3D Modeling of Epithelial Tumors

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Definition

The current statistics on cancer show that 90% of all human cancers originate from epithelial cells. Breast and prostate cancer are examples of common tumors of epithelial origin that would benefit from improved drug treatment strategies. About 90% of preclinically approved drugs fail in clinical trials, partially due to the use of too simplified in vitro models and a lack of mimicking the tumor microenvironment in drug efficacy testing. This entry focuses on the epithelial cancers, followed by experimental models designed to recapitulate the epithelial tumor structure and microenvironment. A specific focus is to put on novel technologies for cell culture of spheroids, organoids, and 3D-printed tissue-like models, utilizing biomaterials of natural or synthetic origins, and how the models could be utilized for nanotechnology-based drug delivery in the future.

1. Introduction

Cancer is the second leading cause of mortality worldwide, and 90% of cancers are of epithelial origin, known as carcinoma. Carcinoma is a malignancy in the epithelial cells, which have a multidisciplinary role in protection, absorption, secretion, excretion, filtration, diffusion, and sensory reception in tissues. The World Health Organization (WHO) conducted a worldwide study in 2018, which showed that in both sexes, the leading cause of cancer deaths is lung cancer (18.4%). In males, prostate, colorectal, and liver cancers show the highest incidence after lung cancer, and stomach cancer shows the highest mortality, whereas in females, breast cancer is the leading cause of cancer deaths, followed by colorectal and lung cancer for incidence [1].

To study the origin, progression, metastasis, and underlying mechanisms of epithelial cancers, various models have been designed, utilizing multidisciplinary fields of science such as biomaterials engineering, nanotechnology, and high-content imaging. They have provided substantial information to improve anti-cancer drug discovery and diagnostics (Figure 1). The most simplistic model of in vitro cancer research is a two-dimensional (2D) monolayer culture of cancer cell lines, which, in spite of being the most utilized model, cannot provide realistic data about the heterogeneous, multicellular tumor microenvironment (TME) in the body. Currently, various models such as animals [2][3], transwells [4], spheroids [5][6][7][8], organoids [8][9][10], and xenografts [11][12] are utilized in cancer research with many advantages and disadvantages [13][14]. Hence, there is a need to develop models that can be utilized for monitoring cell growth, viability, polarization, and differentiation, as well as for and studying migration and invasion of tumor cells into the surrounding TME. Hence, a lot of attention has been focused on developing three-dimensional (3D) model systems containing the extracellular matrix (ECM) of natural, synthetic, or semisynthetic origins using 3D bioprinting technologies that can provide more accurate information about the TME and cancer progression [14][15][16][17][18]. The 3D models can also serve as better choices for high-content screening approaches in the preclinical phase of drug development.

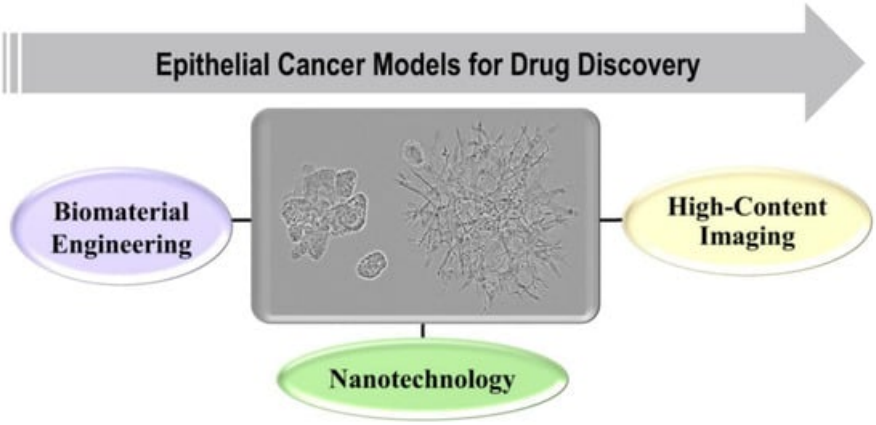

Figure 1. Research areas that are related to epithelial cancer and where 3D cell culture models are useful.

2. In vitro 3D Experimental Models in Cancer Research

In vitro cancer models are the simplified versions in comparison to in vivo models, when studying cancer mechanisms, and the effect of anticancer moieties on tumor growth and progression. Standard 2D cell culture models fail to recapitulate the cellular mechanisms involved in tumor progression such as cell-cell adhesion, polarization, epithelial differentiation, mechanotransduction, invasion and proper signaling of cells within the tumor tissues. Recent developments have shown that 3D in vitro models have tremendous potential in cancer research due to their most promising characteristic of very closely mimicking the in vivo model systems. An ideal in vitro tumor model should be able to recapitulate the 3D in vivo environment along with reproducing the interaction between tumor and stromal cells, thus regulating the cellular functions. Depending upon the method of cell seeding, the 3D in vitro models could be categorized as scaffold-based and scaffold free models. The scaffold-based models utilize the prefabricated ECMs prepared from different materials such as natural or synthetic materials, or decellularized ECM. While in scaffold-free models, cells proliferate as non-adherent floaters without any support material and 3D constructs are formed due to cellular self-assembly [19].

Non-adherent 3D spheroids can to some degree mimic the solid tumor architecture and it is comprised of different cell layers. The core is composed of necrotic cells while the middle layer has mostly senescent cells. The necrotic or senescent cells of the inner layer is dedicated to the absence or deprivation of nutrients and hypoxic environment, which results in the accumulation of lactate in the spheroids same as that of in vivo solid tumors. The outer layer is formed of cells with high proliferating rates due to convenient access to oxygen and nutrients [20][21].

Tumor cells can also be cultured embedded in ECM, where they spontaneously form 3D structures of organotypic nature, which can be called spheroids if they are round or tumoroids if they have an invasive appearance. Here, single cells that are embedded into ECM grow into multicellular, organotypic structures. Each of the functional structures are of clonal nature, but often has characteristic phenotypes that correspond to different tumor stages. Normal epithelial cells or non-aggressive cancer cells can form well differentiated, polarized, round spheroids with functional basement membranes. In contrast, tumoroids formed by aggressive cells mainly result in undifferentiated clusters of cells, or massive invasive structures. In epithelial cancers, invasion through the ECM is typically of the collective type, and ameoeboid invasion is less frequently observed [22][23]. Tumor cells can also be embedded together with stromal cells and be co-cultured in the ECM. Incorporation of stromal cells such as CAFs will promote genuine, functional interactions between the different cell types, which can be observed in vivo [24]. 3D organotypic cell cultures can therefore act as a bridge between traditional 2D cell culture and costly animal models.

Organoids are more advanced 3D in vitro multicellular structures that mimic the corresponding architecture of in vivo organs. The term organoid is mostly used to describe structures obtained in 3D

culture derived from stem cells that are isolated from primary patient samples. The complexity of an organoid is regulated by the developmental potential of the starting stem cells [25]. The organoids can like the spheroids be cultured in non-adherent conditions or embedded in ECM. Organoids are mostly used for translational epithelial research, patient specific treatment planning and disease modelling due to close resemblance to the native tissue composition. However, the 3D organoid culture is advantageous over 3D spheroids due to enhanced physiological and clinical functions. The 3D organoid models of various tumor types have provided concrete evidence to validate the use of these models [26][27][28][29][30]. Thus, in the future, with continuous development, they can provide substantial information in cancer research.

3. 3D Bioprinting Technologies

3D bioprinting is a technology in which 3D structures are fabricated by layer-by-layer precise deposition of biological materials, living cells, and biochemicals. In cancer research, 3D bioprinting technology has provided hope and motivation to design models to recapitulate the in vivo TME to study cancer genesis, mechanisms, and to facilitate drug development screening by conveniently combining patient-derived cells and materials [31]. Ideal material for 3D bioprinting must meet the most important criteria, such as easy handling and deposition by the bioprinter, biocompatibility, structural and mechanical stability, tissue-specific material biomimicry, and minimalistic nontoxic byproduct generation. Herein, we discuss the most studied 3D bioprinting technologies.

3.1. Types of 3D Printing Technologies

In 3D bioprinting, the most important factors to be considered are biological materials used for printing, cell viability, and surface resolution. The current 3D bioprinting technologies are inkjet-based [32], microextrusion [33], stereolithography (SL)-based [34], and laser-assisted printing [35] (Figure 2). The inkjet-based 3D bioprinters were originally modified versions of 2D ink-based printers in which the ink is replaced by biological material. It works on the principle of generating bioink droplets at the printhead with the energy provided either by a heater or a piezoelectric actuator. The major common limitation of inkjet bioprinting is that the biological sample has to be in a liquid state to enable droplet formation followed by self-solidification to form organized 3D structures. Various groups have tried to address the limitation by utilizing cross-linking strategies such as chemical or UV light exposure after droplet formation. However, the cross-linking process may slow down the overall bioprinting process, affect the natural composition of extracellular material, and could also be toxic to the cells [36][37]. Compared to other technologies, the inkjet process also offers advantages such as high speed, high resolution, simplicity of operation, low cost, and compatibility with numerous biological samples.

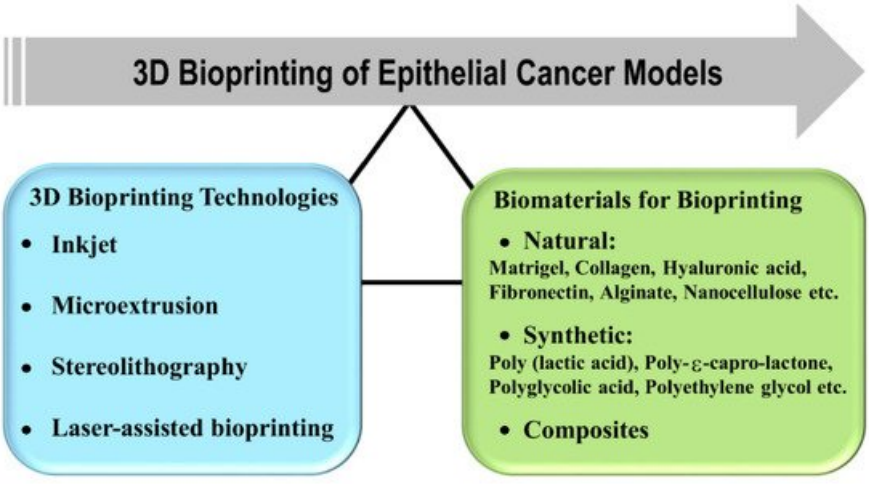

Figure 2. 3D bioprinting technologies and classification of biomaterials in bioprinting to design epithelial cancer models.

In microextrusion 3D bioprinting, the robotically controlled printers extrude a continuous stream of bioink through a nozzle utilizing mechanical or pneumatic forces, thus, resulting in layer-by-layer deposition onto

a substrate by a microextrusion head [38][39]. This technique is often described as direct ink writing (DIW), which may be equipped with UV-led sources to solidify the printed scaffold substrate in situ [40]. The substrate can be a culture dish (solid), growth medium (liquid), or material derived from the gel. The final bioprinted structure characteristics are directly dependent upon various parameters such as nozzle diameter, extrusion pressure, speed, temperature, UV-led curing, and many more. The rheological properties such as viscosity, shear thinning of the polymer or hydrogel employed play a vital role in designing the table 3D construct. It has been shown that the materials showing shear thinning behavior are preferred in microextrusion applications. During biofabrication, the high shear rate at the nozzle tip facilitates the material flow, and upon deposition, the viscosity of the material decreases automatically with a decreased shear rate. The technique has been utilized to fabricate various tissue and tumor models [41][42][43].

SL-based printing mainly includes projection-based digital light processing (DLP) and laser-based stereolithography apparatus (SLA), due to its high printing precision, excellent surface quality, and defect-free printing process has received widespread attention [44][45]. SL-based 3D printing applies light exposure (usually UV light) to convert photosensitive materials into cured solids in a layer-by-layer fashion [44][45]. The photosensitive material can be customized and formulated to have a single component or multiple components, such as biological macromolecules [45], multifunctional nanocomposites [46], and even living cells [34], which can simultaneously integrate the scaffolds' bioactivity and function for various biomedical applications. SL printing technology offers a universal 3D printing platform for tissue engineering with high precision.

Laser-assisted bioprinting (LAB) is another promising 3D bioprinting technology that has displayed successful compatibility with biological molecules such as DNA, as well as cells [47][48]. It has shown the potential to print mammalian cells with a minor negative impact on cellular viability and functions [49]. The technology uses focused laser pulses on the absorbing layer of the ribbon to generate a high-pressure bubble that propels cell-containing materials toward the collector substrate. The LAB resolution performance is affected by the following factors: surface tension, wettability of the substrate, thickness, and viscosity of the biological material layer [50]. Until now, LAB has been explored in the area of mostly tissue engineering, but there are hopes of utilizing the technology to also explore the potential to design tumor models in the future.

4. Biomaterials for Organotypic 3D Cancer Models

The complexity and heterogeneity of tumors present the biggest challenge in modeling the tumor and tumor microenvironment. Biomaterials can be used to create defined macro- and microenvironments, which have the potential to manipulate cells and tissues in vitro and in vivo. In the early 1980s, the concept to utilize biomaterials to study tumor biology was attempted to know how and whether the signals from the extracellular material regulate cellular behavior. The biomaterials based upon origin can be broadly classified into natural, synthetic, and hybrid materials (Figure 2). The natural biomaterials can be further classified into animal and not animal-based. The most used ECM-derived biomaterials in cancer research are collagen, laminin, hyaluronic acid, and reconstituted basement membrane or Matrigel®. These biomaterials have promising characteristics such as cytocompatibility, the ability to be remodeled by cells along with intrinsic cell adhesion properties. Still, there are associated challenges to study the influence of ECM on tumor cells due to uncontrolled degradation of natural biomaterials, batch to batch variability, and complex molecular composition [51][52][53]. Synthetic biomaterials can, therefore, provide more precise control over biochemical and mechanical properties when modeling the ECM of tumors. However, as the synthetic biomaterials lack natural cell adhesion sites, they are not remodeled by cells [54].

5. Conclusions

In epithelial cancer research, the synergy between basic research and technological advancement has provided substantial information about the cancer origin, TME, and cellular mechanisms involved in tumor progression. There is a tremendous potential for 3D in vitro models that very closely mimic the in vivo

model situation and recapitulate the cellular mechanisms involved in tumor progression. The possibility to design cell models by combining patient-derived cells and biomaterials utilizing 3D bioprinting technology has provided hope and motivation when studying cancer genetics and invasion mechanisms, but also facilitating the screening of anti-cancer drugs. In anti-cancer therapeutics, the crucial role of targeted nanotechnology-based drug delivery systems has proven advantageous over conventional treatments, thus enabling the improved biodistribution of anti-cancer drugs via various administration routes with enhanced therapeutic efficacy and reduced side effects. However, the translation from bench to bedside has not been as rapid as initially hoped for, partly due to a lack of suitable platforms for nanomedicine evaluation in a relevant setting. Therefore, the greatest potential relies on using high-content imaging combined with complex 3D culture models including ECM and the generation of a platform that not only addresses the TME combined with physiologically relevant ECM but is also suitable for screening and testing of NP delivered drugs on larger scale.

Nevertheless, the chosen biomaterial has to meet several categories for TME in terms of mechanical and biochemical properties. New materials need to be developed in order to create relevant 3D platforms. The selection of material substrates and further chemical design approaches should keep those categories as prerequisites. One single material cannot meet all the required properties, and thus composites with dual- and multicomponent structures could be applied to tailor optimal systems. The advancement of bioprinting techniques enables the extended possibility to tailor materials in a customized manner and create biomimetic 3D environments. However, the design of biomaterials for ink formulation is still a challenge and thus requires active dialogues between cell biologists and material scientists.

Miniaturized and standardized high-content screening platforms for the investigation of nanoparticle (NP) behavior are still very rare. In the future, quantitative high-content imaging approaches will most likely be utilized more for the profiling of the effect of drug-loaded NPs and the evaluation of anti-tumorigenic effects, and better information on how therapeutics interact with ECM or scaffold and different cell types in tissues. The technological development enabling such in-depth studies tandem with higher throughput capacity has been exceptionally rapid during recent years, spurring high hopes of the likewise rapidly developing 3D models to be utilized to their full potential in the development of new medicines.

In summary, 3D cell models have unique advantages compared to 2D models since they mimic the TME and recapitulate the cellular and ECM crosstalk. In addition, (targeted) nanotechnology-based drug delivery systems have proven advantageous over conventional anti-cancer treatments. Therefore, it is crucial that complex 3D models combined with high-content imaging platforms also will be suitable for screening and testing of NP delivered drugs and evaluation of their anti-tumorigenic effects.

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Keywords

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