

## Mini Review - Semi-solid Media for Transformed Cell Culturing

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**Received:** August 11, 2020**Published:** August 31, 2020

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**Abstract**

Cell culture techniques help us to understand the cell biology in a much better way. Transformed cell culturing will be one of the useful methods to understand the cancer cell physiology and morphology but culturing of transformed cells in-vitro is a challenging task. The cellular outcome read-outs such as cell signals, cellular secretions, gene expression and downregulation, morphology, etc., vary depending upon the microenvironment given to the cells. Therefore, it is essential to culture the cells in an environment similar to in- vivo for understanding the cell biology behind various mechanisms. This mini review aims at summarizing the conventional and advancements in culturing the transformed cells. Different techniques are highly exploited to study transformed cells, with the most commonly used 3-D semi-solid media culturing method. It is a selective media with suitable substrate that supports the growth of anchorage-independent transformed cells and is useful to elucidate results from products of each cell differentially localized in the media. Early research using soft agar helped to understand and characterize the transformed cells whereas advancements in the semi-solid media helps to screen a wide range of anti-cancer drugs, to study gene regulation, and in biosensors. The major setback of this technique remains a hindrance to extract viable cells from the media. There by to overcome the drawback of conventional semi-solid media technique, over the years, various components are incorporated and substituted to provide a within reach in-vivo conditions. More recently, using semi-solid matrix/scaffolds like matrigel/laminin/alginate, co-culturing of other cells with transformed cells provides the *in vivo* like niche.

**Keywords:** Transformed Cell Culture; Semi – Solid Media; Soft Agar; Laminin; Matrigel

**Introduction**

Animal cell culturing is one of the significant technologies with a wide range of application in the field of medicine and biology. This technology involves cell isolation and culturing them in a suitable artificial environment started in the early 19<sup>th</sup> century and the field has advanced in the past few decades [1]. Primary and secondary cultures of tissues have the disadvantage of limited life span (20-100 generations), confluency stage (contact inhibition) and can potentially differentiate into unstable aberrant cells (genetic instability), and these limitations can be overcome by using transformed cell lines. During transformation the cell takes up foreign DNA from the environment and undergoes genomic changes. The acquired DNA can either express the desired characteristics of foreign DNA or help the cells to grow infinitely. Their gene alteration

results in genetically modified, immortalized, anchorage independent and high growth rate cells which can grow on low serum requirement. The significant differences between the primary strain and the transformed cell lines are that the transformed cells gain higher telomerase activity, expression of specific genes responsible for growth and cell division, the abolition of contact inhibition, and genetic changes to bypass senescence [2,3]. The transformation of the primary cell strain occurs in two different ways: either by extending the minimum number of passages of a primary strain creating a selection of specific cells exhibiting transformation or by artificially transforming the cells using carcinogens, ionizing radiations, and transfecting oncogenic viruses or genes [4,5]. These cells grow indefinitely and create an established cell line. One of the best examples of transformed cells is HeLa, the first human immortal

cell line isolated from the cervical cancer biopsy of Henreitta, is now widely used in research. The Human Papilloma virus (HPV) infection of cervical cells suppressed the p53 tumor suppressor gene and resulted in transformed HeLa cells (<https://berkeley-science.com/article/good-bad-hela/>). Transformed cell lines are widely used in therapeutic proteins, vaccine and antibody development; to study drug metabolism and carcinogenicity; tissue culturing and 3D organ development [6]. The importance given to indefinite cell lines is high because they are easy to manipulate and can be maintained with the same phenotype over any number of passages. They display anchorage-independent growth and exhibit the characteristics of a tumour cell. Thereby, it helps us to study niche of tumours as well as to understand the properties of primary cell strain. But for the in-vitro understanding of cell biology mechanisms of different cell types, mimicking the microenvironment to elucidate the cell behavior is necessary [7]. It majorly depends upon the culturing aspects of the particular cell line. It is essential because different cell types differ in their nutrient requirements or stimulus [7]. The transformed cells have the ability to grow on a 3D format, whereas the non-transformed cells grow as 2D monolayer. Semi-solid media like soft agar serves as a gold standard method to study the tumours and their drug targets, the role of tumour suppressor genes involved and to identify chemopreventents. The first 3D culture using soft agar was made by Hamburg and salmon in 1970s [8]. In this mini- review, the early stage research using soft agar and the advancements in the semi-solid media with its application will be discussed.

### Culturing techniques for transformed cell lines

The property of transformed cells like growing as anchorage-independent cells to form spheroids is highly exploited for culturing the transformed cells into different cell lines exhibiting different characteristics [2]. Two major approaches for culturing the transformed cell lines are 2D monolayer cell culture and 3D spheroid cell culture. Mostly, 2D cell culture is utilized in various *in-vitro* studies, but the setback of this method is the cultured cells grow as flat monolayers on the surfaces of the media containing petri dish and hence it is difficult to study the mechanism of cells. Also, it does not resembles the natural microenvironment of tissues *in vivo* [8]. The cells are availed with unlimited access of nutrients all the time, which produces altered morphology, function, secretions, cell signaling and mode of division. The major disadvantage of 2D cell culture is that we can only study a particular cell type per experiment [8,9]. The disadvantages of 2D cell culture were replaced by 3D cell culture methodologies.

The 3D cell culture can be subdivided into two based on the nature of the media utilized: Liquid and semi-solid (or soft agar) media cell culture [8]. Both the media helps to exploits the ability of the transformed cells to grow as anchorage independent cells and form suspensions or bulky spheroids secreting extracellular matrix (ECM) [10]. Cells grow in liquid media as suspensions are majorly used for the storing the transformed cells. In soft agar colony formation assay the cells are immobilized using gelling components like agar, agarose, and methylcellulose [5,8,9,11]. The gelling ingredients differ based on the different factors of study and cell type used. Semi-solid assay helps to differentiate and confirm the presence of transformed cells from primary cell strain because primary cells cannot propagate independently on semi-solid media [2].

### Culturing transformants on the soft agar

The steps include isolation of human or animal cells, that will be treated with chemicals like MNNG, aflatoxin B1, diethyl nitrosamine; or by infecting with viruses like polyomia, SV-40, adeno virus, etc., to obtain transformed cell lines [12-15]. In brief, the primary cells will be cultured in nutrient media like DMEM or MEM and will be subjected to transforming agents and pre-transformants will be obtained. Then, the pre-transformants will gain new property which will be characterized by growing them in semi-solid media and incubated at 37°C at 5% CO<sub>2</sub> incubators and incubation period will vary according to the study (Macpherson, I. 1971). The karyotype and cell morphology-physiology of transformants will be examined [13]. Semi-solid media include majorly soft agar techniques which were widely used till late 20<sup>th</sup> century. The soft agar assay is usually performed by three-layer method. The bottom layer of the culture dish is coated with 0.6-1% of agarose gel to prevent cell from growing on the surface. The second layer is 0.3% agarose with cell suspension and the upper (feeder) layer contains 0.3-1% of agarose with component of choice (depending on the assay method either drug targets or substrates or nutrients can be added) (Figure 1) [2,16,17]. The cultured cells are maintained with different oxygen/ carbon dioxide concentration, serum and specific growth factors based on cell type chosen [3,8].

### Basic research on soft agar colony formation assay

In the late 20<sup>th</sup> century, soft agar method was widely used to study basic characteristics of transformants like transformation ability, serum requirements and their ability to grow as anchor independence colonies. This method was also vastly deployed for studying the mechanism 'anoikis' and its related factors in cell [8]. Bush., *et al.* [18] and Renato Dulbecco [19] studies show that viral

based transformed animal cells can grow under reduced serum conditions and cells will undergo deterioration and death when se-

**Figure 1:** Schematic representation of the soft agar assay.

rum is limited implies that transformed cells require serum mainly for DNA synthesis [18,19]. Huschtscha., *et al.* [14] reported that SV40 transformed human lung fibroblast strains when cultured on bacto agar gave rise to pure and stable cell lines with higher growth rate and colonies could grow on low serum levels. Also, the fibroblast cell line when transformed gave rise to epithelial cell like morphology [14]. Soft agar plates were used for cloning and replica plating of transformed cells as well [20]. Also, this technique serves as good *in vitro* model to study the cellular properties associated with tumorigenicity and different potential carcinogens. Freedman., *et al.* [12] observed that both tumor derived and non-tumour derived cell could grow on semi-solid methyl cellulose media and the degree of growth rate of this media is relatable with tumorigenicity [12]. Freedman again in 1977 studied the characteristics of chemically (MNNG) transformed human diploid cells using methyl cellulose media. Stable diploid variants were grown as colonies on the semi- solid medium [21]. The enzyme activity (Example: Plasminogen activator) in neoplastic hamster cells was studied by growing the cells on soft agar and overlaying the colonies with substrate (fibrin) with agarose [22].

The key advantage of this technique over 2D cell culture is it represents a close mimicry of the cellular niche seen *in-vivo*, and it selectively promotes the growth of cells that can proliferate in an anchorage-independent manner [16]. The efficacy of cell growth is measured using the read-outs of colony count and colony sizes. The semi-solid media helps us to understand the communication and signaling networks present in the cells. It helps us to formulate systems of cell-cell, cell- environment and paracrine mode cell signals [7-9,16]. It also overcomes the 2D cell culture disadvantage on studies of a particular cell type per experiment, by enabling the mode of study using co-cultures to understand the cell differentiation and signaling paradigm as it allows the single progenitor

cell to remain localized [8]. The biggest drawback of this culture technique includes the inability of the user to extract viable cells from the agar-based media. It also hinders the reproducibility of the results. Some cell lines, like cartilage cells, hematopoietic cells, etc., can naturally grow on these media without transformation [7,8,23]. The cellular colonies formed cannot be transplanted in experimental animal models for *in-vivo* studies and remain a challenge to date [8].

#### Recent advancements in soft agar colony formation assay

The cellular growth in the natural environment exhibits tight cellular integrity in the presence of many basement membranes and other ECM compounds [7]. But when cells isolated from tissues, they lose their integrity and undergoes morphological changes according to the stimulus provided through the culture medium. Cells that grow on soft agar as 3D models do not provide the *in vivo* like niche because they grow as suspended colonies rather than spheres/spheroids/organoids\*. Also, the traditional soft agar based 6-24 well plate assays are laborious, costly and inefficient [24]. Most of the clinical trials of anti-cancer drugs fail in phase III - animal trials because the drugs were not screened in the proper *in vitro* cancer cell microenvironment. To lower the chance of drug failure, the 3D models of cancer cells will help to understand the drug dynamics and efficacy, because the cells grown as 3D spheres/spheroids displays cell-cell interaction and cell-ECM interactions [8]. Therefore, to maintain or at least reach a close by mimicry of the cellular niche *in-vitro* by forming spheroids/organoids from transformed cells, many different trials are experimented to formulate appropriate methodologies. The advancements the culturing media are listed below.

Matrigel, a product of Corning Life Sciences, is used for soft agar culture. It contains the ECM secreted by the Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells. This particular ECM provides all the requirements for the maintenance of the cellular niche and promotes *in-vivo* like morphology in the different cell lines. The compound solidifies like other gelling compounds at higher temperatures. The advantage of using this media over traditional soft agar method is that, it facilitates the transplantation of viable colonies from culture plates to *in-vitro* animal model studies and pathological studies (Figure 2). According to the cell type additional basement membrane components such as fibronectin, collagen IV, laminin, proteoglycans, protein kinases, etc. are incorporated in the media to promote *in-vivo* like morphology and cellular integrity [5,10]. Replacement of ordinary agar or agarose with low molecular weight gelators (LMWG) such as Hydrogels provides a matrix

\*The term sphere refers to clustering of transformed cells; spheroid refers to group of clustered spheres; organoid refers to clusters of spheroids

and also can be designed in various scaffolds for the desired cellular outcome. These are mainly used in tissue engineering for the growth of lab organs and other tissues. These matrices are highly porous and are useful in trials of different drug delivery systems [25].

Other commercially available synthetic or natural scaffolds are polyethylene glycol (PEG), polyvinyl alcohol (PVA), polylactide-co-glycolide (PLG), polycaprolactone (PLA), matrigel, basement membrane extract, laminin-rich ECM (IrECM) and hyaluronic acid [8,26]. It is evident that depending on the cell type, the matrix should be chosen because morphology and growth rate depends on the matrix used. For example, breast cancer cells (MDA-MB-231) grow well on alginate-matrigel (50:50) as elongated-stellate morphology [27] (Figure 3A). But the only, single drawback of this compound is that it is an animal ECM and growth of human transformed cell line might perturb the results [8,10]. Renal cell carcinoma (Caki-1 skin metastasis derived cell lines) could grow well on laminin coated plates containing StemXvivo media as large spheroid with fibroblast like morphology (Figure 3B) and less cell viability [28]. Another study on Invasive lobular carcinoma cell lines, a subtype of breast cancer cells- MDA-MB-134, SUM44, MDA-MB-330, BCK4 (Figure 3- C,D,E,F) were cultured on collagen I and matrigel. In the collagen containing plate, MDA-MB-134 and SUM44 cells were exhibiting robust growth, whereas MDA-MB-330 cells formed loose colonies and BCK4 cells formed small colonies. In matrigel, "grape-like" protrusive structures were formed with high growth rate [29].

Growth in Low Attachment (GILA) assay is the latest transformed cell culture method in which the transformed cells will selectively grow on low attachment coating in flasks or Petri dishes (Figure 4). The most commonly used coating is poly HEMA (polyhydroxy ethyl methacrylate), an antiadhesive polymer which inhibits the strong adherence characteristics of cells. Using this assay, researchers can extract viable cells without the downstream processing of removal of agar media [9,30]. For example, ACHN (plural effusion derived cell lines) formed small spheroids with epithelial like morphology when cultured in poly-D-lysine [28].

### Application of semi-solid media

By the end of the 20<sup>th</sup> century the semi solid media based cancer studies have emerged greatly with many advancements and applications. The soft agar based tumorigenesis studies, oncogene studies, drug screening/discovery/resistance, 3D culturing of tissues/cancer stem cells, phenotype studies of tumours, etc.,

**Figure 2:** Schematic representation of culturing transformed cells (red) on matrigel and viable colonies were selected and transplanted into the in-vivo system like mouse model.

**Figure 3:** Different morphology of different cancer cells when grown on scaffold/matrix containing media. A)MDA-MB-331 cell grown on alginate-matrigel, B) Caki-1 cells grown on laminin coated plates, C-F) MDA-MB-134, SUM44, MDA-MB-330, BCK4 cells grown on collagen, G-H) MDA-MB-134, SUM44, MDA-MB-330, BCK4 cells grown on matrigel. Reprint from Cavo., *et al.* 2018; Brodaczewska., *et al.* 2019; Tasdemir., *et al.*

**Figure 4:** Growth of transformed cells on low attachment surfaces. The transformed cells will be initially seeded onto the plates and the cells will grow into aggregates and later it forms 3D spheroids.

has been developed in the recent few decades. Rather than studying the tumours in monolayers it is more suitable to study in the 3D which provides a suitable micro- environment. This method helps to study the mechanistic processes and drug resistances in tumours as well [31]. Beyond cancer cell culturing, the semi-solid media were used in biosensors, tissue/organ development and used for coating them on small chips as well. Also novel methods like co-culturing of cancer cells with stromal cells also been developed. Few robust examples and applications of semi-solid media are tabulated (Table 1).

### Conclusion

Semi-solid media is one of the most useful culturing techniques available for the transformed cells. Though the method was widely

| Semi-solid media   | Area of study  | End point   | Reference                       |
|--|--|---|---------------------------------|
| Agarose gel and 2-Hydroxyethyl agarose   | Anti-cancer drug screening   | Peptidylarginyl deaminase 2 (PADI) can be a potent breast cancer biomarker. BB-Cl-amidine can inhibit PADI over expressing (MCF10DCIS) cells.   | Horibata., <i>et al.</i> [16]   |
| Any kind of Semi-solid media like poly HEMA, agarose, methyl cellulose, etc.,                                      | Assay Technologies   | Culturing 3D culture, it is possible to study cell viability, invasiveness, colony count and size, architectural disruption of 3D cell cultures, spheroid size analysis, apoptosis assessment.  | [2,31,32]                       |
| Soft agar in a sterile plastic tube with lid, containing filter for gas exchange.                                  | Immuno histochemical analysis of gene  | Immunofluorescence analysis of subcellular localization of mTOR kinase in MCF-7 breast cancer cells.  | Khoruzhenko, A. I. [17]         |
| Serum free, xeno free 3D culture media containing growth factors identified – Nutristem, MeSenCultSF and StenXvivo | 3D culture of cancer cells and cancer stem cells which resembles in vivo model | Renal cancer cells and cancer stem cells cultured as spheres and spheroids. Spheres are 3D clusters of cancer stem cells used to identify stem-like characteristics and spheroids are clusters of cancer cells used to study cell-cell, cell-matrix interaction and drug screening. | Klaudia., <i>et al.</i> 2019    |
| High throughput screening Using agar 384 well plates   | To identify chemo preventive drug For obesity associated cancer                | Piropodophillin and fluvastatin could inhibit obesity associated cancer. This study was conducted using skin epithelial JB6 p+ cells.   | Benham., <i>et al.</i> [24]     |
| Synthetic hydrogel (alginate, collagen, matrigel) on flat electrode surface  | Electrode/ electrochemical biosensor   | Study on A549 lung cancer cells to monitor cellular responses when treated with anti-cancer drugs.  | Biosensors., <i>et al.</i> [26] |

**Table 1:** Semi-solid media and its application in medicine and research.

used in the 1960s, deployment of this method for various clinical studies was done much later. In the beginning, the technique was

only used to screen the cancer cells and test compounds which can inhibit tumour formation. But soon the method took a turn in



other aspects to understand different molecular mechanisms. In the past few decades the transformed cell culture techniques have gained more importance. In that culturing those cells on the appropriate media is more important. Only soft agar technique based research was to gain the basic knowledge about the transformed cells like its morphology, characteristics. The current research and recent advancements in this technique have given a considerable contribution to the clinical trials of many drugs and to formulate various drug delivery systems to target the desired cell type minimizing side effects. They are utilized for the treatment of cell line based drug screening before entering into the animal model testing [9,25,33]. The culture technique is nowadays introduced in the field of tissue engineering to study stem cells and different progenitor cells [33]. The frequency and qualitative analysis of cellular differentiation are observed, and the knowledge is applied for the organ development and transplantation units. Despite the new advancements, we still have to focus on reducing the cost and to increase reproducibility with high throughput, standardized validation methods [34-36].

## Bibliography

1. Verma A. "Animal Tissue Culture". *Animal Biotechnology* (2014): 211-231.
2. Borowicz S., et al. "The Soft Agar Colony Formation Assay". *Journal of Visualized Experiments* 92 (2014).
3. Bielecka ZF., et al. "Hypoxic 3D in vitro culture models reveal distinct resistance processes to TKIs in renal cancer cells". *Cell and Bioscience* 7 (2017): 71.
4. Hakomori SI and Murakami WT. "Glycolipids of hamster fibroblasts and derived malignant transformed cell lines". *PNAS* 59.1 (1965): 254-261.
5. Pollack R., et al. "Plasminogen Activator Production Accompanies Loss of Anchorage Regulation in Transformation of Primary Rat Embryo Cells by Simian Virus 40". *Proceedings of the National Academy of Sciences* 71.12 (1974): 4792-4796.
6. Bhatia S. "Introduction to animal tissue culture science". In *Introduction to Pharmaceutical Biotechnology*, IOP Publishing 3 (2019).
7. Lodish H., et al. Section 6.2, Growth of Animal Cells in Culture. *Molecular Cell Biology*. 4th edition. New York: W. H. Freeman.
8. Kapałczyńska M., et al. "2D and 3D cell cultures - a comparison of different types of cancer cell cultures". *Archives of Medical Science* 14.4 (2018): 910-919.
9. Rotem A., et al. "Alternative to the soft-agar assay that permits high-throughput drug and genetic screens for cellular transformation". *Proceedings of the National Academy of Sciences* 112.18 (2015): 5708-5713.
10. Yoshida S., et al. "Stimulatory effect of reconstituted basement membrane components (matrigel) on the colony formation of a panel of human lung cancer cell lines in soft agar". *Journal of Cancer Research and Clinical Oncology* 123.6 (1997): 301-309.
11. Kubota K., et al. "Comparison between Agar and Methylcellulose Cultures of Human Leukemic Cells". *Cancer Research* 41 (1981): 3052-3057.
12. Freedman V. "Cellular tumorigenicity in nude mice: Correlation with cell growth in semi-solid medium". *Cell* 3.4 (1974): 355-359.
13. Graham F L., et al. "Characteristics of a Human Cell Line Transformed by DNA from Human Adenovirus Type 5". *Journal of General Virology* 36.1 (1977): 59-72.
14. Huschtscha L I and Holliday R. "Limited and unlimited growth of sv40- transformed cells from human diploid mrc-5 fibroblasts". 99 (1983): 77-99.
15. Idoine JB., et al. "Rat liver cells in culture: Effect of storage, long-term culture, and transformation on some enzyme levels". *In Vitro* 12.8 (1976): 541-553.
16. Horibata S., et al. "Utilization of the Soft Agar Colony Formation Assay to Identify Inhibitors of Tumorigenicity in Breast Cancer Cells". *Journal of Visualized Experiments (JoVE)* (2015): e52727.
17. Khoruzhenko AI. "Optimization of tumor cell culture conditions in soft agar for subsequent immunohistochemical analysis". *Biopolymers and Cell* 28.4 (2012): 302-305.
18. Bush H and Shodell M. "Cell cycle changes in transformed cells growing under serum-free conditions". *Journal of Cellular Physiology* 90.3 (1977): 573-583.

19. DULBECCO R. "Topoinhibition and Serum Requirement of Transformed and Untransformed Cells". *Nature* 227.5260 (1970): 802-806.
20. Kuroki T. "Colony formation of mammalian cells on agar plates and its application to Lederberg's replica plating". *Experimental Cell Research* 80.1 (1973): 55-62.
21. Freedman V H and Shin S. Brief Communication 1,2. 58.6 (2018): 1873-1875.
22. Barrett JC., *et al.* "Reexamination of the Role of Plasminogen Activator Production for Growth in Semisolid Agar of Neoplastic Hamster Cells". 40 (1980): 1438-1443.
23. Adams A., *et al.* "Sensitivity of the Epstein-Barr virus transformed human lymphoid cell lines to interferon". *Journal of General Virology* 28.2 (1975): 207-217.
24. Benham V., *et al.* "Identifying chemopreventive agents for obesity-associated cancers using an efficient, 3D high-throughput transformation assay". *Scientific Reports* 9.1 (2019).
25. Vashist A., *et al.* "Recent advances in hydrogel based drug delivery systems for the human body". *Journal of Materials Chemistry B* 2.2 (2014): 147-166.
26. Edmondson R., *et al.* "Three-Dimensional Cell Culture Systems and Their Applications in Drug Discovery and Cell-Based Biosensors". *ASSAY and Drug Development Technologies* 12.4 (2014): 207-218.
27. Cavo M., *et al.* "A new cell-laden 3D Alginate-Matrigel hydrogel resembles human breast cancer cell malignant morphology, spread and invasion capability observed "in vivo". *Scientific Reports* 8.1 (2018).
28. Brodaczewska K., *et al.* "Metastatic renal cell carcinoma cells growing in 3D on poly-D-lysine or laminin present a stem-like phenotype and drug resistance". *Oncology Reports* (2019).
29. Tasdemir N., *et al.* "Comprehensive Phenotypic Characterization of Human Invasive Lobular Carcinoma Cell Lines in 2D and 3D Cultures". *Cancer Research* 78.21 (2018): 6209-6222.
30. Fukazawa H., *et al.* "A Microplate Assay for Quantitation of Anchorage-Independent Growth of Transformed Cells". *Analytical Biochemistry* 228.1 (1995): 83-90.
31. Lovitt C., *et al.* "Advanced Cell Culture Techniques for Cancer Drug Discovery". *Biology* 3.2 (2014): 345-367.
32. Macpherson I. "Soft Agar Techniques". *Tissue Culture* (1973): 276-280.
33. Buick N R., *et al.* "Application of in vitro soft agar techniques for growth of tumor cells to the study of colon cancer". *ACS Journals* 45 (1980): 1238-1242.
34. Maliszewska-Olejniczak, K., *et al.* "Development of extracellular matrix supported 3D culture of renal cancer cells and renal cancer stem cells". *Cytotechnology* 71.1 (2018): 149-163.
35. Stoker M., *et al.* "Anchorage and growth regulation in normal and virus-transformed cells". *International Journal of Cancer* 3.5 (1968): 683-693.
36. Technical Library. Guidelines to Maintain Cultured cells | Thermo Fisher Scientific - UK.

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