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Transforming Cancer Studies: State-of-the-Art Technologies for 3D Tumor Spheroid Development and Analysis

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Abstract-Unlike 2D cell culture models, 3D spheroids can more closely resemble the spatial architecture, physiological reactions, release of soluble mediators, patterns of gene expression and medicine resistance pathways of solid tumours. Because of these distinctive qualities, 3D cellular aggregates have the potential to be exploited as small- and large-scale in vitro models for testing new anticancer treatments.

Many techniques used to create 3D tumour spheroids are highlighted in the first section, including scaffold-based methods, hanging drop techniques, and microfluidic systems. Each technique is discussed in terms of its advantages, limitations, and suitability for different applications. Additionally, the incorporation of bioengineered scaffolds, extracellular matrix components, and stromal cells into 3D tumor spheroids is explored for enhanced biomimicry and recapitulation of the tumor microenvironment.

The second section explains the characterization techniques used to assess the physiological and molecular properties of 3D tumor spheroids. These include imaging modalities such as confocal microscopy, multiphoton microscopy, and high-content screening, which enable the visualization and analysis of spheroid morphology, viability, proliferation, and drug response.In the final section, the paper discusses the promising applications of 3D tumor spheroids in research related to cancer and drug development. The potential of 3D tumor spheroids to overcome the gap between traditional in vitro cell culture models and in vivo animal studies is also emphasized.Overall, this review showcases the emerging technologies that have propelled the field of 3D tumor spheroid research, enabling more physiologically relevant models for studying cancer biology and therapeutic interventions. The comprehensive understanding of 3D tumor spheroid development, characterization techniques, and promising applications discussed herein will undoubtedly contribute to advancing cancer research and improving patient outcomes in the future.

Keywords: Spheroids, Tumor, Resistance, Cell Signalling, Organ Engineering

Introduction

2D in vitro cultures, in which cells develop in a monolayer, have been used for many years as a method to assess the biological efficacy of bioactive compounds being researched for use as treatments for various disorders like Parkinson's, cancer, HIV or diabetes. One of the most often used pre-clinical in vitro approaches for drug development is the use of 2D cultures due to their simplicity, affordability, good reproducibility, and capacity to grow a wide variety of distinct cell types.[1,2] Moreover, the usage of animals as

models in laboratory has decreased because to this kind of cell culture technology. Monolayers, on the other hand, typically entail cell seeding on surfaces of polymers, like culture flasks or Petri dishes, which are unable to accurately replicate the true intricacy and 3D structure present in the human. Monolayer cell cultures cannot replicate the structure and drug resistance provided by components from the microenvironment of tumour and its 3D structure in the specific scenario of solid tumours. [1,2,3]

A tumor micro-region is made up of a diverse population of cancer cells arranged in a 3D (3 dimensional) structure, where interactions with the microenvironment have an impact on the proliferation of the cells. The extracellular matrix (ECM), fibroblast cells, endothelial tissue, and immune cells are micro-environmental elements that interact with tumour cells in a way that promotes tumour growth and medication resistance.[4,5] In such a complicated environment, mechanical cues as well as biochemical elements like hypoxia, growth factors, enzymes, hormones, or cytokines affect tumour initiation and spread.[6,7] In fact, mechano-sensing, or the ability to detect compression and tension pressures, is a crucial aspect of cell physiology, and changes to the mechanical homeostasis of tissues are seen during tumour development.[6,8]

Multicellular tumour spheroids (MCTS), which are ideal models for studying this problem because cancer cells are cultivated as 3D structured aggregates, are one solution. These intricate multicellular structures mimic the interactions between tumour's cells and the tumour's matrix. [9]

Additionally, MCTS can expand to a diameter of up to several hundred micrometres and gradually exhibit a gradient of proliferating cells that is comparable to that seen in tumour micro-regions. Particularly, in big spheroids, quiescent cells are found more centrally in hypoxic and nutrient-poor regions and proliferating cells are found in the outermost layers. [10,11]

Spheroid manufacturing can now be accomplished using a number of methods, including liquid overlay, hanging drops, microfluidic-based assembly, and spinner flasks. Large-scale spheroids may now be produced under extremely reproducible conditions because to significant work put into optimising these procedures. To enable high throughput screening (HTS) of anticancer treatments, various products are also available commercially that can enhance the repeatability of 3D spheroid generation. [1,12]

Internal Structure Of 3d Tumor Spheroids

Spheroids internal structure is made up of several cell layers, just like solid tumours do. High-proliferating cells make up the outside layer, quiescent cells make up the intermediate layer, and necrotic cells make up the centre of the structure (Fig.1). Because they have increased access to oxygen and nutrients, cells on the spheroid perimeter proliferate at a high rate. In contrast, the lack of oxygen (hypoxia) and nourishment causes the cells of spheroids to remain in a dormant or necrotic state. Additionally, through a mechanism known as the Warburg effect, cancerous cells produces the lactate by converting the pyruvate to gain energy in hypoxic conditions. The interior of spheroids becomes more acidic (pH of 6.5-7.2) due to the buildup of lactate.[13-17]

Anticancer medications or drug-loaded nanocarriers have been shown to have reduced therapeutic efficacy due to spheroid arrangement in cell layers. Some drugs encourage necrosis through the generation of reactive oxygen species (e.g., doxorobicin and cisplatin) and it is less effective therapeutically in the innermost layer of the spheroid, or the hypoxic area.[18,19,20] However, because the core areas of the spheroids are made up of senescent and necrotic cells, medications that are most successful in rapidly dividing cells (like paclitaxel) have limited therapeutic impact there. Additionally, the typical low pH of the spheroid core might cause modifications in the net charge of the drug (such as melphalan, mitoxantrone and methotrexate) and further affect tumour penetration and cellular uptake.[15,16,21]

Collagen,laminin, proteoglycans,
fibronectin, tenascin, and other ECM components are deposited by cells inside 3D spheroids. Additionally, limited mass transport, also known as the barrier formed by extracellular matrix (ECM)-cell and cell to cell physical interactions in 3Dspheroids, inhibits the penetration and

distribution of anticancer substances in the tumour mass. However, as spheroid density rises due to the deposition of extracellular matrix proteins and intimate physical contacts, IFP also rises. The reduced convectional penetration of medicines is caused by this interstitial fluid pressure. Additionally, ECM-associated signalling pathways (i.e., interactions between ECM and cells) can affect the development of tumours. Proteinases that can alter the structure of the ECM and facilitate cancer cell migration may specifically be expressed as a result of ECM-cell exchanges.[22,23,24]

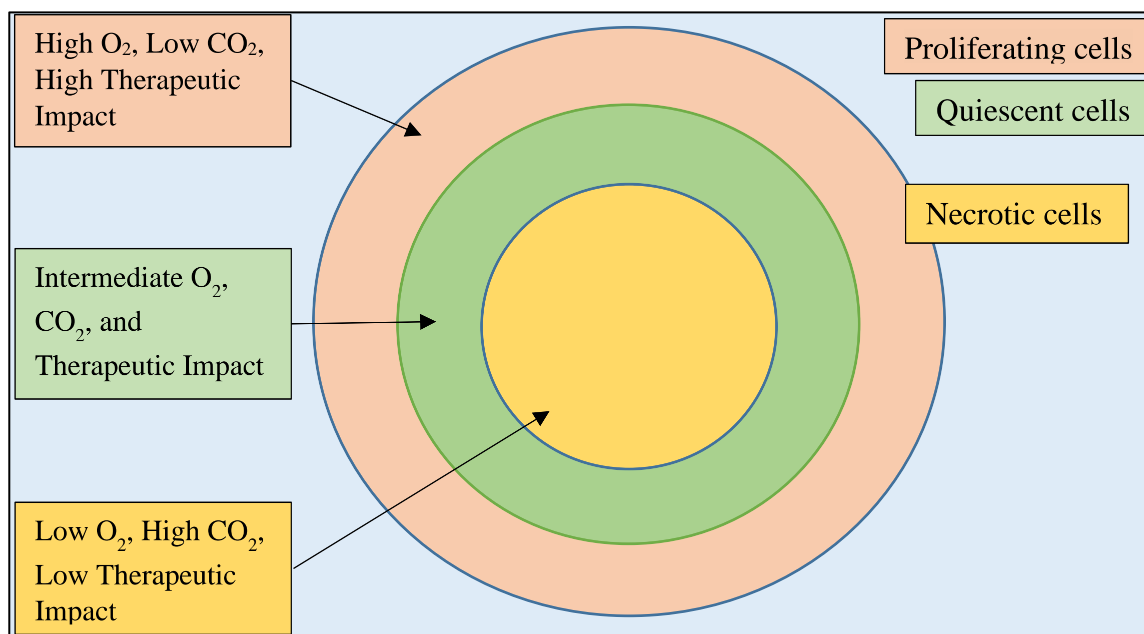

Fig.1 Schematic representation of internal cell layers and gradients in 3D tumor spheroids

Techniques For Developing Tumour Spheroids

There are various no. of commercial and non-commercial in vitro methods for spheroid development which meet the demands of researchers like control in size and cellular composition of spheroids. Given this, it is crucial to carefully choose the 3D tumour spheroid development technique that will best serve the requirements of each therapeutic investigation. [25]

1. Hanging Drop Method

The Hanging Drop technology falls under the category of aggregate-based technologies. Cells are cultivated in a drop of medium that is put in the lid of a petri plates that are made expressly to make small droplets of media containing the cells easier to form. Surface tension keeps the drops in place, and microgravity prevents the cells in the droplets from dispersing. As a result, the cells interact and aggregate, which encourages the creation of a 3D spheroid inside the droplet of the air-liquid interface.[26] Spheroids can have varying sizes depending on the properties of the cells, the volume of the medium, and the initial number of cells hanging in the drops, with most of them being small (200–500 m in diameter). A variety of homotypic and heterotypic tumour spheroids can be produced using the hanging drop approach because of its high degree of adaptability [26,27]. GravityPLUS™ Hanging Drop System and Perfecta3D® Hanging Drop Plate, two commercially available hanging drop systems, were created by Perkin Elmer and 3D Biomatrix, respectively. Both systems are compatible with numerous HTS systems and are offered in 96- and 384-well configurations. To produce reliable findings, a high level of technical proficiency might be required. When conducting the tests, some technical difficulties could arise, such as evaporation from mediium and challenges with the pipetting procedures (during the production of the first drop and during change in medium. It is also necessary to consider the less homogeneity in the structure of the tumour spheroids produced by this procedure.[25,27]

2. Low Adhesion Microplate

This process is categorised as an aggregate-based technology, just like the Hanging Drop technique. Cells are injected into 96- or 384-well plates with round or "v-shaped" bases and coated surfaces (ideal for HTS). Typically, polystyrene is used to create the plates, which are then coated with hydrophobic or hydrophilic materials such poly-2-hydroxyethyl methacrylate, agarose, or poly(N-isopropylacrylamide). Thermo Scientific Nunclon Sphera, Lipidure®-COAT dishes, Corning® Ultra-Low Attachment Surface plates, Nexcelom3D Ultra-low attachment plates, Sphericalplate5D® , EZSPHERETM , and PrimeSurface are just a few examples of the commercially available low adhesion plates.[28,29] Cellular aggregation, spheroid size, and structure homogeneity are affected by culture parameters as cell density, cell type, and medium composition. Low adhesion plates are easier to use than the Hanging Drop technique because they require fewer steps for spheroid creation and no special safety measures during medium changes (s). The long-term culture of spheroids made using other techniques can also be done on low adhesion plates, allowing for the examination of spheroid behaviour and growth over time.[30]

3. Magnetic Levitation And Bioprinting

Magnetic bioprinting and magnetic levitation are two innovative and exciting methods for creating 3D spheroids without the use of scaffolds. Spheroids are created in a 2D system using magnetic nanoparticles which is loaded previously in the wells of the cells, and forced clustering at the air-liquid interface brought on by the induction of a magnetic field applied above the plate.[31] This device, which is available in 6- to 384-well plate configurations and can be used to create homotypic and heterotypic spheroids using several types of tumour cells, is a promising new method for drug development.[32]

Spheroids' size and shape are determined by the amount of cells seeded, the length of culture, and the type of well plate utilised. Using biocompatible magnetic nanoparticles, such as gold or magnetic nanoparticles encapsulated with bovine serum albumin, and positioning the magnet at the below level of plate, which causes spheroid production at the bottom of the well, distinguishes the bioprinting technology from conventional magnetic levitation.[33]

4. pellet culture

To differentiate chondrogenic mesenchymal cells from rabbit bone marrow into 3D aggregates for tumour spheroids, the pellet culture technique was first employed. Cells are pelleted in a tube at the required density using low-speed centrifugation, which causes the cells to cluster and interact with one another. The cellular aggregation formed at the bottom of the tube is transferred to ultra-low attachment well plates for long-term culture after being incubated at 37°C for at least 24 hours to allow spheroid formation. Although the pellet culture approach produces spheroids that are uniform in size and shape in a short amount of time, it takes a significant number of vials to completely cover multiwell plates, making it inappropriate for HTS.[25,34]

5. Rotatory Methods/Microgravity Bioreactors

Since 1970, considerable quantities of spheroids have been produced using spinner flasks and roller bottles with treated surfaces. The constant mobility of the cells reduces adhesion and fosters cell-cell communication. Cellular physiology can be changed by mechanical stress and intense shear forces. The American National Aeronautics and Space Administration (NASA) created an improved rotary cell culture system with low shear force and high mass transfer rates to solve these issues. This Culture System is a bioreactor technique that avoids wall chamber collisions and simulates microgravity by seeding suspended cells in volume-defined rotating chambers that are continuously rotated. The resolution of gravitational, centrifugal, and Coriolis forces keeps cells suspended and encourages aggregation. The progressive increase in rotational speed counterbalances the gradual increase in cell aggregate size . Depending on the cells utilised, a sizable number of big spheroids are produced in about 15 days. Spheroids are transferred into ultra-low attachment well plates, where they undergo a process known as "spheroidization time" that causes them to become more uniform in size and shape and acceptable for drug screening after around 7 days. This process can produce a lot of spheroids, but it takes a lot of

time, costs a lot of money, and demands a high level of technical skill.[30,35,36,37]

Analysis And Characterization Of 3d Tumor Spheroids

However, there are methods for homogenous assays as well as HTS and HCS procedures to assess anticancer medication activity in tumour spheroids, it is not always simple to get repeatable and reliable data. The extraction of pertinent biological information and the standardisation of techniques, methods, and methodologies for spheroid model evaluation in preclinical experiments depend on a thorough characterisation of these complex cellular aggregates. Spheroids can be analysed using a variety of methods based on their morphology, cellular organisation, cellular size, cellular shape, expression of genes and proteins, metabolic status, responsiveness to anticancer medications, and migration behaviour. [25,38]

1. Optical Microscopy

Characterizing the shape, size and internal structure of the spheroids can be done best using optical and fluorescent microscopic techniques. It is common practise to employ optical microscopes with built-in digital cameras to track the development of 3D tumour spheroids. Additionally, this kind of microscope enables observation and study of the internal structure of spheroid as well as the condition of the cells in each layer. To do this, the cellular microenvironment (hypoxia) is characterised using antibodies that selectively target proteins (e.g., caspase-3, HIF, Ki-67) or biomarkers (e.g., EF5, pimonidazole). Fluorescence-based live/dead tests can be employed with fluorescent microscopy, another type of optical microscopy, to ascertain the dispersion of dead and alive cells within 3D tumour spheroids. For this, stains like propidium iodide (PI) and calcein AM (Cal AM) are frequently employed. Additionally, optical microscopy can be used to monitor the results of additional stains for histological investigation, such as hematoxylin and eosin (H&E), toluidine blue, and Masson's trichrome. [39,40,41]

2. Drug Screening Assay

The methods of colorimetry, fluorometry, and luminescence frequently employed to examine efficacy of drug in 2D cell cultures and typically assess the performance of certain enzymes or the metabolite concentrations that are associated with the condition of cells' state of health. Several of these techniques were created for 2D cultures are employed in the 3D models for the assessment of medication cytotoxicity, however, they might be a cause of diversity so the observed data should be properly assessed because the techniques employed are designed for monolayer culturing and not for 3D models. Actually, the limited mass movement and the compactness of spheroids impede the solutes' uniform distribution, notably in the deepest regions of the spheroids, and they give false results. However, several of the tests are used to assess the effectiveness of tumor therapy, the tests include MTT, AlamarBlue, Trypan Blue and lactate dehydrogenase. [25,30,42]

3. Electron Microscopy

Techniques used by electron microscopy produce substantially higher resolution images of samples than the images produced by techniques of light microscopy. Techniques like TEM (transmission electron microscopy) and SEM (scanning electron microscopy) employs electron beams to produce sample's images in great detail with nanoscale resolution. The TEM method employs an electron beam with a high voltage to illuminate the sample and construct a picture. This approach is frequently used to improve spheroids by supplying cell-cell information about connections between physical factors and on the reaction of a cell to physical or chemical interventions. [43] The SEM process involves scanning the samples' surfaces with a concentrated electron beam, and the resulting images provide details on the specimens' topography and surface makeup. Spheroid analysis with SEM is a really intriguing method. The 3D context of the spheroid can be used to analyse cellular protrusions (such as lamellipodia and filopodia), permutations in cell-cell interactions (such as loss of cell-to-cell contact, pits in spheroid structure), and morphology of cell surface (such as membrane blebbing condensation, protrusion of cellular membrane). [44,45]

4. Flow Cytometry

A common cell biology method that allows for the continuous multiparametric study of several physical and chemical properties of cells and

particles is flow cytometry. It is a very efficient technique that offers accurate and reliable results. The utilisation of single cells in suspension necessitates the disaggregation of whole 3D spheroids. Trypsin or a combination of less harmful enzymes (such as Accutase®) can be used in the disaggregation process, which can also be carried out physically and enzyme-wise. The same procedures used for 2D cell cultures can be utilised to highlight recovered cells. [46,47] In most cases, flow cytometry is utilised to assess the cytotoxic and/or cytostatic actions of anticancer medications in spheroids. The percentage of living and dead cells, proliferating or quiescent cells, and the cell cycle phase can all be determined using a variety of conventional fluorescent dyes, such as 5-bromo-2'-deoxyuridine (BrdU) and calcein for live cells and ethidium for dead cells. The metabolic state of the cells making up the spheroid or the proportion of cells expressing a given protein, for example, can be evaluated and quantified using fluorescent antibodies or probes towards specific proteins or cellular compartments of concern. [48]

5. Western Blot And Qrt-Pcr

Conventional cell biology methods like Western blot (WB) and qRT-PCR are used to evaluate the expression of proteins and genes. Variation in conventional 2D cultures can likewise be applied to 3D spheroids. The process utilised to harvest protein from the 3D cell culture is the significant problem with these approaches. In reality the connection between chemical buffers and detergents like SDS via mechanical pipetting or sonication, additionally, lengthier incubation periods are required to disrupt the spheroids' compact structure. [49] Extracted proteins are then partially measured via conventional western blot analysis. Spheroids, however with regard to western blot, a suitable lysis technique is a need to guarantee that RNA is obtained from all cells putting together the spheroid. Scientists have made use of these two combining several approaches to discover various genes and proteins in spheroids involved in tumour growth treatment response. [50,51]

Applications Of 3D Tumor Spheroids

3D models are broadly used for studying the biology of tumor, interactions between cells, genetic profile and microenvironment. It is worth mentioning that these 3D tumour models are essential for the development of anticancer medications and can aid in the discovery of novel targeted cancer therapies. Doxorubicin, 5-Fluorouracil, Taxol and Cis-Platin are those cytotoxic drugs which shows resistance to the various cell lines of spheroids. It can be assumed that Paclitaxel shows the resistance to the spheroid's cell line of MDA-MB-157. [52,53] It can be inferred that inadequate drug penetration and drug distribution in the spheroid cell mass is the primary cause of drug resistance. Therefore, it is important to consider the gradient structure of spheroids while screening anticancer drugs, including the existence of hypoxia and cell growth areas.

The hypoxia inducible factor (HIF) 1 is one gene that can be changed by hypoxia and cause alterations in tumour cells. [54] It has been demonstrated that inhibiting HIF-1 causes doxorubicin to accumulate in breast tumour spheroids, lowering treatment resistance. Additionally, this addition boosted caspase-9 activity, which is crucial for cell apoptosis. Tumor spheroids can also be utilised to assess the efficacy of the drug delivery method. [55]

Spheroids made from tumour tissue can be used for drug testing. One study held the comparative study to compare the therapeutic efficacy of drugs on cell line of breast tumor and spheroids formed from tissue of tumor and this study revealed the significant efficacy difference between these two groups. [56] Spheroids of primary colorectal cancer cells from five individuals were used for chemosensitivity screening, which revealed unique response profiles. Spheroids nevertheless showed variations in patient sensitivity to conventional medication and combination regimens for the treatment colorectal cancer. [57] Tumor spheroids can thus play a key role in drug testing prior to their clinical application due to their easy production, low cost, and possession of some characteristics of a tumour. [58]

1. Drug Testing

Spheroids that have been co-cultured with stem cells have improved protein, RNA, and in vitro production capabilities. In co-culture with fibroblasts, hepatic cancer cells have an invasive

phenotype and produce tissues that resemble primary hepatic cancer tissues in terms of the expression of certain proteins. The angiogenic and metastatic capacity of tumour cells was assessed using a three-dimensional tumour-endothelial model. The spheroids' vascularized structure did not improve medication penetration, but the tumour cells resisted chemotherapy and radiation treatment more. [59’60] This substantial structure, which was thicker than the cell membrane, effectively stopped enzymes and antibodies from penetrating the drug-coated surface. This demonstrates the tumor-like structure of spheroids with varying cell phenotypes, varying rates of proliferation, and even varying levels of gene expression throughout the relatively large spheroids. As a result, the drug penetrability of spheroids will be a popular research subject in the coming ten years. Fluorescence or autoradiography of radio-labeled medicines like Doxorubicin, Anthracyclines have been used to test the three-dimensional penetrability of drugs in tumour spheroids. [61]

2. Tissue Engineering

In tissue engineering, a lot of cells are used to replicate organs or tissues in a dish before being transplanted into a patient's body. But if the ideal is pudgy, the fact is bony. The main disadvantage of tissue engineering is the lack of a high density of living cells. For instance, the mono-spheroid diameter is only 200–400 m due to oxygen and nutrition limitations. Endothelial cells are frequently utilised to vascularize cell spheroids in order to create a network of resembling capillaries in order to get around the size restriction of spheroids. [62] All of the suitable endothelialized spheroids utilised in clinical trials are small (2 mm) tissues where oxygen and nutrients are distributed through simple diffusion. These "endothelialized" spheroids can be combined further to create a bigger tissue that, once implanted, can merge with the host vascular system. Pre-vascularization is a critical step before implant. In order to try and manufacture organs in vitro, the burgeoning area of bioprinting and bio-fabrication is looking for big clusters of cells to chnage with conventional cells. Extracellular matrix (ECM) with different shapes, such as cell rings and cell sheets, has been utilised to print tissue utilising bio-printers, such as inkjet printers and other robotic-based intruments. Spheroid cultivation using magnetic levitation is appealing because the magnetic field can easily control the structures of spheroids. [63, 64]

3. In Vivo Applications

Spheroids are increasingly being used in a variety of pathological and metabolic research in the biomedical field with the ultimate goal of implantation due to their numerous good characteristics. Cell size is measured prior to tissue transplantation. Spheroids can be implanted into mammalian animals on an individual basis, in contrast to 2D cells. Additionally, there have been numerous translational studies looking at the in vivo implantation of spheroids into different animal models and tissue production because the knowledge regarding the creation and maintenance of spheroids has evolved. Spheroid transplants have been used in nearly every human system, including the skin, musculoskeletal system, digestive system, and cardiovascular system. Spheroid implantation, however, is still a relatively new topic with few published in vivo results. Using spheroids in animal models have a number of limitations in spite of their outstanding characteristics (Fig.2). [65,66]

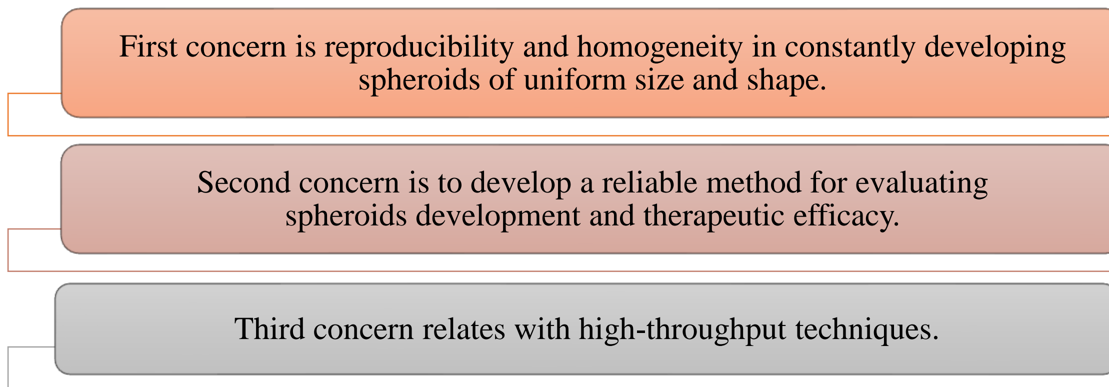

Fig.2 Explains about the limitations of 3D spheroids in preclinical phase

4. Drug Delivery Vehicles

Applying spheroids as a delivery method is one of the projects looking to expand the limited application of cell spheroids. The ability of mesenchymal stem cells (MSC) to colonise tumours is well established. Only a few cells and medications are employed in MCS therapy to specifically target tumours. To overcome these restrictions, a hybrid spheroid/ nanomedicine system made of MSC spheroids encasing a drug-loaded nanocomposite was developed. MSC cell generation in spheroids increases the payload of anticancer medicines and their capacity to target tumours. With the help of this technology, which actively seeks out and targets glioblastoma cells, chemotherapy medications can be administered to patients in an efficient manner. Microfluidic tools were used to create MSC hybrid nanoparticles. This creative use of spheroids offers a fresh method. There may be a use for spheroid-loaded pharmaceuticals as a "cruise missile" in the future. Additionally, this suggests a potential strategy for evaluating the penetration of medicines from the centre of spheroids. [67]

5. Biosensors

As interest in 3D cell culture systems grows, it becomes more challenging to employ in vivo-like spheroids as a model for straightforward biomedical toxicological tests. Spheroids' distinctive construction makes it difficult for drugs to enter them and for antibodies to work properly. The conversion of various signals into equivalent legitimate values is difficult when creating in vitro sensors for toxicological studies. A biosensor was recently created is concerned about the electrochemical monitoring of the enzyme based activities of live cells' non-specific esterases. It was simple to translate the enzyme activity into the viability of the spheroids. It is simple to assess the viability of both 2D and 3D cell culture models using this enzyme-based biosensor and to give more crucial data for drug or toxicity screening. A other kind of biosensor operates by gathering quantifiable analytical signals. The detected signals are molecular recognition events taking place inside the spheroids at the molecular and cellular levels. [68, 69]

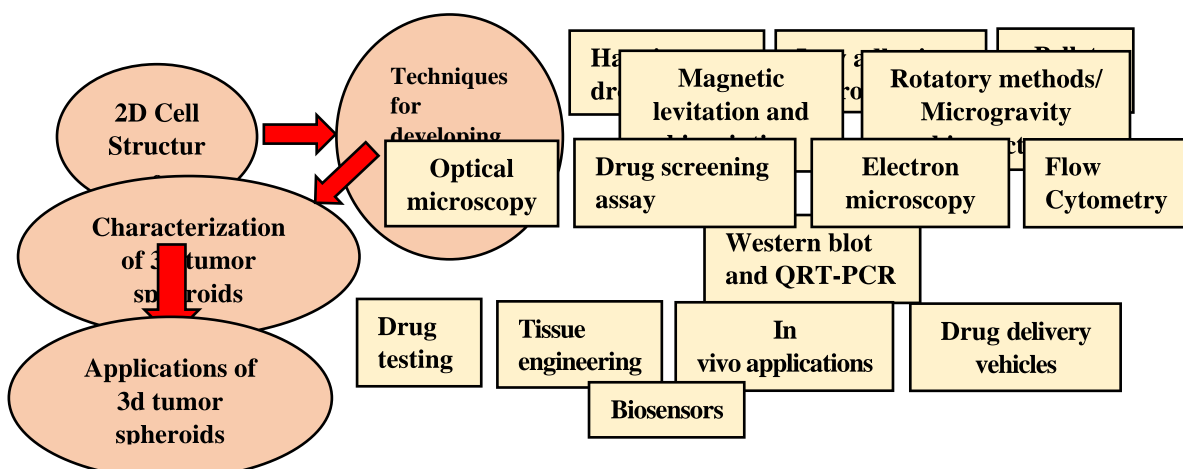

Fig.3 Schematic representation of techniques, characterization and applications of 3D tumor spheroids

Conclusion

For evaluating the complete impact of a certain anticancer drug, regulatory bodies now accept animals as models because it is the only pre-clinical platform. In fact, the only appropriate method for obtaining the results of complete effects of a treatment on a living organism is through the use of animals in laboratories. However, the use of in vitro models such as 2D and 3D models for the assessment of therapies has grown over the past few decades as a result of the cost and ethical/legal issues associated with using animals in lab experiments.

Spheroid-based models seem to be the major intriguing tools to date for the creation of high-throughput screening platforms and reasonably priced mimetic tumor models in the constantly changing environment of 3D cell culture approaches created for in vitro tumour modelling. Another advantage of adopting these models is the potential to characterise and evaluate data using current biomolecular and imaging tools. Even when they incorporate components of the tumour microenvironment, the relative simplicity of spheroid models compared to actual malignancies must warn researchers against the danger of overestimating their accuracy in foretelling therapeutic response.

In the end, researchers may be able to employ the microsphere model for various applications, such as tissue engineering and studies related to toxicity. By altering the osmotic pressure for MCS creation and growth to simulate in vivo settings, the techniques may simply change the microenvironment. Additionally, the development in size and yield control made possible by improvements in the techniques we've already discussed, like magnetic levitation and microgravity bioreactora, opens up new opportunities for the in vitro application of MCS, which is essential for regenerative medicine and other clinical related applications. This ensures that this exciting field of research continues to be efficient for patient care. For 3D spheroid culture systems, more sophisticated designs are required, such as co-culture/tri-culture MCS creation, to provide physiologically realistic micro- and macro-environments.

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