for animal cell culture. *Chemical Engineering Science*. 2020, V.219:115606. https://doi.org/10.1016/j.ces.2020.115606
7. *Dumont J., Euwart D., Mei B., Estes. S, Kshirsagar R.* Human cell lines for biopharmaceutical manufacturing: history, status, and future perspectives. *Crit Rev Biotechnol*. 2016, 36(6):1110–1122: https://doi.org/10.3109/07388551.2015.1084266
8. *Kuystermans D., Al-Rubeai M.* Biopharmaceutical Products from Animal Cell Culture. *Cell Engineering*. Cham, 2014, P. 717–757. https://doi.org/10.1007/978-3-319-10320-4_23
9. *Mitra S., Tomar P. C.* Hybridoma technology; advancements, clinical significance, and future aspects. *Journal of Genetic Engineering and Biotechnology*. 2021, V. 19:1. https://doi.org/10.1186/s43141-021-00264-6
10. *Bayer V.* An Overview of Monoclonal Antibodies. *Seminars in Oncology Nursing*. 2019, V.35:150927. https://doi.org/10.1016/j.soncn.2019.08.006
11. *Maoz B. M.* Brain-on-a-Chip: Characterizing the next generation of advanced *in vitro* platforms for modeling the central nervous system. *APL Bioengineering*. 2021, V.5:030902. https://doi.org/10.1063/5.0055812
12. *Bang S., Jeong S., Choi N., Kim H. N.* Brain-on-a-chip: A history of development and future perspective. *Biomicrofluidics*. 2019, 13(5), 051301. https://doi.org/10.1063/1.5120555
13. *Mofazzal Jahromi, M. A., Abdoli, A., Rahmanian, M., Bardania, H., Bayandori, M., Moosavi Basri, S. M., Kalbasi, A., Aref, A. R., Karimi, M., & Hamblin, M. R.* Microfluidic Brain-on-a-Chip: Perspectives for Mimicking Neural System Disorders. *Molecular neurobiology*, 2019, 56(12):8489— 8512. https://doi.org/10.1007/s12035-019-01653-2
14. *Franzen N., van Harten W. H., Retèl V. P., Loskill P.,van den Eijnden-van Raaij J., IJzerman M.* Impact of organ-on-a-chip technology on pharmaceutical R&D costs. *Drug Discov Today*. 2019, 24(9):1720–1724. https://doi.org/10.1016/j.drudis.2019.06.003
15. *Ofenbauer A., Tursun B.* Strategies for *in vivo* reprogramming. *Current Opinion in Cell Biology*. 2019, V.61:9–15. https://doi.org/10.1016/j.ceb.2019.06.002
16. *Mei Y., Wang Y., Chen H., Sun Z. S., Ju X. D.* Recent Progress in CRISPR/Cas9 Technology. *J. Genet. Genomics*. 2016, 43(2):63–75. https://doi.org/10.1016/j.jgg.2016.01.001
17. *Horie M., Yamano-Adachi N., Kawabe Y., Kaneoka H., Fujita H., Nagamori E., Iwai R., Sato Y., Kanie K., Ohta S., Somiya M., Ino K.* Recent advances in animal cell technologies for industrial and medical applications. *J. Biosci. Bioeng*. 2022, 133(6):509–514. https://doi.org/10.1016/j.jbiosc.2022.03.005

НАПРЯМИ СТВОРЕННЯ НОВИХ ТЕХНОЛОГІЙ НА ОСНОВІ КУЛЬТИВУВАННЯ КЛІТИН І ТКАНИН ТВАРИН

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Культури тваринних клітин знайшли застосування в різних сферах, від фундаментальних до прикладних досліджень. Це включає вивчення основ біології клітин, механізмів клітинного циклу, спеціалізованих функцій клітини, взаємодій клітина-клітина та клітина-матрикс, тестування на токсичність для вивчення нових ліків, генної терапії для заміни нефункціональних генів функціональними клітинами, характеристики ракових клітин, роль різних хімічних речовин, вірусів і радіації в ракових клітинах, виробництва вакцин, моноклональних антитіл і фармацевтичних препаратів, продукування вірусів для використання у виробництві вакцин, вакцин проти сказу, гепатиту В, кору.

Метою дослідження було проаналізувати дані літератури щодо використання клітинних ліній тварин у генній інженерії, терапії, ксенотрансплантації, біофармацевтиці, харчовій промисловості та наукових дослідженнях.

Методи. Аналітичний огляд даних літератури проводили з використанням інформаційного аналізу баз даних Medline (PubMed), Web of Science і Scopus, Google Scholar і Кокранівського центрального реєстру контрольованих досліджень (CENTRAL) та інших джерел до 2023 року включно за ключовими словами: «клітинні лінії тварин», «імунобіологічні препарати», «ксенотрансплантація», «біофармацевтика», «генна інженерія».

Результати. Проведено аналіз результатів досліджень, пов'язаних із використанням тваринних клітин у біофармацевтичній промисловості, та викладено міркування щодо перспектив використання їх у різних наукових і виробничих технологіях.

Висновки. Технологія культивування тваринних клітин стала фундаментальним інструментом у розвитку досліджень у різних галузях біотехнології. Здатність культивувати клітини тварин *in vitro* дозволила розробити інноваційні методи, такі як iPSC і моделі органу на чіпі, які надали цінну інформацію про механізми захворювання та потенційні терапевтичні цілі. Хоча існують деякі проблеми з використанням тваринних клітин, пов'язані з варіабельністю ефективності диференціювання та занепокоєння щодо безпеки та ефективності, необхідні подальші дослідження для оптимізації протоколів та подолання цих обмежень. Загалом технологія культивування тваринних клітин залишається важливою складовою сучасних біомедичних досліджень і має потенціал здійснити революційні зміни в галузі регенеративної медицини.

Ключові слова: клітинні лінії тварин, імунобіологічні препарати, ксенотрансплантація,біофармацевтика, генна інженерія.