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REVIEW

PRIMARY TUMOR CELL CULTURES: CURRENT METHODS OF OBTAINING AND SUBCULTIVATION

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ABSTRACT

Over the past decades, transplantable cell lines have been an affordable model for studying the biology and effect of chemotherapeutic drugs on tumors. However, numerous studies have shown that these cell lines are not heterogeneous enough and cannot reflect the drug resistance of tumors that occurs in some patients. Primary cell line cultures isolated from solid tumors have become widespread in personalized cancer therapy. This review discusses the basic methods for the preparation and cultivation of primary cell lines. A brief description is given of the methods for the disaggregation of tumor material using enzymatic, chemical and mechanical dissociation. The systems of cultivation of primary cell cultures. The selection of an appropriate dissociation method and cultivation is important to preserve the benefits of primary culture in preclinical studies.

Keywords:
primary cell cultures, cell lines, method of cell dissociation, 2-D culture, 3-D culture, microfluidic platforms, explants

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ОБЗОР

ПЕРВИЧНЫЕ КУЛЬТУРЫ ОПУХОЛЕВЫХ КЛЕТОК: СОВРЕМЕННЫЕ МЕТОДЫ ПОЛУЧЕНИЯ И ПОДДЕРЖАНИЯ *IN VITRO*

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РЕЗЮМЕ

В течение последних десятилетий перевиваемые клеточные линии являлись доступной моделью для изучения биологии и влияния химиотерапевтических препаратов на опухоли. Однако, многочисленные исследования показали, что данные клеточные линии недостаточно гетерогенны и не могут отражать лекарственную резистентность опухолей, возникающую у некоторых пациентов. Культуры первичных клеточных линий, выделенные из солидных опухолей, получили значительное распространение для определения химиочувствительности опухолей к препаратам, применяемым в химиотерапии. В данном обзоре рассматриваются основные методы получения и культивирования первичных клеточных линий. Дается краткая характеристика методикам дезагрегации опухолевого материала при помощи ферментативной, химической и механической диссоциации. Рассмотрены различные системы культивирования первичных клеточных культур. Выбор подходящего метода диссоциации и культивирования имеет важное значение для сохранения преимуществ первичной культуры в доклинических исследованиях.

Ключевые слова:
первичные культуры клеток, клеточные линии, методы диссоциации клеток, 2-D культуры, 3-D культуры, микрофлюидные платформы, эксплантаты

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RELEVANCE

A primary culture is called cell culture at the stage which is immediately after cell isolation from samples and before the first seeding [1]. The primary tumor cell cultures are *ex vivo* populations isolated by surgical resection of tumor tissue fragments [2]. Primary cell lines include both tumor cells and microenvironment cells (fibroblasts, T-cells, vascular endothelial cells) that are relevant in the physiology, structure, and functioning of the tumor [3]. The tumor and it's microenvironment can cause mutual changes in the phenotype and functions that support the continuous process of carcinogenesis. Transferable cell lines derived from a small proportion of tumors, usually very aggressive, are the most common *in vitro* model for research in Oncology. However, such models do not provide a representation of the entire spectrum of tumor subpopulations. Primary cell cultures reflect the high heterogeneity of tumor cells, and represent an important tool for research into the biology of tumors, and new opportunities in personalized medicine in General. While preserving cells with phenotypes similar to those of the original tumor, primary cell lines play an important role in the study of mechanisms of chemoresistance, the search for new drug candidates, which is particularly relevant in preclinical research. The study of interactions between tumor cells and its microenvironment includes the development of optimal models for the study of tumor migration and proliferation [4]. In the era of personalized therapy, researchers need to create more primary tumor lines from patients, which will provide high-quality data for translating *in vitro* results into *in vivo* models and, ultimately, implementation into the clinic. In this review, we will look at the methods currently available for generating and culturing primary tumor cell lines.

TUMOR CELLS ISOLATION METHODS

Isolation and cultivation of tumor cells *in vitro* conditions similar to the microenvironment of the original tumor is a complex task and requires certain methods. Successful isolation of tumor cells with the use of appropriate technologies depends on the method of destruction of the extracellular matrix, which consists of a variety of related factors (connective tissue fibers, glycoproteins and tissue-specific proteins). Additional difficulties in isolating primary cell culture include the presence of tumor material in the samples:

1. cell debris and non tumor cells, that affect the proliferation of tumor cells, often slowing down the proliferative activity of the primary culture;
2. a small number of viable cells due to resection in the necrotic area;
3. fibroblasts, that actively proliferate during the cultivation [5].

The selection of a good method of tumor dissociation material affects the selection of a sufficient number of viable cells and introduction into the primary culture. There are several methods for dissociating material and obtaining primary cell lines from tumors; however, very few methods have been recognized as promising. It is necessary to develop modern techniques adapted to each type of tumor tissue for reproducible generation of primary cell lines from tumors. Currently, such methods as mechanical, chemical and enzymatic disaggregation are used for dissociation of tumor samples [6].

Fermentative Dissociation

The fermentative dissociation is the most usable method for disaggregating tissue and producing a suspension of individual tumor cells, while maintaining their viability and integrity. Usually, proteolytic enzymes are used for dissociation of tumors, including trypsin, papain, elastase, hyaluronidase, collagenase, pronase, and deoxyribonuclease [7]. Some researchers use a mixture of enzymes, such as collagenase/hyaluronidase combinations and a solution of dyspase and DNA-ase to dissociate breast tumor samples [8]. In a study by Volovitz et al. neutral protease (NP) from Clostridium histolyticum, an enzyme not previously used in the field of neuro-

biology, was used for enzymatic dissociation of brain tissues and tumors. Dissociation under the influence of protease allowed to obtain a cell suspension for introduction into the primary culture with significantly higher cell viability, compared to the enzymatic action of collagenase, DNA-ase, and papain [9].

Use of trypsin and accutase in the Skog study et.al on autodermal grafts, it was also shown to produce cells with greater viability after trypsinization, but samples treated with accutase later proliferated better in the primary culture. There was no significant difference between the average intensity of fluorescence of stem cell markers, both after trypsinization and after accutase treatment [10]. For the research of primary cultures of breast cancer Nishikata et.al we used the Explant method and obtained a suspension of cells. For dissociation of a fragment of the tumor used dispute II. The most effective method for obtaining primary culture of breast tumors was considered to be obtaining a suspension of cells after dissociation with a dyspase II [11]. Models for obtaining primary cultures of brain neurons using enzymatic papain dissociation are being actively developed to study cellular and molecular genetic features of brain functioning [12]. Protocols for perfusion of mouse liver fibrous tissue with pronase/collagenase solutions and isolation of mouse liver stellate cells into primary culture are being implemented [13].

Chemical dissociation

Various types of cations maintain the integrity of the cell surface and the intracellular structural matrix [7]. Chemical dissociation is a process in which Ca2 + and Mg2 + cations are washed out of epithelial cells, reducing intercellular interactions. Removal of Ca2 + and Mg2 + is best achieved when exposed to EDTA (ethylenediaminetetraacetic acid) or tetraphenylboron complexes with potassium ions, which are used for dissociation of liver tissues, intestinal crypt cells, and solid breast tumors [14]. Hypertonic solutions of sucrose, maltose, and lactose affect the slit contacts and areas of dense contacts, which causes the presence of cell clusters after enzymatic tissue cleavage [15]. Some researchers perform double perfusion of liver cells with EDTA / collagenase to isolate and culture primary hepatocytes, culturing them with the addition of insulin and glucose [16]. Trojaneck and his colleagues successfully obtained 14 primary melanoma lines using tumor material obtained from 45 patients with melanoma. Tumor samples were affected by EDTA and DTT (dithiotreitol) [17].

Mechanic dissociation

There are several options for mechanical dissociation of tumors: conventional manual homogenization and various automatic dissociators for suspensions of individual cells. Mechanical dissociation of tissue involves shredding a resected tumor sample with scissors or sharp blades, homogenization (using BD Medimachine, Becton Dickinson), filtration through nylon filters or steel mesh filters (with different pore diameters), shaking, re-aspiration through serological pipettes, or any combination of these methods. Usually, tumor samples are first crushed into small pieces (nearly 1–2 mm), and then washed in a tissue-specific medium or salt solutions (Hanks ' solution, Dalbecco's solution) to remove loosely bound cells or non-specific debris by light mixing. In this way, a suspension of individual cells is obtained. Mechanical dissociation is a simple but effective method for removing primary colorectal cancer cell lines obtained from primary tumors with an efficiency of 39.4%, as well as cell lines isolated from corresponding lymph node metastases with an efficiency up to 70% [18]. However, some researchers believe that this type of dissociation of tumor tissue using mechanical methods leads to significant cell death and is not suitable for obtaining tumor cells and introducing them into primary culture [19].

Comparing mechanical and enzymatic dissociation of primary glioblastoma, some researchers prefer enzymatic methods to obtain cells with higher migration activity [20]. Qiu X et.al we have developed a microfluidic device that allows for a softer mechanical cell disaggregation using

a network of channels and "hydrodynamic scalpels" [21]. In the Kar et.al study, the primary ovarian cancer cell lines were successfully obtained both by mechanical tissue dissociation and enzymatically by a Dispase II [22].

The own data

The laboratory of cell technologies National Medical Research Centre for Oncology of the Ministry of Health of Russia studied the possibility of using collagenase from crab hepatopancreas to isolate breast cancer stem cells. After studying the effect of collagenase in three concentrations and a variant without the use of collagenase (using only the mechanical method of disaggregation), we obtained a higher concentration of living cells when applying enzymatic disaggregation [23].

In The next study was used the tumor material obtained from patients with astrocytic tumors. Dissection of the tumor was performed by surgeons of the Department of neuroncology National Medical Research Centre for Oncology of the Ministry of Health of Russia under visual control using the Blue E400 unit of the Opmi Pentero ™ microscope and 5-ALA (5-aminolevulinic acid). The tumor tissue was disaggregated at room temperature on BD Medimachine (Becton Dickinson, USA) in sterile Medicons (Becton Dickinson, USA) with a pore diameter of 50 microns. The study was produced cell lines of low-grade astrocytic tumor material after dissection of the tumor, has also been shown that this method is effective, as it enables selection of a material with viable cells [24].

Isolation of tumor stem cells from brain tumors was performed to obtain primary cell lines. Initially, the noo formation tissue was subjected to mechanical or enzymatic dissociation. Mechanical dissociation of tumor tissue was performed in BD Medimachine (Becton Dickinson) in Hanks solution at room temperature. Enzymatic dissociation was performed using a set of reagents Brain Tumor Dissociation Kit (Miltenyi Biotec) in accordance with the manufacturer's instructions. Based on the results of tests of various combinations of techniques for tissue dissociation: the use of a set or the use of mechanical dissociation, it was found that for brain tumors, the optimal result was achieved when using an enzymatic set. The number of living cells in this case was on average less than in mechanical dissociation, but the proportion of living cells was 2 times higher. In addition, enzymatic treatment of the tissue allowed to obtain a more homogeneous suspension, in which the cells were well separated and did not form conglomerates during subsequent cycles of centrifugation and resuspending.

THE PRIMARY CELL LINES CULTIVATION METHODS

There are several types of cultivation of primary cell lines after dissociation of tumor tissue. The suspension of tumor cells can be cultured in 2D culture (monolayer of cells), 3D culture (spheroids, gels, scaffolds), microfluidic technologies, explants (cultivation of small tumor fragments). After disaggregating the material, the cells are cultured in a nutrient medium, making the necessary tissue-specific additives, fetal bovine serum, amino acids, and antibiotics. Nutrient media are supplemented by various factors detected *in vivo*. In order to maintain the viability and ensure the safety of the genotype and phenotype of tumor cells *in vitro*, mitogenic growth factors are introduced into the nutrient media [25].

The 2D cultures

The 2D cultures are ordinary monolayer cultures, grown under conditions that do not reflect *in vivo* conditions: tissue physiology, tumor microenvironment. The suspension of tumor cells is sown on Petri dishes, culture tablets or vials, conducting passages as the primary culture forms a monolayer. After separation from the tissue and transition to 2D conditions, the morphology of cells changes, as well as the way they divide. Changes in the cell phenotype are the result of 2D culture, which can affect their function, organization of intracellular struc-

tures, cytokine secretion, and cell signaling. Due to violations in interaction with the external environment, cells attached to the plastic surface lose their polarity, which leads to a change in the response of these cells to apoptosis inducers. Another disadvantage of 2D culture is that cells in the monolayer have unlimited access to the environment's ingredients, such as oxygen, nutrients, metabolites, and signaling molecules. For tumor cells *in vivo*, the availability of nutrients, oxygen, etc. is more variable due to the natural architecture of the tumor mass. It is noted that the 2D system changes gene expression and cell biochemistry. Due to many disadvantages of 2D cell line culture systems, it became necessary to search for alternative models. The advantages of 2D crops include ease of operation and their low cost. To grow 2D cultures of tumor cells, a special plastic coated for monolayer cell lines or plastic coated with collagen, D-lysine, or a mixture of various components is used [25].

The 3D cultures

3-D tumor cell culture is currently used in research, both in personalized medicine and in regenerative medicine. This technology most accurately displays the processes occurring in the tumor *in vivo* and recreates the tumor phenotype, which is a valuable tool for studying the biology of the tumor, and will also allow preclinical evaluation of anti-tumor drug candidates on primary cell lines. Currently, the most commonly used model includes small cell aggregates-spheroids, which have been used by oncologists for decades [26]. The use of cell culture spheroids to evaluate the effectiveness of anti-cancer drugs is not a new concept. For almost 50 years, colony formation analysis in soft agar has been the gold standard *in vitro* method used to determine the status of cell transformation, as well as testing new candidate drugs with low throughput [27]. Figure 1 shows an example of spheroids obtained in our own research In the laboratory of cell technologies National Medical Research Centre for Oncology of the Ministry of Health of Russia.

Tumor spheroids are formed using various methods and techniques: the "hanging drop" method; cultivation on plastic with a non-adhesive coating; using scaffolds: hydrogels; using magnetic mixers and bioprinting.

The "hanging drop" method was originally used in Microbiology to study and cultivate bacteria. A suspension of tumor cells is placed on the inside of the Petri dish lid and covered with a Cup containing a phosphate-salt buffer to prevent the droplets from drying out. At the tip of the drop, cells aggregate at the air-liquid interface and then spheroids are formed [28]. This method does not require the use of any substances as a matrix or framework. However, the size of the drops should not be too large — drops with a liquid volume of more than 50 ml will not attach to the Petri plates, because the surface tension of the liquid is overcome by gravity. Replacement of the nutrient medium must be carried out carefully, so as not to damage the resulting spheroid.

An example of the "hanging drop" method Protocol for creating spheroids (permanent lines) is shown in table 1 [29].

Using the "hanging drop" method, spheroids can be maintained in culture for up to several weeks.

Researchers Jeppesen et. al. developed a protocol for obtaining spheroids from colorectal cancer tissue samples (table 2).

From 18 adenocarcinomas, spheroids were successfully created for 15 samples, using this

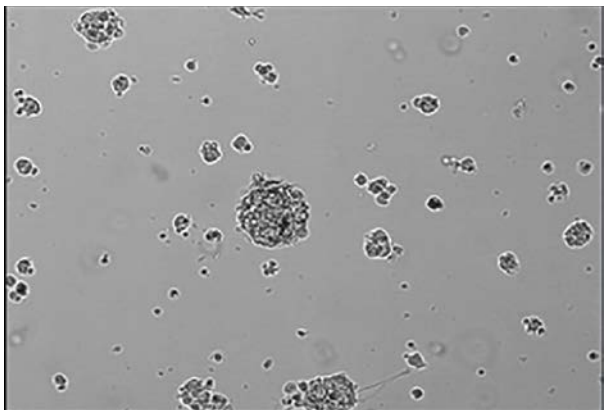

Fig. 1. A culture of spheroids obtained from a glial tumor. Magnification ×100

protocol. We also assessed if the primary culture spheroids of colorectal cancer belong to the histotype of the original tumor. One approach to improving the effectiveness of treatment is to determine the chemosensitivity of tumor cells obtained from the patient's material. Comparison of spheroids with a sample of the original tumor showed that the cells in the culture preserved the histology of the adenocarcinoma and the patterns of expression of cytokeratin 20 and

Table 1. The protocol for the "hanging drop" method.

№ p/p	Steps
Cell suspension making	
1	Cultivate cell lines to form a monolayer. Wash the cells twice with dobs buffer and decant the liquid. Add 2 ml of 0.05% trypsin - 1mm EDTA, incubate at 37 ° C. Control cell detachment. Add 2 ml of the complete nutrient medium to inactivate the trypsin. Resuspend the cells. Transfer to a 15 ml centrifuge tube.
2	Add 40 mcl 10 mg / ml of DNAase, incubate for 5 minutes at room temperature. Shake the test tube and centrifuge at 200 g for 5 minutes.
3	Remove the supernatant, wash the sediment with 1 ml of full nutrient medium. Repeat, then resuspend the cells in 2 ml of full nutrient medium.
4	Count the cells using a hemocytometer or an automatic cell counter. The required cell concentration is 2.5 × 106 in 1 ml.
Formation of "hanging drops"	
5	Add 50 ml of phosphate-salt buffer to the bottom of a 60 mm Petri platelet.
6	Remove and flip the lid off the Petri platelet. With a 20-ml dispenser, place 10-ml drops of the nutrient medium with the cells at the bottom of the lid, so that they do not touch each other. At least 20 drops are placed on one lid.
7	Carefully flip the lid and close the Petri platelets. Incubate at 37°C, 5% CO2, 95% humidity. Daily do microscopy of the platelets, cultivate up to the formation of cell aggregates.
8	After forming of the aggregates, they can be transferred to round-bottomed glass shakers in 3 ml of the complete nutrient medium. Incubate in a shaken water bath at 37 °C, 5% CO2 until spheroids form.

Table 2. The protocol for obtaining spheroids from colorectal cancer tissue samples

№ p/p	Steps
1	Wash the tumor tissue in a phosphate-salt buffer containing antibiotics. Remove fat and necrotic areas with sterile tools (scalpel or scissors).
2	Split the tumor material into 1 ± 2 mm pieces.
3	Add a phosphate-salt buffer containing 1 mg / ml of type II collagenase (Gibco) and antibiotics. Incubate the sample with enzymes for 20 minutes at 37°C.
4	Pass the tissue suspension through several filters in the following sequence: 230 μm filter (Sigma-Aldrich), 100 μm filter (BD Biosciences), 40 μm filter (BD Biosciences) and 30 μm pre-separation filter (MACS, Miltenyi Biotec).
5	Tissue samples,that have not passed through the filter 230 microns, collect and incubate with collagenase (item 2) for 10 minutes at 37°C. Pass it through the filters
6	Collect the tumor fragments with the use of filters of 100.40 and 30 microns and divide them into three fractions according to the size of the filtered cells.
7	Isolated tumor fragments are cultured in a stem cell medium (Thermo Fisher) with the addition of antibiotics (200 u/ml of penicillin, 200 u / ml of streptomycin, 100 u/ml of gentamicin and 2.5 u / ml of amphotericin B) in Petri dishes coated with agarose (Sigma-Aldrich) at 37°C, 5% CO2.

cancer-embryonic antigen. In this paper, chemosensitivity screening using spheroid cultures of five patients showed individual drug response profiles, which is a promising *in vitro* model for use in personalized medicine [30].

Monocultural spheroids obtained in breast cancer research *in vitro* are called mammospheres. There is evidence that the source of metastases are breast cancer cells with a phenotype similar to stem cells. Mammosphere culture is often used to study breast cancer stem cells [31].

Researchers Lombardo and others have developed the protocol for obtaining primary mammospheres from human breast tumor tissue after mastectomy (table 3) [32].

Halfter et al. Have compared the chemosensitivity of spheroids obtained from HER2 — positive breast cancer cell lines with spheroids from 120 fresh tissue samples. Their results showed greater yield efficiency and lower metabolic activity of spheroids obtained from primary cultures compared to spheroids obtained from cell lines [33].

Qureshy-Baig et al. it was reported that primary colorectal cancer spheroids retained their chemoresistance and genetic mutations in relation to the tumor tissue from which they were isolated [34]. In the Weiswald colorectal cancer study et.al. created "colospheres" using the technique of mechanical disaggregation of a sample of tumor tissue with a scalpel and crushing it using a syringe piston. The researchers obtained this model of "colospheres" in 95% of patients.the success of culturing this type of spheroid was associated with the aggressiveness of the tumor [35]. Jaganathan and his colleagues created a cartless 3D model *in vitro* using lines of breast cancer epithelial cells and fibroblasts cultured in a magnetic stirrer incubator in collaboration with NanoshuttlesTM. Fibroblasts were found more on the periphery of three-dimensional structures, while epithelial cells were located in the center. In this model, the authors tried to reproduce the heterogeneity of the tumor environment observed *in vivo*, so they used fibroblasts, thus simulating the extracellular matrix. Treatment of the tumor with doxorubicin resulted in inhibition of the growth of the resulting 3D model [36].

Cultivation in gels

The interaction of the cell with the extracellular matrix (ECM) can modify cellular organization, cell function, and response to therapy. In this regard, there is a need to create a three-dimensional culture model that repeats the role of ECM *in vivo*.

In this context, natural or synthetic hydrogels are used [27] of natural origin (for example, Matri-

Table 3. The protocol for obtaining mammospheres from human breast tumor tissue

№ p/p	Steps
Keep the tumor material in a cold place	
1	Put the sample into 100mm Petri platelets. Remove adipose tissue using sterile tools.
2	Add 2-3 ml of DMEM/F12 and chop the sample into pieces about 1 mm^3 in size with a sterile scalpel.
3	Resuspend tumor samples in 10 ml of DMEM containing proteolytic enzymes (3000 E / ml collagenase and 1000 E / ml hyaluronidase). Incubate at 37°C in a rotary shaker until all tissue fragments dissociate. Complete dissociation takes from 1 to 3 hours. Time of dissociation vary depending on the tissue. (For example, breast adenocarcinoma is usually more difficult to dissociate compared to mucosal carcinoma). Evaluate the degree of dissociation in the hemocytometer every half hour.
4	Precipitate the fragments for 5 minutes, then transfer the supernatant into a 15 ml conical polypropylene tube and centrifuge at 200 g for 10 minutes at room temperature. Remove the capillary fluid and resuspend the cells in 1-5 ml of mammospheric culture medium (it is possible to use specialized culture media for growing tumor stem cells or mesenchymal stem cells).

gel ™, collagen, alginate and fibrin), synthetic (for example, polyethylene glycol or PEG) and some semi-synthetic hydrogels that are a combination of synthetic and natural polymers (for example, hyaluronan, polypeptides) [30]. The cells are inserted into the upper part of the matrix after it has solidified or mixed with a liquid hydrogel. In both methods, tablets for cell culture are pre-coated with hydrogel [37].

Examples of natural scaffolds are Matrigel ™ and collagen. Matrigel ™ is a commercial ECM that includes basal membrane proteins from mouse tumor cells Engelbreth-Holm-Swarm (EHS), such as collagen IV, entactin, laminin perlecan, matrix metalloproteinase-2, and growth factors necessary for polarization, growth regulation, chemotherapeutic resistance, and cell adhesion [38]. Collagen — the most frequent fibrous protein — in the composition of ECM provides strength, regulates cell adhesion, and participates in cell migration and chemotaxis. In three-dimensional cultures, type I collagen is often used, but type II and III collagen can also be used [39]. Like Matrigel ™, collagen varies from batch to batch and has a low stiffness. In addition, natural hydrogels can cause immunogenic reactions [40]. Variability in properties may affect the reproducibility of results and limit the use of such frameworks for drug screening. To overcome the disadvantages of natural hydrogels, synthetic alginate hydrogels have been developed. The use of synthetic hydrogel allows you to control the biochemical and mechanical properties of ECM. There are PEG-based hydrogels, which may include cell adhesion molecules, peptides, or bioactive natural polymers (collagen, fibrin) to enhance cellular activity [41]. Natural and synthetic hydrogels have their limitations for re-determining tumor ECM. Alternatively, semi-synthetic hydrogels can be used. Semi-synthetic hydrogels can provide a controlled environment. Hyaluronan-the main component of natural ECM-is a biocompatible, biodegradable polymer that does not cause immune reactions. It has a high affinity for cell surface receptors involved in cell proliferation, adhesion, migration, and differentiation [42].

Spontaneous formation of spheroids: the method of non-adhesive surface

For this method, pre-coated plates are used, in which the lower surface is hydrophilic, charged neutrally, and covalently bound to the surface of a polystyrene vessel. This coating prevents the cells from adhering to the surface, causing the cells to be suspended and therefore form three-dimensional spheroids. The coating is stable, non-cytotoxic and does not decompose. However, there is a problem associated with the formation of inhomogeneous spheroids [37].

The 3D frame systems

De et.al a new 3D system for *ex vivo* culture of circulating tumor cells (CTCs) from blood samples of breast cancer patients using poly-ε-caprolactone (PCL) scaffolds was presented. It has been shown that this 3D PCL-based frame system can be used to study circulating tumor cells [43].

Since breast cancer cell lines lose their original tumor gene expression profiles when cultured in a monolayer, a model was developed for culturing primary breast cancer cells by decellularization of tumor-associated fibroblasts on three-dimensional polymer scaffolds. The presence of an extracellular matrix derived from tumor fibroblasts deposited on a polycaprolactone scaffold promotes cell attachment and viability, which is associated with higher levels of phosphorylated kinase, which provides cell attachment via integrins. Individual cells of primary breast cancer self-organize into tumor spheroids during long-term cultivation. In this model, the response of tumors received from different patients to chemotherapy drugs differed significantly from sample to sample. The authors suggest using this model as an *ex vivo* platform for culturing primary cell lines to develop effective and personalized chemotherapy regimens [44].

The magnetic levitation method

In this method, cells are grown to 80% confluence, treated with hydrogels containing magnetic iron oxide (MIO), and cultured over-

night [45]. The treated cells are trypsinized and placed in an ultra-low attached plate. Simultaneously, a cover with a neodymium magnet is attached to the top of the plate. Spheroids begin to form within a few hours at the air-liquid phase boundary due to attraction to a magnet. When cells aggregate with each other, they begin to synthesize ECM proteins such as collagen, fibronectin, and laminin. Spheroids can be incubated for several days until they reach the required size for research. This method has many advantages: the growth rate of spheroids is high compared to more common methods; spheroids form their own ECM (there is no need for an artificial framework), spheroids have a size in the mm^2 range (this size better reproduces the necrotic and hypoxic regions found in tumors) and, finally, they do not require a specialized nutrient medium. Disadvantages include the high cost of MIO, as well as it's possible cytotoxicity.

Microfluidic platforms

Microfluidic platforms are devices in which living cells can be cultured and permanently inserted into micrometer-sized chambers. This method allows precise control of the cellular microenvironment, ensuring continuous isolation of growth factors or nutrients [46]. In the simplest system, one microfluidic chamber contains one type of cultured cell. It is also possible to study the interaction between different cell types to recreate the boundaries between different tissues. To do this, micro-channels are connected to each other through porous membranes lined on opposite sides of different cell types (tumor/organ on a chip). The goal is to create an environment in which different types of cells can interact with each other. The organs on the chip allowed us to recreate the entire complex structure and environment, such as skin and hair [47], lungs [48], liver [49] and intestines [50]. This method is convenient for high-performance testing for various drugs, but requires special equipment. Recently, a two-layer microfluidic device has been developed that allows forming, cultivating and testing drugs on 5000 spheroids of tumors of the same size with different geometry of the culture chamber (200x200 m^2 and 300x300 m^2) [51].

Explantates

The method of cell explant culture is suitable for the development of primary tumor cell lines. This technique is the cultivation of small pieces of tumor tissue (2–5 mm in size) in a culture medium. This method significantly facilitates the preservation of the native architecture of tissues and microenvironment, which more fully reflects the interactions in the tumor *in vivo*. However, genetic variation can also occur in the culture being re-grafted and persist in media containing serum. In addition, it is possible to change the cell phenotype due to incorrect orientation of the explant in the culture medium. This method requires sequential subcultivation to produce primary tumor cell lines [6].

By direct explantation, Indian researchers were able to obtain primary epithelial cells of the oral cavity with a yield of up to 90% without microbial expansion in the primary culture [52].

In the research Goldman et.al. suggest the presence of dynamic phenotypic heterogeneity in tumor cells, resulting from the use of chemotherapy, causing resistance to chemotherapy drugs. The authors used explants extracted from the biopsy material of breast cancer patients to analyze the clinical consequences of metabolic reprogramming [53].

Baird et. Al. conducted a study of the STING-ligand (the ligand that stimulates the interferon gene) on an Explant model from patients undergoing resection for head and neck cancer to assess the patient's tumor response to the ligand. Treatment with sting ligands resulted in a statistically significant increase in IFN-α secretion in the Explant [54].

Muff researchers et.al it is believed that the Explant method is suitable for creating primary cell lines of bone and soft tissue sarcoma obtained from a patient, which opens up opportunities for molecular analysis and drug testing for such a heterogeneous group of tumors [55].

Solid tumor culture based on patient-derived explants (PDE) is increasingly being used for preclinical evaluation of new therapeutics and for the detection of biomarkers. Using mass spectrometry, a group of Australian scientists determined the degree of absorption of enzalutamide in 11 explants from prostate tumor samples obtained from 8 patients. At the same time, inhomogeneous intensity of the chemo drug signal was observed in all samples, while a higher area of the drug signal was recorded in the epithelial tissue of the sample with the highest concentration of the drug [56].

The PDE model is also used to study hormone-dependent tumors, such as prostate cancer and breast cancer. PDE cultures obtained from patients with breast or prostate cancer were grown on a gelatin sponge, which is a high-performance and cost-effective method that preserves the natural tissue architecture, microenvironment, and key oncogenic factors [57].

In the Ricciardelli study et.al we used tissue fragments about 5 mm^3 in size of the ovarian tumor after cryopreservation, obtained from patients. It has been shown that this method of cultivating explants using even pre-cryopreserved tissue allows obtaining viable tumor cells with the initial tumor microenvironment for introduction into the primary culture [58].

Karekla et.al have developed a platform for evaluating the response to drugs in non-small cell lung cancer, which will allow conducting preclinical trials of new drug candidates. The researchers propose to use the samples of tumor tissues obtained immediately after surgery. The authors described an optimized model of *ex vivo* explant culture that allows evaluating the response of non-small cell lung cancer to therapy while preserving the tumor microenvironment [59].

CONCLUSION

For decades, the gold standard for preclinical research has been the use of cell lines. However, the long time that cells are maintained in a monolayer, the subcultivation of cell lines used to produce a stable phenotype contributes to the change in the original phenotype of the cell population. Closer collaboration between clinicians and researchers, along with improved laboratory and methodological approaches, has led to the fact that primary cell lines have become a promising model in the field of tumor biology research, as well as opened up wide prospects for the use of these cultures in personalized medicine for preclinical evaluation of chemotherapy drugs. Primary cell lines have the advantages of preserving the original phenotype and features of the tumor, its microenvironment. Obtaining primary cultures is a rather complex process due to the small number of initial tumor cells, as well as the partial loss of cell viability after resection of the tumor and the use of methods of material disaggregation. Many researchers prefer enzymatic methods of dissociation of tumor tissues, since mechanical dissociation is a more "rough" method, while it is possible to obtain the necessary number of viable cells when using two methods simultaneously. The traditional 2D culture systems help to study the morphology and function of tumor cells, while losing important components of the intercellular matrix and intercellular interactions important for cell differentiation and proliferation. The 3D cultivation of primary tumor lines allows you to create cultivation conditions close to those of *in vivo*. The cell culture in Matrigel improves the integration of signaling pathways in cells, increases the expression of biomarkers. Scaffold-based methods for culturing primary cell lines have become important, especially in the past two decades. These methods can potentially overcome some of the limitations of modern three-dimensional cell culture methods, such as uneven cell distribution, inadequate nutrient diffusion, and uncontrolled size of cell aggregates. The use of scaffolds allows obtaining a membrane for attachment, proliferation and migration of tumor cells. Explant culture is a promising method for obtaining primary cell lines for use in personalized medicine and for use in preclinical studies to evaluate the tumor response to new candidate drugs. New methods

and approaches are being developed to isolate and obtain primary cell lines from tumor samples. The choice of the method of the tumor material dissociation and the method of culturing the primary cell line, provides an opportunity to study the biology of the tumor in its various aspects and is an excellent preclinical tool for the study of tumors in the *in vitro* systems.

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References

1. Freshney J. Animal cell culture. Practical guide. Translated from the 5th English edition. Moscow: Binom.Laboratory of knowledge, 2011.
2. Leithner K, Wohlkoenig C, Stacher E, Lindenmann J, Hofmann NA, Gallé B, et al. Hypoxia increases membrane metallo-endopeptidase expression in a novel lung cancer *ex vivo* model — role of tumor stroma cells. BMC Cancer. 2014 Jan 25;14:40. https://doi.org/10.1186/1471–2407–14–40
3. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell. 2012 Mar 20;21(3):309–322. https://doi.org/10.1016/j.ccr.2012.02.022
4. Shibue T, Weinberg RA. EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. Nat Rev Clin Oncol. 2017 Oct;14(10):611–629. https://doi.org/10.1038/nrclinonc.2017.44
5. Hirata E, Sahai E. Tumor Microenvironment and Differential Responses to Therapy. Cold Spring Harb Perspect Med. 2017 Jul 5;7(7). https://doi.org/10.1101/cshperspect.a026781
6. Mitra A, Mishra L, Li S. Technologies for deriving primary tumor cells for use in personalized cancer therapy. Trends Biotechnol. 2013 Jun;31(6):347–354. https://doi.org/10.1016/j.tibtech.2013.03.006
7. Li W-C, Ralphs KL, Tosh D. Isolation and culture of adult mouse hepatocytes. Methods Mol Biol. 2010;633:185–196. https://doi.org/10.1007/978–1–59745–019–5_13
8. Janik K, Popeda M, Peciak J, Rosiak K, Smolarz M, Treda C, et al. Efficient and simple approach to *in vitro* culture of primary epithelial cancer cells. Biosci Rep. 2016;36(6). https://doi.org/10.1042/BSR20160208
9. Volovitz I, Shapira N, Ezer H, Gafni A, Lustgarten M, Alter T, et al. A non-aggressive, highly efficient, enzymatic method for dissociation of human brain-tumors and brain-tissues to viable single-cells. BMC Neurosci. 2016 Jun 1;17(1):30. https://doi.org/10.1186/s12868–016–0262-y
10. Skog M, Sivlér P, Steinvall I, Aili D, Sjöberg F, Elmasry M. The Effect of Enzymatic Digestion on Cultured Epithelial Autografts. Cell Transplant. 2019;28(5):638–644. https://doi.org/10.1177/0963689719833305
11. Nishikata T, Ishikawa M, Matsuyama T, Takamatsu K, Fukuhara T, Konishi Y. Primary culture of breast cancer: a model system for epithelial-mesenchymal transition and cancer stem cells. Anticancer Res. 2013 Jul;33(7):2867–2874.
12. Spaethling JM, Na Y-J, Lee J, Ulyanova AV, Baltuch GH, Bell TJ, et al. Primary Cell Culture of Live Neurosurgically Resected Aged Adult Human Brain Cells and Single Cell Transcriptomics. Cell Rep. 2017 Jan 17;18(3):791–803. https://doi.org/10.1016/j.celrep.2016.12.066
13. Mederacke I, Dapito DH, Affò S, Uchinami H, Schwabe RF. High-yield and high-purity isolation of hepatic stellate cells from normal and fibrotic mouse livers. Nat Protoc. 2015 Feb;10(2):305–315. https://doi.org/10.1038/nprot.2015.017
14. Castell JV, Gómez-Lechón MJ. Liver cell culture techniques. Methods Mol Biol. 2009;481:35–46. https://doi.org/10.1007/978–1–59745–201–4_4
15. Ribatti D. A milestone in the study of the vascular system: Wilhelm Roux's doctoral thesis on the bifurcation of blood vessels. Haematologica. 2002 Jul;87(7):677–678.
16. Damm G, Schicht G, Zimmermann A, Rennert C, Fischer N, Kießig M, et al. Effect of glucose and insulin supplementation on the isolation of primary human hepatocytes. EXCLI J. 2019;18:1071–1091. https://doi.org/10.17179/excli2019–1782
17. Trojaneck B, Niemitz S, Micka B, Lefterova P, Blasczyk R, Scheffold C, et al. Establishment and characterization of colon carcinoma and renal cell carcinoma primary cultures. Cancer Biother Radiopharm. 2000 Apr;15(2):169–174. https://doi.org/10.1089/cbr.2000.15.169
18. Krbala L, Soukup J, Stanislav J, Hanusova V. Derivation and basic characterization of colorectal carcinoma primary

cell lines. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2017 Dec;161(4):360–368.
https://doi.org/10.5507/bp.2017.040
19. Cunningham RE. Tissue disaggregation. Methods Mol. Biol. 2010;588:327–330.
https://doi.org/10.1007/978–1–59745–324–0_32
20. Skarkova V, Krupova M, Vitovcova B, Skarka A, Kasparova P, Krupa P, et al. The Evaluation of Glioblastoma Cell Dissociation and Its Influence on Its Behavior. Int J Mol Sci. 2019 Sep 18;20(18):4630. https://doi.org/10.3390/ijms20184630
21. Qiu X, De Jesus J, Pennell M, Troiani M, Haun JB. Microfluidic device for mechanical dissociation of cancer cell aggregates into single cells. Lab Chip. 2015 Jan 7;15(1):339–350. https://doi.org/10.1039/c4lc01126k
22. Kar R, Chawla D, Gupta B, Mehndiratta M, Wadhwa N, Agarwal R. Establishment of Primary Cell Culture From Ascitic Fluid and Solid Tumor Obtained From Epithelial Ovarian Carcinoma Patients. Int J Gynecol Cancer. 2017;27(9):2000–2005.
https://doi.org/10.1097/igc.0000000000001087
23. Filippova SY, Sitkovskaya AO, Sagakyants AB, Bondarenko ES, Vashchenko LN, Kechedzhieva EE, et al. Breast cancer stem cells isolation with application of collagenase from crab hepatopancreas. Modern problems of science and education. 2019;6:147. (In Russian).
24. Mezhevova IV, Sitkovskaya AO, Rostorguev EHE, Filippova SYu, Nistratova OV, Kuznetsova NS, et al. Neurosurgical approach for obtaining primary cell lines of glial tumors. Research and Practical Medicine Journal (Issled. prakt. med.). 2019;6(S):191. (In Russian).
25. Kapałczyńska M, Kolenda T, Przybyła W, Zajączkowska M, Teresiak A, Filas V, et al. 2D and 3D cell cultures — a comparison of different types of cancer cell cultures. Arch Med Sci. 2018 Jun;14(4):910–919.
https://doi.org/10.5114/aoms.2016.63743
26. Burdett E, Kasper FK, Mikos AG, Ludwig JA. Engineering tumors: a tissue engineering perspective in cancer biology. Tissue Eng Part B Rev. 2010 Jun;16(3):351–359.
https://doi.org/10.1089/ten.teb.2009.0676
27. Sant S, Johnston PA. The production of 3D tumor spheroids for cancer drug discovery. Drug Discov Today Technol. 2017 Mar;23:27–36. https://doi.org/10.1016/j.ddtec.2017.03.002
28. Jørgensen A, Young J, Nielsen JE, Joensen UN, Toft BG, Rajpert-De Meyts E, et al. Hanging drop cultures of human testis and testis cancer samples: a model used to investigate activin treatment effects in a preserved niche. Br J Cancer. 2014 May 13;110(10):2604–2014.
https://doi.org/10.1038/bjc.2014.160
29. Foty R. A simple hanging drop cell culture protocol for generation of 3D spheroids. J Vis Exp. 2011 May 6;(51).
https://doi.org/10.3791/2720
30. Jeppesen M, Hagel G, Glenthoj A, Vainer B, Ibsen P, Harling H, et al. Short-term spheroid culture of primary colorectal cancer cells as an *in vitro* model for personalizing cancer medicine. PLoS ONE. 2017;12(9):e0183074.
https://doi.org/10.1371/journal.pone.0183074
31. Ahmad A. Breast Cancer Metastasis and Drug Resistance. Challenges and Progress. Advances in Experimental Medicine and Biology. 2019;1115: 1–7.
https://doi.org/10.1007/978–3–030–20301–6
32. Lombardo Y, de Giorgio A, Coombes CR, Stebbing J, Castellano L. Mammosphere formation assay from human breast cancer tissues and cell lines. J Vis Exp. 2015 Mar 22;(97): 52671.
https://doi.org/10.3791/52671
33. Hoffmann O, Ditsch N, Ahne M, Arnold F, Paepke S, et al. Testing chemotherapy efficacy in HER2 negative breast cancer using patient-derived spheroids. J Transl Med. 2016;14(1):112.
https://doi.org/10.1186/s12967–016–0855–3
34. Qureshi- Baig K, Ullmann P, Rodriguez F, Frasquilho S, Nazarov PV, Haan S, et al. What Do We Learn from Spheroid Culture Systems? Insights from Tumorspheres Derived from Primary Colon Cancer Tissue. PLoS ONE. 2016;11(1):e0146052.
https://doi.org/10.1371/journal.pone.0146052
35. Weiswald L-B, Bellet D, Dangles-Marie V. Spherical cancer models in tumor biology. Neoplasia. 2015 Jan;17(1):1–15.
https://doi.org/10.1016/j.neo.2014.12.004
36. Jaganathan H, Gage J, Leonard F, Srinivasan S, Souza GR, Dave B, et al. Three-dimensional *in vitro* co-culture model of breast tumor using magnetic levitation. Sci Rep. 2014 Oct 1;4:6468. https://doi.org/10.1038/srep06468
37. Hoarau- Véchot J, Rafii A, Touboul C, Pasquier J. Halfway between 2D and Animal Models: Are 3D Cultures the Ideal Tool to Study Cancer-Microenvironment Interactions? Int J Mol Sci. 2018 Jan 18;19(1):181.
https://doi.org/10.3390/ijms19010181
38. Kleinman HK, Martin GR. Matrigel: basement membrane matrix with biological activity. Semin Cancer Biol. 2005 Oct;15(5):378–386.
https://doi.org/10.1016/j.semcancer.2005.05.004
39. Doyle AD, Carvajal N, Jin A, Matsumoto K, Yamada KM. Local 3D matrix microenvironment regulates cell migration through spatiotemporal dynamics of contractility-dependent adhesions. Nat Commun. 2015 Nov 9;6:8720.
https://doi.org/10.1038/ncomms9720
40. Tibbitt MW, Anseth KS. Hydrogels as extracellular matrix mimics for 3D cell culture. Biotechnol Bioeng. 2009 Jul 1;103(4):655–663. https://doi.org/10.1002/bit.22361
41. Tokuda EY, Jones CE, Anseth KS. PEG-peptide hydrogels reveal differential effects of matrix microenvironmental cues on melanoma drug sensitivity. Integr Biol (Camb). 2017 Jan 23;9(1):76–87. https://doi.org/10.1039/c6ib00229c

42. Yu M, Jambhrunkar S, Thorn P, Chen J, Gu W, Yu C. Hyaluronic acid modified mesoporous silica nanoparticles for targeted drug delivery to CD44-overexpressing cancer cells. Nanoscale. 2013 Jan 7;5(1):178–183. https://doi.org/10.1039/c2nr32145a

43. De T, Goyal S, Balachander G, Chatterjee K, Kumar P, Babu K G, et al. A Novel *Ex Vivo* System Using 3D Polymer Scaffold to Culture Circulating Tumor Cells from Breast Cancer Patients Exhibits Dynamic E-M Phenotypes. J Clin Med. 2019 Sep 16;8(9):1473. https://doi.org/10.3390/jcm8091473

44. Nayak B, Balachander GM, Manjunath S, Rangarajan A, Chatterjee K. Tissue mimetic 3D scaffold for breast tumor-derived organoid culture toward personalized chemotherapy. Colloids Surf B Biointerfaces. 2019 Aug 1;180:334–343. https://doi.org/10.1016/j.colsurfb.2019.04.056

45. Nath S, Devi GR. Three-dimensional culture systems in cancer research: Focus on tumor spheroid model. Pharmacol Ther. 2016;163:94–108. https://doi.org/10.1016/j.pharmthera.2016.03.013

46. Whitesides GM. The origins and the future of microfluidics. Nature. 2006 Jul 27;442(7101):368–373. https://doi.org/10.1038/nature05058

47. Ataç B, Wagner I, Horland R, Lauster R, Marx U, Tonevitsky AG, et al. Skin and hair on-a-chip: *in vitro* skin models versus *ex vivo* tissue maintenance with dynamic perfusion. Lab Chip. 2013 Sep 21;13(18):3555–3561. https://doi.org/10.1039/c3lc50227a

48. Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE. Reconstituting organ-level lung functions on a chip. Science. 2010 Jun 25;328(5986):1662–1668. https://doi.org/10.1126/science.1188302

49. Powers MJ, Domansky K, Kaazempur-Mofrad MR, Kalezi A, Capitano A, Upadhyaya A, et al. A microfabricated array bioreactor for perfused 3D liver culture. Biotechnol Bioeng. 2002 May 5;78(3):257–269. https://doi.org/10.1002/bit.10143

50. Kimura H, Yamamoto T, Sakai H, Sakai Y, Fujii T. An integrated microfluidic system for long-term perfusion culture and on-line monitoring of intestinal tissue models. Lab Chip. 2008 May;8(5):741–746. https://doi.org/10.1039/b717091b

51. Patra B, Peng C–C, Liao W-H, Lee C-H, Tung Y-C. Drug testing and flow cytometry analysis on a large number of uniform sized tumor spheroids using a microfluidic device. Sci Rep. 2016 Feb 15;6:21061. https://doi.org/10.1038/srep21061

52. Shwetha HR, Kotrashetti VS, Babu NC, Kumbar V, Bhat K, Reddy R. *Ex vivo* culture of oral keratinocytes using direct explant cell culture technique. J Oral Maxillofac Pathol. 2019 Aug;23(2):243–247. https://doi.org/10.4103/jomfp.JOMFP_105_19

53. Goldman A, Khiste S, Freinkman E, Dhawan A, Majumder B, Mondal J, et al. Targeting tumor phenotypic plasticity and metabolic remodeling in adaptive cross-drug tolerance. Sci Signal. 2019 Aug 20;12(595). https://doi.org/10.1126/scisignal.aas8779

54. Baird JR, Bell RB, Troesch V, Friedman D, Bambina S, Kramer G, et al. Evaluation of Explant Responses to STING Ligands: Personalized Immunosurgical Therapy for Head and Neck Squamous Cell Carcinoma. Cancer Res. 2018 Nov 1;78(21):6308–6319. https://doi.org/10.1158/0008–5472.can-18–1652

55. Muff R, Botter SM, Husmann K, Tchinda J, Selvam P, Seeli-Maduz F, et al. Explant culture of sarcoma patients' tissue. Lab Invest. 2016;96(7):752–762. https://doi.org/10.1038/labinvest.2016.49

56. Mutuku SM, Trim PJ, Prabhala BK, Irani S, Bremert KL, Logan JM, et al. Evaluation of Small Molecule Drug Uptake in Patient-Derived Prostate Cancer Explants by Mass Spectrometry. Sci Rep. 2019 Oct 18;9(1):15008. https://doi.org/10.1038/s41598–019–51549–3

57. Centenera MM, Hickey TE, Jindal S, Ryan NK, Ravindranathan P, Mohammed H, et al. A patient-derived explant (PDE) model of hormone-dependent cancer. Mol Oncol. 2018;12(9):1608–1622. https://doi.org/10.1002/1878–0261.12354

58. Ricciardelli C, Lokman NA, Sabit I, Gunasegaran K, Bonner WM, Pyragius CE, et al. Novel *ex vivo* ovarian cancer tissue explant assay for prediction of chemosensitivity and response to novel therapeutics. Cancer Lett. 2018 May 1;421:51–58. https://doi.org/10.1016/j.canlet.2018.02.006

59. Karekla E, Liao W-J, Sharp B, Pugh J, Reid H, Quesne JL, et al. *Ex Vivo* Explant Cultures of Non-Small Cell Lung Carcinoma Enable Evaluation of Primary Tumor Responses to Anticancer Therapy. Cancer Res. 2017 Apr 15;77(8):2029–2039. https://doi.org/10.1158/0008–5472.can-16–1121

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