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A Review on Biomaterials for Organoid Modeling and Tumor Spheroids

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ABSTRACT

Organoids are miniature forms of organs to demonstrate spatio-temporal cellular structure and tissue function. The organoids creation revolutionized developmental biology and provided the opportunity to study and modify human development and disease in laboratory setting. Recently, new biomaterial-guided culture systems have represented the versatility for designing and producing of organoids in a constant and reproducible manner. Since 2D cell culture models often lack *in vivo* tissue architecture, recent detailed research has allowed many 3D culture models development demonstrating the characteristics of *in vivo* organ structure and function. Organoid models are able to create 3D constructions complex that maintain various cell types and also hide the relevant organ functions *in vivo*, and therefore, the development of organoids in particular has reformed drug discovery, disease modeling, and developmental biology.

The new biomaterials production has been important for improving of *in vitro* 3D patterns. Future work with biomaterials has been about the creation of hybrid polymers combining the benefits of both synthetic and natural polymers to take place of communal supplies including Matrigel and polydimethylsiloxane (PDMS). The creation of 3D culture systems has also revolutionized *in vitro* drug testing. Furthermore, recreating the three-dimensional tumors microenvironment and the proper regulation of cancer cells have been encouraging to develop new tumor models. Under known culture conditions, cancer cells are able to form three-dimensional structures known as spheroids and self-organize to further study advances in embryonic development in three-dimensional cultures known as organoids. These newly designed biomaterials using for tumor modeling will make an important contribution to understand the main mechanisms of cancer. This review aimed to focus on studies examining advances in 3D culture systems in disease pathology modeling and new drug discovery studies, focusing on the active roles of biomaterials.

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1. INTRODUCTION

According to the research published by the World Health Organization's International Agency for Research on Cancer (IARC) in 2021, the number of cancer deaths worldwide in 2020 is 9958133, and the number of new cases is 19292789 by the World Health Organization [1]. Their heterogeneous nature gives rise to the complexity of tumors and therefore the development of new treatments is of constant importance. Cancer research mostly relies on different cell-based designs *in vitro* to examine the mechanisms and signaling pathways underlying the various functions and phenotypes of cancer cells which include growth, metabolism, drug resistance, matrix invasion and migration [2, 3]. In addition, cell-based disease models are commonly used in cancer drug discovery efforts to reveal the mechanisms and off-target interactions of drug effects against cancer cells, the toxicity profiles of compounds, and their biological activities [4, 5]. Monolayer two-dimensional (2D) *in vitro* cultures have been used for these applications, ignoring the complexity of interactions seen *in vivo* in tumors [6]. The ease of starting and maintaining two-dimensional (2D) cell cultures, their fitness with various culture dishes such as petri dishes, flasks, and a wide variety of biochemical analyzes have made 2D culture systems necessary for cancer research [7]. Despite this, it is now well established that 2D cultures have not been successful in demonstrating the pathophysiological properties of human tumors. In the 2D culture method, interactions of cell-cell and cell-extracellular matrix (ECM), which are responsible for cellular functions such as cell proliferation, differentiation,

viability, gene and protein expression, response to stimuli, and drug metabolism, are not represented as in physiological conditions [8-10]. After being isolated from tissue and transferred to 2D conditions, the morphology of cells changes and the loss of different phenotypes is a result of 2D culturing [11, 12]. This changing cell morphology can affect the functions of cells, the organization of cell structures, and cell signaling [13-16]. In addition, adherent cells can lose their polarity in a 2D culture medium, which can change the cell response to different mechanisms such as apoptosis [17-19]. On the other hand, *in vivo* for cancer cells, the presence of oxygen, nutrients, metabolites, and signaling molecules is more changeable due to the natural structure of tumor tissues, while cells in the monolayer have unrestricted access to these environmental components [8]. It has also been observed that in 2D systems, the cell's gene expression and splicing change its topology and biochemistry [20-23]. In addition, adherent cultures that allow only one cell type to function are often monocultures, resulting in a lack of the tumor microenvironment that cancer-initiating cells need *in vivo* in particular [24-26]. Due to the many disadvantages of 2D systems mentioned above, such studies are needed to develop alternative models that can better mimic a natural tumor structure, such as three-dimensional (3D) culture systems, to provide the credibility of the data obtained. These models have been demonstrated by different approaches known as the multicellular tumor spheroid model (MCTS) [27], organotypic cancer tissue segments [28], multilayer cell cultures [29] and scaffolds [24]. Further research in tissue engineering which include the various 3D scaffolds development and bioreactor systems, has put there the need to thoroughly evaluate the fidelity, capacity and diversity of culture models using for in cancer research [30].

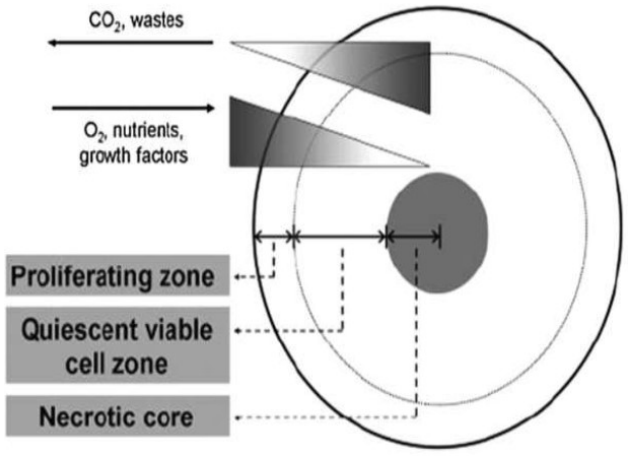

Figure 1 Schematic demonstration of the multilayer spherical structure. Reproduction is higher on the outside because of the higher levels of nutrients and oxygen. Towards the center of the spheroid structure, oxygen and nutrient decrease causes growth arrest or cell necrosis [31].

The 3D spherical structure is based on the emergence of spherical structures that simulate the physical and biochemical properties of the tumor tissue and form various layers of cells (Figure 1). Morphological analysis of different types of tumor cell lines cultured in 3D spherical conditions were defined as tight spheroids, compact aggregates and loose aggregates according to the structure of the spherical shapes [32, 33]. Cells obtained from donor tissues are cultured in 3D- environment and tissue structure is more accurately mimicked than 2D models as a result of appropriate cell-cell and cell-ECM interactions composed to simulate tissue structure [34]. Cells can adopt stimuli from their environment *in vivo* [35, 36] and the cell polarity and morphology are preserved [12, 37, 38]. Another considerable 3D culture feature is its close resemblance to growing cells *in vivo* in terms of gene expression, metabolism, and signaling [39- 43].

Biomaterial-based 3D cancer models

The need for better cancer models *in vitro* has triggered both basic cancer and drug discovery research which is lead to the 3D models development [44]. These models are created using various technologies, and cancer cells such as tumor spheres, cellular spheres, matrix-mediated assembled cellular clusters, organotypic spheres, multilayered cancer cell cultures or tumor slices, [45] microfluidics- and microfabrication-mediated cancer cells cultures and organoids presents a variety of complex structures, including self-assembled and independent globular clusters [46- 50].

The main difference between 2D and 3D tissue models is that biomaterials use to better mimic *in vivo* physiological conditions, and the discovery and advancement of biomaterials has led to the new *in vitro* 3D models development. Each biomaterial to be used in 3D tissue modeling has its own characteristics to satisfy certain requirements [51]. These progresses have empowered the improving and usage of synthetic and natural materials (Figure 2) for various applications in tissue engineering, including 3D cancer models [52-55]. These materials are utilized to construct scaffolds with biochemical and mechanical properties that are characterized to physically support cell growth and adhesion and facilitate the self-organization of cells into 3-dimensional clusters. Tissue derived cancer models also allow homotypic interactions of cancer cells with particular biochemical factors integrated to scaffolds and signaling between stromal cells, matrix protein and cancer cells [51]. Natural materials (e.g. hyaluronic acid (HA), laminin-rich extracellular matrix (lrECM), collagen, alginate and chitosan) have been utilized individually or in different combinations to preserve supporting constructions for stromal and cancer cells and let cells to re-modify the matrix [24, 37, 51]. Synthetic materials [eg., poly-lactic-glycolic acid (PLGA), polyethylene glycol (PEG) and polycaprolactone (PCL)] can be suitably designed with the described features such as porosity, stiffness, and introducing of signaling molecules including in the tumor microenvironment. On the other hand, native matrix proteins have limitations such as variations of bulk-to-batch composition and variations in crosslinking and gathering density, tissue sources deriving from which materials, and the cost of producing and purifying them [56]. In contrast, the use of 3D scaffolds originating from natural polymers has advantages such as being very close to the structural complexity of the extracellular matrix *in vivo*, ligands availability and cell surface receptors for cell signaling and attachment, and ease of production. These characteristics are crucial for cultured cells to growth and compose a tissue on a biocompatible scaffolding. Also, natural

polymers, unlike synthetic polymers, do not need to modification of post-production to require ligands or peptides. However, there are several disadvantages such as poor immunogenicity, mechanical properties and limited control of degradation rates [57, 58]. Synthetic polymers can be produced to provide desired mechanical properties and breakdown rates with advanced recurrence. It can also integrate drugs or proteins as needed. On the other hand, it has disadvantages such as the absence of identified ligands for cell surface receptors and the potentially toxic byproducts produced by polymer degradation [59]. Before crosslinking, the polymer can submit to chemical modification with peptides like GPQG-AGQ to improve proteolytic deterioration or RGD (arg-gly-asp) to improve cell-specific adherence or signaling ligands such as bone morphogenetic protein (BMP) and vascular endothelial growth factor (VEGF) can control cellular process [60, 61].

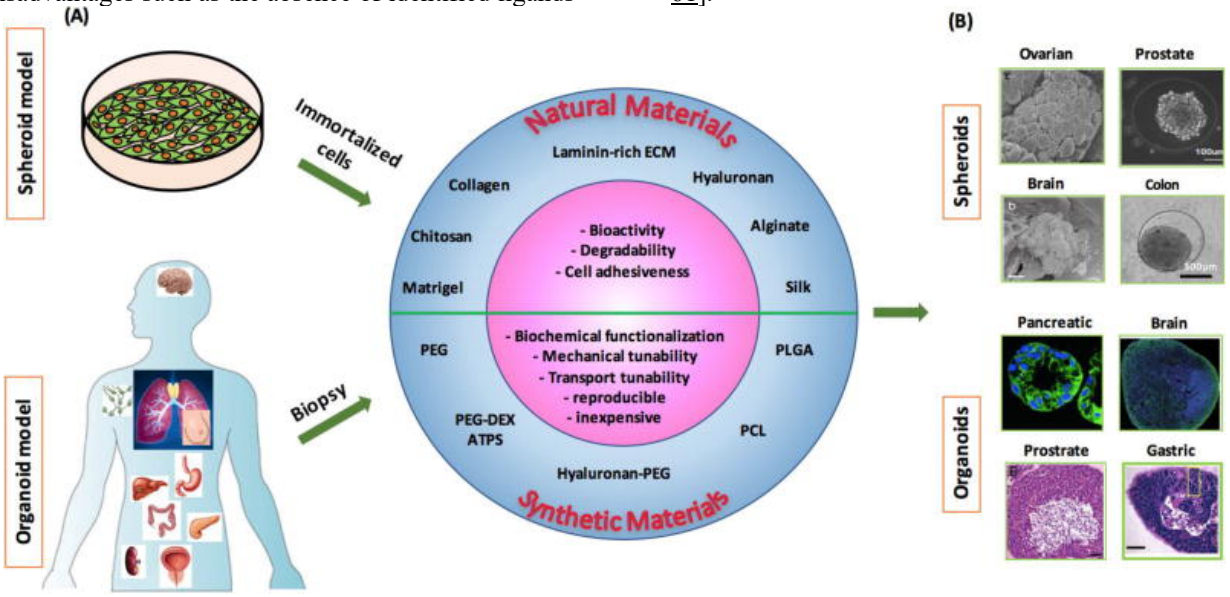

Figure 2 Spherical and organoid modeling base on biomaterials. (A) Perspectives to improve 3D cancer models from cell lines as biopsies, organoids, and spheroids. (B) Typical images of organoids and spheroids generated using cells from various cancers [56].

Organoid and spheroid models

Organoid models are three-dimensional (3D) cell culture systems that mimic the *in vivo* organ or tissue from which they originated. Organoids derived from primary tissue or stem cells exhibit organ functionality. Organoids: 1) show primary tissue-like composition and architecture, containing a small self-renewing population of stem cells that can differentiate into cells of all major cell lines with similar frequency as in the physiological state 2) biologically closely related and niche components and gene sequence to any model system It includes in-vivo conditional models that are suitable for manipulating 3) biobanks and self-renewal has stable system that can be frozen and self-organized indefinitely expanded as differentiation ability of stem cell [61]. This results in the emergence of a multicellular 3D construction composed of diverse cell lines revealing crucial functional and structural features of organs. [62].

While internal developmental stages direct formation of organoid, spheroids growth primarily via adhesion of cell-to-cell. The other important consideration is that the long-term cell growth of in culture requires an undifferentiated stem cell to replace with dead cells. Furthermore, organoids are initiated and maintained from a stem cell population during *in vitro* culture, ensuring their long-term viability. These two important situations make up the major difference between spheroids and organoids [63]. In addition, during the organoids passage, they serve as "living biobanks" that are preserved *ex vivo*, as they preserve the genetic characteristics of the organ of origin for several generations. On the other hand, there may be difficulties in obtaining and preserving viable cells during long-term culture of spheroids obtained from tissue [63].

Spheroids and organoids can be derived from a diversity of diseased and healthy tissues and cell types and have been broadly searched for their usage in drug discovery. However, there are some significant distinctions in the production of spheroids and organoids from patient-derived tumors [63- 65].

Patient-originated tumor organoids

3D spheroid cultures more accurately reflect the patient's primary tumor than 2D monolayer cultures. In addition to producing 3D spheroid structures from patient-derived tumors, healthy tissue replicas can also be created. This allows for joint comparison of drug response between healthy and tumor organoid from the same patient and broadens the prediction path for personalized therapy [64; 66]. Another important point is that tumor organoids produced from patient is more cost-effective compared to patient-derived xenograft (PDX) models, as they require less resources and time [67]. Besides these advantages of organoids, it has the limitation of lack of communication between organs found in complex *in vivo systems*. Since this will be an important element in both tumor growth and treatment response, recent studies have aimed to mimic the tumor microenvironment by incorporating immune cells into the tumor microenvironment [66]. What is critical and important here is to optimize the co-culture protocols of immune cells and organoids, and research is still ongoing to achieve this successfully. Thus, it allows *in vitro*

investigation of niche-tumor interactions. Instance of patient-originated tumor organoid types improved by now contain glioblastoma multiform (GBM), colorectal cancer (CRC), lung and breast cancer models [68]. These enhanced tumor organoid models will combine 3D culture systems with patient-originated primary cells to more definitely pattern the tumor environment compared to conventional cancer cell lines, allowing for advanced drug discovery and personalized medicine [64].

Patient-derived tumor spheroids (tumor spheres)

These are floating spheres that are ordinary utilized to assess cancer stem cell (CSC)-related features *in vitro* [65]. Since cells with high replication potential are similar in content to CSC properties *in vivo*, making tumor spheres a good model for CSC expansion. Furthermore, they are simple to maintain and clonal, and easy to genetically manipulate, making them extremely suitable for screening drugs targeting CSCs. Tumor spheres mimic clinical drug resistance, are more accurately identified than in 2D culture, and response profiles may develop in passages losing their predictability. Moreover, the high rate of CSC in a tumor spheroid culture can be considered a limitation when trying to reflect a particularly heterogeneous primary tumor structure in 3D culture systems. In addition, tumor spheres often show little histological resemblance to the primary cancer from which they are derived [69].

2. CONCLUSIONS

Intense research in recent years has led to the development of many 3D culture models that allow to investigate the properties of *in vivo* organ function and structure *in vitro*. The development of organoids has a major impact in drug research, disease modeling, and developmental biology, as organoid models can produce complex 3D formations including multiple cell types and preserve some organ functions *in vivo*. The production of new biomaterials used in the creation of these organoids and spheroids has been very important for *in vitro* 3D model development. Recent work on biomaterials has focused on the production of more effective hybrid polymers that combine the benefits of both synthetic and natural polymers. Furthermore, revealing multi-organ designs for *in vitro* disease modeling and drug experiments in a body-on-a-chip system using bioreactors will be crucial. With all these developments, 3D systems will replace 2D culture systems, leading to more clinical applications due to their improved physiological suitability. More importantly, the improving of 3D models allowing *in vitro* trial of drug metabolism, absorption, and toxicity will also reduce the necessity for high-priced preclinical animal studies and will also be critical for clinicians and scientists to better diagnose and treat or mitigate disease

Competing interests

The authors declare that they have no competing interests.

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