REVIEW

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# Cancer Cell Culture Techniques, Micronutrients, and Protocols in Health Sciences: A Comprehensive Review

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Cancer cell culture techniques play a pivotal role in advancing our understanding of cancer biology, drug development, and personalized medicine in the field of health sciences. In order to understand how micronutrients affect the activity, viability, and genomic stability of cells in cancer research, these nutrients must be added to procedures for growing cancer cells. Essential elements that impact DNA metabolism, cell viability, and genomic integrity are micronutrients, which comprise minerals and vitamins. Research revealed that in cancer cell cultures, micronutrients can improve cell viability and preserve genomic integrity. Micronutrients, including vitamins and minerals, are essential components of cell culture media that are designed to promote cell growth and maintain the cells in an *in vitro* environment. These micronutrients play crucial roles in various cellular functions, including metabolism, DNA synthesis, and antioxidant defense. While micronutrients are generally beneficial for cancer cell growth, it's important to carefully balance their concentrations in cell culture media to avoid adverse effects such as apoptosis. Micronutrients and cancer outcomes have been found to have complex relationships, with varying but significant effects on cancer types and cancer outcomes. Additionally, micronutrients have a critical role in the pathophysiology of cancer by modifying signaling pathways, adding to the complexity of the disease. This review aims to explore the significance of cancer cell culture techniques and the role of micronutrients in cancer research and therapy. Micronutrients must be incorporated into cancer cell growth techniques in health sciences labs in order to preserve genome integrity and cellular viability. Studies revealed that micronutrients play a major role in cellular health through their impact on DNA metabolic pathways. The acknowledgement of their significance in experimental procedures highlights the progress in comprehending their function. As a result, improving the micronutrient content of cell culture supplements has the potential to increase the validity of results and expand knowledge of cancer cell biology.

***Key Words:*** *Cancer, micronutrients, cell line, proliferation, cell culture techniques*

## Sağlık Bilimlerinde Kanser Hücre Kültürü Teknikleri, Mikro Besinler ve Protokoller: Kapsamlı Bir İnceleme

Kanser hücre kültürü teknikleri, sağlık bilimleri alanında kanser biyolojisi, ilaç geliştirme ve kişiselleştirilmiş tıp anlayışımızı ilerletmede çok önemli bir rol oynamaktadır. Kanser araştırmalarında mikro besinlerin hücresel aktivite, canlılık ve genomik stabilite üzerindeki etkileri, bu besinlerin kanser hücre kültürü prosedürlerine entegrasyonu ile birlikte ele alınmaktadır. DNA metabolizmasını, hücre canlılığını ve genomik bütünlüğü etkileyen temel unsurlar, mineraller ve vitaminlerden oluşan mikro besinlerdir. Araştırmalar, kanser hücre kültürlerinde mikro besinlerin hücre canlılığını artırabildiğini ve genomik bütünlüğü koruyabildiğini ortaya koymaktadır. Vitaminler ve mineraller de dâhil olmak üzere mikro besinler, hücrelerin *in vitro* ortamda büyümesini teşvik etmek ve korumak için tasarlanmış hücre kültürü ortamının temel bileşenleridir. Bu mikro besinler metabolizma, DNA sentezi ve antioksidan savunma dâhil olmak üzere çeşitli hücresel işlevlerde önemli roller oynar. Mikro besinler genellikle kanserli hücre büyümesi için faydalı olsa da, apoptozis gibi etkilerden kaçınmak için hücre kültürü ortamındaki konsantrasyonlarını dikkatlice dengelemek önemlidir. Mikro besinler ve kanser sonuçları arasında karmaşık ilişkiler olduğu, kanser türleri ve kanser sonuçları üzerinde çeşitli ancak önemli etkilerin olduğu bulunmuştur. Ek olarak, mikro besinler sinyal yollarını değiştirerek kanser patofizyolojisinde kritik bir rol sahibidir ve bu da hastalığın kompleksliğine katkıda bulunur. Bu derleme, kanser hücresi kültürü tekniklerinin önemini ve mikro besinlerin kanser araştırmalarındaki rolünü araştırmayı amaçlamaktadır. Genom bütünlüğünü ve hücresel canlılığı korumak için mikro besinler sağlık bilimleri laboratuvarlarında kanser hücresi büyütme tekniklerine dâhil edilmelidir. Çalışmalar, mikro besinlerin DNA metabolik yolları üzerindeki etkileri yoluyla hücresel sağlıkta önemli bir rol oynadığını ortaya koymaktadır. Deneysel prosedürlerdeki önemlerinin kabul edilmesi, işlevlerinin anlaşılmasındaki ilerlemeyi vurgulamaktadır. Sonuç olarak, hücre kültürü takviyelerinin mikro besin içeriğinin iyileştirilmesi, sonuçların geçerliliğini artırma ve kanser hücresi biyolojisi hakkındaki bilgileri genişletme potansiyeline sahiptir.

***Anahtar Kelimeler:*** *Kanser, mikrobesinler, hücre hattı, proliferasyon, hücre kültürü teknikleri*

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## Introduction

Cancer is a multi-faceted collection of diseases defined by unregulated cell proliferation and the potential of these cells to infiltrate surrounding tissues (1). Tumor formation is caused by genetic alterations that interfere with the regular control of cell division (2). Cancer can occur in virtually any organ or tissue in the body and is one of the major causes of different diseases and deaths globally. The incidence of cancer has increased substantially, emerging as a prominent global public health issue. The World

Health Organization (WHO) has declared that cancer is responsible for millions of fatalities annually. Various factors, including age, genetics, lifestyle, and environmental exposures, can influence the occurrence of cancer (3). As populations age and lifestyles change, the burden of cancer is expected to increase (4).

Understanding the fundamental aspects of cancer biology is crucial for developing effective prevention, diagnosis, and treatment strategies. Cancer cells exhibit unique characteristics, and studying them *in vitro* provides significant findings about the molecular processes leading to tumorigenesis (5). Cell culture techniques play a vital role in cancer research, allowing scientists to manipulate and observe cancer cells in a controlled environment (6). Cancer cell culture stands as a crucial tool in cancer research, enabling researchers to study cancer cells in a controlled environment (7, 8). The *in vitro* model's usage enables the isolation and manipulation of cancer cells, providing insights into their biology and behavior. Cell lines derived from various cancer types are extensively employed to investigate specific molecular pathways, signaling cascades, and therapeutic responses (9). For example, in breast cancer research, well-known cell lines like MCF-7 and SKBR3 have been used to figure out how estrogen receptor signaling works at the molecular level and how well targeted therapies work (10). The versatility of *in vitro* models extends to various cancer types, contributing to a deeper understanding of the heterogeneity within and across tumors. Cancer cell culture techniques play a pivotal role in advancing and understanding tumor biology and drug responses (11). Maintaining cancer cells outside their natural context allows for detailed investigations into the molecular mechanisms driving tumorigenesis and metastasis (12).

Adherent cultures, where cells grow on a substrate, mimic the interaction between cancer cells and the extracellular matrix (13). Suspension cultures, on the other hand, simulate conditions where cells are not attached, resembling aspects of hematogenous dissemination in cancers like leukemia. Three-dimensional (3D) culture systems have gained prominence for better recapitulating the intricate nature of the tumor microenvironment *in vivo* (14). These systems foster the formation of spheroids or organoids, providing a more precise depiction of how cancer cells behave and respond to therapeutic agents (15).

**Rationale for the Use of Cell Culture Techniques in Cancer Research**

Cancer research has witnessed profound advancements in recent decades, and cell culture techniques stand at the forefront of these transformative endeavors. The application of in vitro models has become indispensable in unraveling the intricacies of cancer biology, offering researchers a controlled environment to investigate cellular behaviors, molecular pathways, and potential therapeutic interventions. Cell culture techniques enable researchers to dissect and isolate specific molecular mechanisms underlying cancer initiation, progression, and metastasis. By manipulating the cellular environment, scientists can delineate the roles of various genes, proteins, and signaling pathways in a controlled setting. This precision is essential for comprehending the complex molecular interactions that propel cancer progression (1). Cancer is characterized by substantial heterogeneity, even within a specific tumor type. Cell culture provides a platform to study and compare different cancer cell lines, allowing researchers to explore the diverse genetic and phenotypic variations. This has proven essential for tailoring treatments to individual patients based on the unique characteristics of their tumors (9). Cell culture techniques play an essential role in assessing the efficacy of potential anti-cancer drugs. Researchers can expose cultured cancer cells to different therapeutic agents, monitoring how cells respond to treatment. This approach aids in identifying promising candidates for further preclinical and clinical studies (16). Resistance to chemotherapy and targeted therapies is a major challenge in cancer treatment. Cell culture studies allow scientists to simulate and understand the mechanisms behind drug resistance. This knowledge is essential for devising strategies to overcome obstacles and improve the effectiveness of therapeutic interventions (17). The era of personalized medicine in cancer treatment relies heavily on cell culture techniques. By culturing cancer cells from individual patients, researchers can assess the specific response of these cells to various treatments. This strategy has enormous potential for customizing treatments according to the distinct genetic and molecular characteristics of each patient's cancer (15). Cancer cells interact with the surrounding microenvironment *in vivo*, which affects the development and course of tumors. Cell culture techniques, particularly three-dimensional (3D) culture systems, enable researchers to mimic aspects of the tumor microenvironment. This enables a more precise depiction of cancer cell behavior and responses to treatments. While the models of animals have conventionally been utilized in cancer research, ethical concerns and the need for more relevant models have shifted focus toward *in vitro* systems. Cell culture techniques provide an ethical alternative that offers more control over experimental conditions, reducing the reliance on animal studies (18). Cell culture allows for high-throughput screening of potential therapeutics, making it a cost-effective method for early-stage drug discovery. This efficiency is crucial for rapidly identifying promising compounds and moving them through the drug development pipeline (19).

**Fundamental Principles of Cancer Cell Culture**

The culture of cancer cells is a foundational technique in cancer research, providing a controlled environment for analyzing the behavior of cancer cells outside the human body. This approach involves the propagation and maintenance of cancer cells *in vitro*, allowing researchers to investigate various aspects of cancer biology. Understanding the basic principles involved in cell culture is crucial for ensuring the reliability and reproducibility of experimental findings.

One of the initial steps in cancer cell culture involves establishing a cell line that can be obtained from a tumor biopsy or a cancerous tissue sample. Verification of the cell line's authenticity is important to ensure its fidelity and relevance to the original tumor. Cell line repositories and databases have significant functions in verifying the identity of cancer cell lines, preventing cross-contamination, and maintaining research reproducibility (20). Choosing an appropriate cell culture medium is crucial for the optimal growth and maintenance of cancerous cells. The medium must contain all the essential nutrients including vitamins, and minerals required for cell proliferation. The commonly used media in cell culture labs are Dulbecco's Modified Eagle Medium (DMEM) and RPMI-1640. Fetal bovine serum (FBS) or alternatives like bovine serum albumin (BSA) are added to enhance cell growth and provide necessary factors for cell survival (21). Maintaining optimal incubation conditions is essential for the success of cancer cell culture. Cells are typically cultured in a humidified incubator with controlled temperature (usually 37°C) and a specific level of CO2 (commonly 5%). These conditions mimic the physiological environment of the human body and support cell viability and proliferation. As cancer cells proliferate, they reach confluency, and subculturing is necessary to prevent overgrowth and maintain healthy cells. Subculturing involves detaching cells from the culture vessel, usually using enzymes like trypsin, and seeding them into new flasks or plates. Regular passaging ensures that the cell population remains actively growing and genetically stable (21). Maintaining sterility during cancer cell culture is crucial to prevent contamination, which can compromise experimental results. Aseptic techniques, including working in a laminar flow hood, proper handwashing, and routine cleaning of the laboratory environment, are essential to ensure the purity of the cell culture (22). To maintain a renewable source of cells and ensure experimental consistency, cryopreservation is employed. Cancer cells are frozen using cryoprotectants and stored in liquid nitrogen. Establishing cell banks provides researchers with a consistent supply of cells for future experiments (23). Regular monitoring of the culture to ensure cell viability and proliferation is crucial for assessing overall health and behavior of the cancer cells. Various methods, including trypan blue exclusion assays and automated cell counting devices, help researchers quantify viable cells and track their growth kinetics (24). Cancer cell culture allows for a range of experimental manipulations and assays to explore different aspects of cancer biology. These include drug screening assays, gene expression analyses, and the study of how cells respond to diverse stimuli. Techniques like immunocytochemistry and flow cytometry provide valuable insights into the molecular characteristics of cancer cells (6).

## Cancer Cell Cultures as a Model for Research

The use of models of animals and the methods of cell culture enables us to study the processes involved in the development, function, and disease of tissues and organs more efficiently (25). In 1907, Harrison conducted the first cell cultures as part of his investigation into the origins of nerve fibers (26). Subsequently, the technique has been refined and applied to observe how cells grow and differentiate in *in vitro* environment (27). These days, primary cells that have been extracted straight from donor tissue or established cultures that are stored in cell banks can be used for research (28).

Primary cultures typically consist of various types of cells found in the originating tissue and are cultured separately from living things. So, it is very important to extract the appropriate and accurate cell type (28). Although the difficulty of extraction and brief lifespan are the characteristic attributes of primary cell lines, they contain the genetic characteristics of *in vivo* tumors that enable them to perform some functional experiments.

Understanding cell biology, tissue morphology, disease processes, protein synthesis, and the advancement of tissue engineering are all made feasible by cell cultures (29). They are frequently employed in studies on gene function, cancer research, and preclinical testing of numerous medications (28).

## Methods of Cancer Cell cultures

In the field of cancer research, selecting the best suitable cell culture techniques may help us better understand the biology of tumors, which may lead to improved radiotherapy and chemotherapy outcomes or even the development of novel therapeutic approaches (30). Under adherent circumstances, the cells are affixed to a plastic dish or a glass plate, or they can be cultured in a manner, that more closely resembles the natural environment (such as lymphocyte cultures) (31). The 2D model is the most frequently utilized form of cell culture, although 3D culture also becoming more and more popular recently (32). Numerous factors influence how a cell behaves, depending on the culture type selected (29).

### *1. Adherent Monolayer*

In 2D adherent cultures, cells proliferate as a single layer within a culture flask or on a flat petri dish, adhering to the surface (33). Despite the simplicity and cost-effectiveness of maintaining these cultures and conducting functional tests, they come with significant drawbacks. Firstly, cells in 2D cultures fail to replicate the natural structures of tissues or tumors. This approach lacks representation of essential cell interactions with both neighboring cells and the extracellular environment, critical for processes such as, proliferation, differentiation, gene and protein expression, response to various stimuli, metabolism of drug, and other cellular processes (34). Secondly, when isolated and subjected to 2D conditions, cells undergo alteration in the shape and mode of replication, leading to a change in phenotypes (35). Changes in cell morphology can impact cellular function, internal structure organization, secretion, and cell signaling (36,37). The loss of polarity due to disrupted interactions with the external environment in adherent cultures can

alter cellular responses, including apoptosis (38). Additionally, 2D cultures provide cells in the monolayer unrestricted access to the components of medium like nutrients, oxygen, metabolites, and signaling molecules, unlike the variable conditions experienced by cancer cells *in vivo* because of the inherent structure of tumor masses. The 2D system has been observed to induce alterations in topology, cellular biochemistry, splicing, and gene expressions (38–41). Also, adherent cultures typically consist of monocultures, limiting studies to a single cell type and lacking the tumor microenvironment or niches essential for cancer-initiating cells *in vivo* (42,43).

### *2. Suspension Cultures*

Salmon and Hamburg pioneered one of the initial 3D cultures in the 1970s using a soft agar solution (30). Subsequently, extensive documentation has highlighted remarkable similarities in morphology and behavior between the cells that are cultured in 3D environment and those within a tumor mass (9,31,41). Based on preparation methods, 3D models can be categorized into three types of Suspension cultures on non-adherent plates, Cultures in concentrated medium or gel-like substances and Cultures on a scaffold. In suspension cultures on non-adherent plates (30,44,45) the single layer of cells is placed on the plates without adhesion-promoting surfaces, and 3D structures emerge after 3 days of culture. This method is valued for its simplicity, ease, and rapidity. While the bacterial coated plates or non-adherent culture plates are alternative options for culturing cells, they are suitable only for certain cell lines and also another advantage is that the cells can be readily harvested from the medium for subsequent experiments. But some cell lines require costly plates that are coated with particular materials, like covalently bound hydrogel or polystyrene, due to strong binding tendencies also the formation of aggregate can occur because of the cells moving in the suspension medium. In cultures with concentrated medium or gel-like substances (44–47) single cells proliferate in a medium that contains substances exhibiting gelling properties. There are two main approaches, first to make a lower layer, dissolve low-melting agarose, mix it with cell medium, pour the mixture onto a plate, and then incubate until the lower layer solidifies. Next, add a top layer that consists of agarose and medium containing single cells and the second is use of Matrigel, the cells are immersed in the multiprotein hydrogel. 3D structures become observable after seven days of culture. The advantages include soft agar enables the examination of single-cell growth independent of adhesion and the process of evading anoikic and the cells cultured in Matrigel can be readily retrieved for additional examination, showcasing 3D engagements with the surrounding environment, constructing tissue-like formations. This method is particularly useful for studying cell aggressiveness, metastatic potential, and analyzing the structure's influence. However, it is difficult to obtain spheres for specific cell lines, and it is not easy to prepare the two layers of agar, it consumes a lot of time. The immunofluorescence staining of the spheres and the extraction of cells from the agar can also be problematic along with the poor reproducibility of the outcomes and the Matrigel which contains innate bioactive components affect the formation of structures. In cultures on a scaffold (44,48–50) cells have the capacity to migrate among fibers, adhere to the scaffolds crafted from biodegradable materials and fill the space between fibers, enabling growth and division. It is useful because the system is highly compatible with the functionals tests that are commercially available and other DNA/RNA and protein isolation kits, also the analysis for immunohistochemical is easy to prepare. But in the scaffold cultures the cells become flattened and spread out in shape like the cells that are cultured under the adherent conditions. The size of scaffolds and the arrangement of cells can result in different cell behaviors and the choice materials for the scaffold construction the scaffold can affect cell adhesion.

## Micronutrients Usage in Cancer Cell Culture Techniques

Micronutrients are the essential nutrients that are needed in small amounts for living organisms and are very important for life. All the vitamins, including A, E, and D, and minerals, including calcium, zinc, and iron, are considered micronutrients. Micronutrients have a well-established role *in vivo*, and their effects on genomic integrity have been the subject of numerous research. (51) The human diet needs about forty micronutrients, and adequate consumption of each element is necessary for proper metabolism. A shortage of micronutrients affects metabolism in a variety of intricate ways, some of which might cause damage to DNA. Because of their vital function in intermediate metabolism, micronutrients are necessary for the best possible metabolism of macronutrients. Every time metabolism occurs, one or more vitamins and minerals must also be involved simultaneously. Thus, the etiology of chronic degenerative diseases and the pace of pathogenesis are closely linked to abnormalities in micronutrients. Recent studies on nutrition have brought attention to the various nutrients' roles in controlling genetic machinery (52). To be more precise, certain minerals and vitamins serve as substrates and/or cofactors in the various metabolic pathways that control gene expression, DNA synthesis, and/or repair. (53) Micronutrients, including minerals and vitamins, are vital components of DNA metabolic pathways, influencing cellular processes vital for life (51). The dynamic interplay between micronutrients and DNA regulation underscores their importance in cancer cell culture. Selected micronutrients, such as vitamins C, D, and E, along with folic acid, polyphenols, metals like copper, iron, zinc etc. iodine, and fatty acids, exhibit varying effects on breast cancer progression (54). A comprehensive guide detailing the connection between 22 cancer related outcomes and the 14 micronutrients revealed the complex relationship between vitamins and minerals and cancer (55). However, inconsistencies in reported effects warrant further exploration in cancer cell culture.

Recent studies highlight the role of micronutrients in cancer cell induction and pathways (56). Vitamin D supplementation has shown significant potential in influencing cellular responses. The influence of micronutrients on cellular pathways, as revealed by recent studies, provides valuable insights into their role in cancer cell culture. Understanding these pathways is essential for targeted interventions. Exploring specific micronutrients, such as Selenomethionine and selenocysteine, unveils their potential impact in cancer cell culture (57). These insights contribute to the nuanced understanding of the use of micronutrients in labs. Certain vitamins and minerals are included in the composition of the culture media enabling the creation of *in vitro* models that can mimic a cell's reaction to various stimuli that replicate *in vivo* environment. Micronutrients like calcium, iron, magnesium, folate, and folate have been identified as essential for various functions of cells such as survival, cellular growth, and multiplication in cell culture (35–38). However, distinct reactions may be elicited by the kind of cell and the specific concentration of micronutrients in a culture. The types and roles of micronutrients in cancer cell culture is essential for creating optimized culture conditions. These micronutrients influence various cellular processes, providing researchers with tools to study cancer cell behavior and responses *in vitro.* The selection and concentration of micronutrients should be carefully considered based on the specific requirements of the cancer cell type under investigation. Micronutrients have the ability to act directly on the genome, preventing mutations or indirectly protecting the genome by serving as cofactors of the enzymes in the cellular processes that modulate transformation (1,58). Consequently, any variation can produce DNA damage. The essential vitamins are vitamin A, vitamin B, $B_7$, $B_9$, $B_{12}$, C and E and essential minerals include copper, iron, magnesium, selenium, and zinc.

## Role of Micronutrients in Cancer Cell Culture

Micronutrients, including vitamins and minerals, are essential components of cell culture media that is designed to promote the growth and maintain the cells *in vitro* environment. These micronutrients play crucial roles in various cellular functions, including metabolism, DNA synthesis, and antioxidant defense. While micronutrients are generally beneficial for cell growth, it's important to carefully balance their concentrations in cell culture media to avoid adverse effects such as apoptosis. The use of micronutrients in cell culture reagents is justified for a number of reasons, and their quantities need to be taken into account.

Micronutrients serve as indispensable components for cellular machinery, participating in a plethora of biochemical pathways critical for cell growth and metabolism. The intricate dance of cellular life relies on the harmonious interplay of these micronutrients, ensuring the structural stability of cells and the dynamic equilibrium of biochemical processes in Cell Culture Media. Vitamin A, an essential fat-soluble micronutrient, has emerged as a key player in cellular identity and differentiation. Recent studies suggest that vitamin A and its derivatives contribute to the regulation of cellular retinol-binding proteins, exerting control over the fate of cells and influencing their growth patterns (59). Vitamin D, often referred to as "sunshine vitamin," extends its influence beyond calcium homeostasis. Research indicates its involvement in cellular processes, including immune modulation and cancer prevention. The intricate web of vitamin D signaling pathways contributes to the regulation of cell proliferation, emphasizing its importance in maintaining cellular harmony (60). Vitamins C and E, renowned for their antioxidant prowess, stand as guardians against the perils of oxidative stress. Recent studies highlight their role in safeguarding the cells against reactive oxygen species, thereby preventing oxidative damage. As integral components of the cellular defense system, these vitamins contribute to the maintenance of cellular health and metabolic balance (61,62) The B-vitamin complex including B7 (Biotin), B9 (Folate), and B12 (Cobalamin), serves as the navigators of intricate metabolic pathways within the cell. Biotin, an essential cofactor, participates in carboxylation reactions crucial for fatty acid synthesis and energy production (63). Folate, on the other hand, contributes to nucleotide synthesis and DNA methylation, while Cobalamin aids in the metabolism of amino acids and nucleotides (64).

Beyond vitamins, minerals such as zinc, iron, and selenium act as the foundation of cellular infrastructure. Iron, an integral component of hemoglobin, ensures oxygen transport and cellular respiration. (65) Zinc participates in the structure of enzymes and DNA-binding proteins, while selenium, incorporated into selenoproteins, functions as an antioxidant, protecting cells from oxidative damage (66).

## Protocols of Cancer Cell Culture

### *1. Primary Culture*

A primary cell culture is the first setup derived directly from body tissues, including solid tumor fragments or cell suspensions like aspirates (e.g., peritoneal ascites or pleural effusions). The cellular composition of primary cultures can be variable, often including hematopoietic and stromal cell types. Fibroblasts, with their strong attachment abilities, can outgrow cancer cell populations, posing a challenge. Cancer cells typically adhere to substrates before proliferating, but they can also grow in suspension, such as in agar. Strategies involving mechanical and enzymatic methods are employed for tissue fragment dispersion.

### *2. Routine Feeding and Maintenance*

Routine examination of cell cultures, both macroscopically and microscopically, is crucial. This involves checking cell morphology, density, and the presence of contaminants. Regular media changes are essential to prevent nutrient depletion and acidification. The pH of the medium, monitored by color indicators like phenol red, provides insights. The number of time you

need to change the media depends on the culture's growth rate, with faster-growing cultures requiring more regular changes.

***3. Subculture of Cells***

When a culture occupies the entire surface of a flask (for a monolayer culture) or depletes nutrients (for a suspension culture), subculture is necessary to maintain healthy growth. This process involves reducing cell density, optimizing growth conditions. The detachment of adherent monolayers, termed "harvesting," often involves proteolytic enzymes like trypsin. Subculturing a secondary culture from a primary culture result in a "cell line," characterized and tracked by the number of passages.

***4. Cloning***

Cloning involves isolating an individual cell and developing its progeny, producing genetically homogeneous cultures. Colony-forming efficiency (CFE) measures a culture's capacity for colony formation. Primary cultures typically have low CFE values (<1%), while cell lines generally exhibit higher values (10–100%). Methods for cloning cells on plastic and within agar are detailed.

***5. Cell Counting***

Accurate cell counting is crucial for experiments and monitoring cell responses. Two protocols are presented: one using a hemocytometer for small sample sizes and minimal equipment outlay, and the other using an electronic counter (e.g., Z2 from Beckman Coulter) for quick and precise counting of large cell numbers.

***6. Cryopreservation***

Cryopreservation, storing cells below –130°C, enables long-term storage. Cryoprotective agents like dimethyl sulfoxide (DMSO) prevent ice crystal formation. Freezing rates of approximately 1°C/min are optimal, while rapid thawing is accomplished in a water bath set at 37°C. Cells are preserved in a liquid nitrogen at a temperature of –196°C, but short-term viability is possible at –80°C.

***7. Troubleshooting***

Several common challenges frequently arise cell culture investigations like Suspension cells clumping together, too acidic pH, too basic pH, Cell death and Poor growth in culture. To get rid of the problems potential solutions are implemented (61-68).

**Characterization of Cancer Cell Lines**

Cancer cell lines provide a versatile platform for studying different aspects of cancer, ranging from understanding genetic mutations to exploring potential therapeutic interventions (69). Cell lines derived from human cancer are extensively utilized to explore the biology of malignancy and evaluate hypotheses aimed at improving treatment efficacy. These models contribute crucial insights into the clinical relevance of experimental findings (70). A critical examination of cancer cell lines revealed challenges, including the constant generation of variants with phenotypic and/or genotypic differences. This genetic heterogeneity raises questions about the reliability of established cancer cell lines for research purposes (71).

Cancer cell lines must undergo molecular characterization to be deemed reliable models for cancer research. Many researchers depend on the assurances offered by cell repositories such as ECACC or ATCC; however, it is observed that misidentification of cell lines or cross-contamination by HeLa cells occur. Allen and colleagues recently discussed the lack of characterization. One conclusion reached by the authors was that the ATCC's U87MGglioblastoma cell line is not identical to the original cell line (72).

The method most frequently used to confirm the authenticity of cancer cell lines is short tandem repeat (STR) profiling (73). Labs can validate their cancer cell lines for free using this technique. Using commercial primers, polymorphic STR loci are amplified in this technique. The validated samples or donor tissue are compared with the PCR product obtained.

The application of STR profiling in cancer cell lines is with restrictions. It is true that cancer cell lines have genetic alterations and are extremely heterogeneous. Furthermore, over an extended period, genetic drift may happen. Redefining the STR profiling analysis parameters is therefore necessary. It will be necessary to look for a close match rather than a perfect fit, and most studies indicate that a match equal to 80% is sufficient.

**Conclusion**

The utilization of micronutrients in cancer cell culture techniques has emerged as a crucial aspect of experimental design. Micronutrients, encompassing minerals and vitamins, play indispensable roles in DNA metabolic pathways, exerting a significant impact on cell viability and genomic stability. Studies, as reflected in reviews and reflections on cell-culture supplementation, emphasize the ability of micronutrients to enhance cell viability and maintain genomic integrity in cancer cell cultures. In conclusion, cancer cell culture techniques and micronutrients play integral roles in advancing cancer research and therapy in health sciences. By employing sophisticated culture models and understanding the impact of micronutrients on cellular processes, researchers can uncover novel therapeutic targets and strategies for cancer prevention and treatment.

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