CellPress

Special Focus on Materials

Review

Evaluating Biomaterial- and Microfluidic-Based 3D Tumor Models

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Cancer is a major cause of morbidity and mortality worldwide, with a disease burden estimated to increase over the coming decades. Disease heterogeneity and limited information on cancer biology and disease mechanisms are aspects that 2D cell cultures fail to address. Here, we review the current 'state-of-the-art' in 3D tissue-engineering (TE) models developed for, and used in, cancer research. We assess the potential for scaffold-based TE models and microfluidics to fill the gap between 2D models and clinical application. We also discuss recent advances in combining the principles of 3D TE models and microfluidics, with a special focus on biomaterials and the most promising chip-based 3D models.

The Importance of 3D *In Vitro* Tissue Models for Advanced Cancer Research

Conventional approaches used in cancer research involve culturing tumor cells on 2D surfaces and the use of animal models, which both poorly correlate with human disease states. 2D cell cultures oversimplify the biological context of a tumor, which is influenced by intrinsic molecular features and external cues from its surrounding microenvironment [1]. Unlike cancer cells grown in 2D, those grown in 3D adopt a rounded shape, forming clusters that are suggestive of tumors *in vivo* [2,3]. Cancer cells grown in 2D versus 3D also exhibit differential gene expression profiles for key genes involved in angiogenesis, cell migration, and invasion [4–8]. *Ex vivo* models or *in vivo* models, such as animal or patient-derived xenograft (PDX) models, are also popular tools for cancer research. Such models have advantages over cell cultures and do not suffer from the lack of 3D context, although they have their own set of limitations (Box 1).

To address the limitations of conventional approaches, the 3D microenvironment of tumors must be taken into account to improve the physiological relevance of *in vitro* models [9,10]. The integration of TE strategies and microfluidic technologies has recently sparked a breakthrough in the design of *in vitro* microfluidic culture models that better adapt to morphological changes in tissue structure and function over time, providing a level of precision control that could not be achieved previously [11]. Here, we review the current 'state-of-the-art' of 3D TE models that have been developed and used in cancer research. We critically assess the relevance of 3D cell models in cancer studies, and discuss the main advantages and limitations, with special emphasis on the biomaterials used. We also highlight new approaches that integrate bioreactors and microfluidic technology, along with the potential impact of 3D TE models on the cancer drug discovery process.

Classical 3D culture systems can be broadly subdivided as scaffold-free or scaffold-based methods [12]. Although scaffold-free 3D cancer models are best exemplified by tumor spheroids

Trends

Conventional 2D approaches used in cancer research poorly correlate with the human condition when compared with the possibilities of 3D models.

We examine how microfluidics can fulfil gaps in 3D models and their other significant advantages, with a special focus on biomaterials.

Using the combination of 3D TE and microfluidics on a multiomics-based approach (i.e., genomics and proteomics) in cancer research can advance our understanding of cancer biology as well as lead to the discovery of novel biomarkers, promising a revolution in the cancer research field. Further development of technologies that are appropriate and sensitive enough to make the most of the new features of microfluidics assays are essential. The development of chip-based 3D cell cultures in cancer research will also be largely dependent on the improvement of biomaterials that emulate the extracellular matrix.

While the physiological architecture of human organs currently exceeds the complexity of all *in vitro* culture systems, microfluidic cell culture devices can be fabricated that capture some of this architectural complexity.

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Box 1. Advantages and Disadvantages of *Ex Vivo* Models, Animal Models, and PDX Models

***Ex Vivo* Models**

Ex vivo tumor culture is performed using a thin slice of tumor tissue collected from human or animal sources and cultured on porous substrates or embedded in ECM-like matrices [55,56]. These models generally preserve the native complex and differentiated 3D cell-matrix architecture, cell phenotype, and complex architecture, logically providing a more accurate mimic of cell behavior.

However, the main drawback of this type of model may be the absence of mechanical forces, such as shear stress and perfusion, as well as surrounding tissue, which may result in changes in the structure and cell behavior compared with the original *in vivo* microenvironment. Another drawback is the need to harvest tissue from human or animal subjects.

Animal Models

Mouse models have proven essential in cancer research. These models yield better prediction of drug behavior and efficacy in humans compared with 2D conventional culture. They are used to understand the genetic basis of tumor development and cancer progression. They can also be used to test the efficacies of different anticancer agents because of their intrinsic microenvironmental complexity. Animal models enable studies of defined mutations, including the analysis of the effects of these mutations on many genetic backgrounds.

However, there is rowing demand from the public to reduce the use of animals as experimental subjects [57–59]. Other limitations involve the inability to mimic human-specific features relating to tumors, autoimmune conditions, stem cell differentiation, and, ultimately, their responses to therapeutic drugs. This is because the physiology, metabolism, tumor cell interactions with the innate immune system, proliferation, metastasis, and the cells themselves are different from those in humans [60,61].

PDX Models

PDX models are models where surgically resected primary tumor samples are engrafted directly from patients onto immunodeficient mice. These enable the molecular, genetic, and histological heterogeneity of their parental tumors to be preserved for longer [62]. PDX models offer a powerful tool for cancer research and a route toward personalized medicine for patients with cancer. They also enable the discovery of biomarkers predicting drug sensitivity and resistance, and possibly the monitoring of the initiation and progression of metastasis as well as the fate of circulating tumor cells using *in vivo* flow cytometry of implanted humor tumors [63].

(Box 2), we focus here on scaffold-based methods because they offer more opportunities for combination with other technologies. Scaffold materials can be synthetic or natural in origin [13]. Synthetic materials typically display better mechanical properties compared with natural ones (Table 1), but we focus our discussion on scaffolds made from naturally derived materials due to their greater physiological relevance. Biomaterials are broadly used for their marked similarities to the extracellular matrix (ECM), and typically have advantageous features, such as biocompatibility, biodegradability, and bioavailability, and also the capability to interact with cells. Additionally, natural polymers can be engineered and their properties tuned to obtain desirable mechanical and physical characteristics [14].

In Vitro 3D Scaffold-Based TE Tumor Models

Scaffold-based models have the advantage of allowing the study of tumor interactions with the microenvironment, in particular, phenomena such as tumor migration and invasion. Another advantage is the possible functionalization of the scaffold materials to obtain desired physicochemical and biological characteristics. For example, it is possible to incorporate bioactive molecules that promote cell adhesion or matrix metalloproteinase (MMP) substrates that render the materials susceptible to degradation by cell-secreted proteases, thus mimicking the naturally occurring interactions of cells with ECM and its consequent remodeling [15]. Great care and attention are required when choosing the biomaterial for culturing cancer cells, to better emulate the physiology of their original ECM, since this facet alone is able to influence tissue organization [11,16].

Models using Matrigel® as reconstituted basement membrane [17,18] can mimic the pathophysiological context of cancer and have enabled advances in 3D tissue engineering. The development of Matrigel grew from pioneering work on the isolation and purification of proteins

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