In Vitro Cancer Models

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In vitro cancer models are envisioned as high-throughput screening platforms for potential new therapeutic discovery and/or validation. They also serve as tools to achieve personalized treatment strategies or real-time monitoring of disease propagation, providing effective treatments to patients. To battle the fatality of metastatic cancers, the development and commercialization of predictive and robust preclinical in vitro cancer models are of urgent need.

cancer 3D cancer models point-of-care modelling tool commercialization

1. 3D Cancer Models: Product Segments, Commercial Tools, Prototypes, and Patents

Looking back, the modelling of cancer using animals is more than 100 years old. The Ehrlich ascites tumor cells, the spontaneous murine mammary adenocarcinoma cells that rapidly grow in almost all mouse strains, are considered one of the most primitive cancer models [1]. Over the past century, the advancements in cancer cell biology, 3D culture techniques, biomaterials, microfabrication, tissue engineering, and microfluidics have promoted the development of different types of in vitro cancer models. In this section, the current state of the art of commercially available in vitro 3D culture platforms, involved in modelling the different stages of cancer (from initiation, for example, spheroid formation, migration, invasion, intravasation, extravasation) is summarized. For convenience, they are categorized as follows.

1.1. Surfaces and 3D Culture Plates

The major limitations related to spheroids' development, maintenance and analysis are controlling the size, uniform production, and difficulties in manipulation and handling. These commercially available solutions are already addressing some of these issues.

1.2. Scaffolds/Matrices

An alternative approach likely to continue to emerge in cancer modelling is the use of ECM-like elements, such as scaffolds or matrices (for example, hydrogels, porous sponges, and so on). These ECM components encapsulate the cells, providing cells with structural, mechanical, and physical cues and supporting migration in all three (x, y, and z) directions—closely mimicking the physiological niche. The matrices with variable physiological stiffness ranging from 0.2 to 64 kPa are also available (CytoSoft® Rigidity plates) to mimic the stiffness of cancerous tissue at different disease stages. These scaffolds/matrices are used to investigate the formation of solid tumor-like

structures, tumorous growth/proliferation, tumor cell activation, invasion, intravasation, and matrix remodeling. The extracellular matrix (ECM) mimetic components can be synthetic (such as Alvetex®, Biogelx™-S, CytoSoft® Rigidity plates) or natural (for example, Matrigel®, PuraMatrix™, HyStem® hydrogels) in origin.

1.3. Patient-Derived and Cell Line-Based Assays/Services, Prototypes

The primary tumor site is preserved in patient-derived models, more specifically, patient-derived tumor xenograft models. PD3D® [2] offers a genomic library of over 200 diverse patient-derived cancer cell strains of 12 different tissue origins for multi-parametric drug response. By incorporating high content imaging, simultaneous recognition of pharmacodynamic biomarkers and anticancer activity can be carried out. Apart from patients' cells, cell line-based phenotype libraries are also commercially available for therapeutic screening. InSphero's 3D InSight™ tumor microtissues [3] is a collaborative approach (the service can also be obtained for a fee) to developing advanced 3D tumor/stromal models for therapeutic screening using cell-line derived or PDX-derived tumor microtissues. The OncoPanel™ service of Eurofins Discovery [4] offers a 3D-spheroid based platform with 100 different types of cancer cell lines. However, as an irreversible genetic mutation generates cell lines, this assay platform has limited predictive therapy value in precision medicine. BioIVT's Tissue Microarrays (TMAs) are another screening tool for identifying new genetic or protein markers for diagnostic purposes, comprising multiple donors (both diseased and healthy). TMA also includes donor and clinical demographics. Fresh human cancer tissue from non-small cell lung cancer is collected and adapted into the 3D culture platform of BioIVT's, termed the 3D Cancer ORGANDOT® model [5]. Another commercial collection of patient-derived cancer cells is the Kiyatec® ex vivo 3D cell culture platform [6], focusing on ovarian, breast, and glioblastoma. Apart from providing patient-specific physiological tumorous and immune microenvironment to investigate and approve specific cancer therapies, Kiyatec® is also presently involved in the development of 25 different classes of anticancer therapeutic molecules, including checkpoint inhibitors (immune-oncology). Crown Bioscience provides the service and access to the World's Largest Commercial Collection of Patient-Relevant Models derived from HuPrime® and HuKemia®, which are generated from highly characterized PDX models [7]. Their services include tumor growth assays, tumor microarrays, biomarker discoveries, immune-oncology, oncology databases, pharmacological and bioanalytical parameters for high-throughput molecular analysis of cancer tissues and therapeutic development. Besides in vivo models and other in vitro services, Charles River offers pre-defined or customized 3D tumor models for screening. These models can be selected from a library of over 55 Human Cell Line Derived (CDX) models, and 425 patient-derived Xenografts (PDX) models [8]. PharmaLegacy, besides offering a repository of in vivo models, also identifies and validates biomarkers and drug sensitivities through ex vivo assay platforms that employ 3D cultures of patient-derived tumors [9].

1.4. Microfluidic Platforms

A microfluidic platform is an add-on robotic biofabrication technology to obtain functional tissue-organ constructs, primarily used to investigate cancer cell migration, invasion, intravasation, and extravasation [10]. The SynVivo® [11] offers cell-based, more realistic, microchip services for cell–cell and cell–drug interaction. SynTumor of SynVivo® is an idealized network configuration with 2 μM or 8 μM pore sizes, enabling circulation in the microvasculature and

across the vessel walls in the tumor niche (created using tumor cell lines or patient-derived cells). The services of SynVivo® also include target validation, compound screening, biomarker analysis, ADME/Tox and mechanism ones. OrganoPlate® technology developed by Mimetas [12] allows the vascularization of 3D engineered tissue constructs such as organoids, spheroids or tumors in vitro. Tissues (including the PDX tumors) are placed into the chips connected to blood vessels, forming in vitro vascularized 3D construct. The vasculatures are then used to administer the drug in order to ensure that the new anticancer therapeutics have efficient, realistic pharmacokinetics. Further, by incorporating live-cell microscopy (time-lapse-enabled microscope) with the microfluidic platform, the real-time dynamic cellular response within a perfusion-based system can be carried out. This platform is commercially known as CellASIC™ ONIX [13] and is marketed by Merck Millipore.

1.5. In Vitro Cancer Models: Patents

The global cancer therapy market has been estimated at USD 13,625,435 million in 2018 and is predicted to be valued at USD 22,070,126 million in 2024 [14]. Factors that drive the market's growth include Patient Assistance Programs, R&D initiatives from key pharmaceutical industry players, and initiatives increasing cancer awareness.

The "3D Cell Culture Market by Product, Application, and End User: Global Opportunity Analysis and Industry Forecast, 2020–2027" analyses the market trends and provides future estimations between 2019 and 2027 [15]. In 2019, this market has been evaluated at $1234.86 million. In 2027, estimates predict it will reach $3721 million. In this analysis, cancer research is predicted as the highest growth segment [15].

Patents are a way of protecting intellectual property, with an essential role in translating scientific knowledge into diagnostic means or therapeutic approaches that can help patients [16]. In 1980, the changes in US government policy regarding government-sponsored research's intellectual property rights marked a new beginning in the commercialization of research results [17]. According to the new policy, the results of federally sponsored research would need to be patented and made available to the private sector for the development of commercial products. Although patents have a vital and unavoidable role in transforming scientific knowledge, they also allow monopolies to operate, blocking products from getting to the market [16]. This particularly concerns the field of unexpansive, affordable health care.

Patenting is a prolonged and extensive process. The pilot programs, such as The Cancer Moonshot Initiative, are created to avoid it; patents4Patients is proposed to expedite cancer research [18]. The Cancer Immunotherapy Pilot Program started in 2016 with the goal of accelerating, without extra fees, patent protection for inventions related to immunotherapy for cancer treatment [18].

2. Limitations and Challenges of Existing Models

The last decade has seen an increasing amount of scientific ones [19][20][21][22][23][24][25][26], book chapters and letters [27][28]. Several were propose using spheroids as one of the rapid preliminary screening strategies to investigate the potential of anticancer therapeutic molecules [29][30][31][32][33]. Others are new advancement in 3D

models, such as in fabrication processes, biomaterial development, and improvements in assay methods and strategies [34][35][36][37][38][39][40][41][42][43][44][45][46][47].

Although over the past decades several scientific and technological advances have been reported in cancer modelling, neo-anticancer therapeutics commercialization does not follow a similar trend [48][49][50]. The number of new therapeutics entering the market per billion US dollars spent on R&D is declining. This trend is called “Eroom’s Law” [48][50]. One possible explanation is the lack of funding to the fundamental research to unwind the unknown biological mechanisms that lead to high failure risk [51]. The current reductionist approaches in cancer models, which include complexity but lack “whole-istic biology” [52], further attributes to it. In 2016, Scannell and Bosley hypothesized that the predictive validity of models has a significant impact on R&D productivity [50]. Hence, the lack of reliable predictive models is a great setback for R&D efficiency [50].

In the early stages of the drug discovery pipeline, when a high number of compounds are screened, simplistic models, such as 2D cell culture, have been used [53]. They are reproducible and less expensive compared to complex models [54]. In the later stages, the use of animal models is required [53][55]. Although 2D cell culture is a convenient model, it does not represent the 3D organization and extracellular matrix (ECM) found in vivo [56]. Animal models also have their limitations, such as the low predictivity of human responses to drugs due to different genomic make up [56]. Also, these models have high costs associated with the animals, and their care and ethical concerns which have been well discussed [55]. For instance, the Transgenic Knockout and Tumor Model Center of Stanford Medicine charges approximately $13,885.49 for a single tumor animal model involving 30 mice from external investigators [57]. The service includes the injection of tumor cells, measuring the tumor growth 10 times, and collecting tumors at the end of the study. The cost of animal housing is separate. In contrast, Merck’s ready-to-use 3dGROTM Human iPSC Derived Colon Organoids cost only €2220 [58], which is nearly a sixth of the cost of the animal trial.

The reasons for the decline in neo-therapeutics entering the market have been identified [48][50][51], and pharmaceutical companies are analyzing their projects, trying to find affordable solutions to improve the productivity of R&D [49][59]. To achieve this goal, the Project Data Sphere has been created. This initiative aims to develop a repository of data from cancer trials in order to help improve new trials and accelerate drug discovery [60]. AstraZeneca has come up with five key factors contributing to project failure, calling them the 5 R’s: the right target, the right tissue, the right safety, the right patient, and the right commercial potential [49]. Decision-making and team behavior driven by volume-based goals, instead of emphasizing the understanding of target biology, seem to negatively impact the outcome [49]. As such, a sixth factor arose: the right culture. The failure of neo-anticancer therapeutics in phase 2 of clinical trials is mainly due to a lack of efficacy [61]. In cancer research, target confidence is lower than in other research fields, due to frequent inadequate translation of preclinical screenings to clinics.

In 2020, the cancer burden was estimated at 2.7 million new cases and 1.3 million deaths [62]. However, there is still a lack of recreation of endless genetic mutations and chaotic molecular involvement during disease progression in in vitro tumor models. The high failure rate (~90% ± 5) of cancer chemotherapies, including site-

specific targeted therapy (such as missile therapy) or molecular targeting drugs (for example, inhibitors of growth factor receptors or enzymes) is attributed to the highly reductionist in vitro models [63]. More realistic models that consider factors such as age, disease, or immune-compromised conditions of patients and complex immune-signaling pathways specific to patient are required. The cost–benefit ratio of the current therapies, the scaling up of some of the available novel modelling approaches, the ready adaptation of complex lab-techniques in clinical practice and the need of high-skilled researchers or technicians to operate the high throughput platforms are the other critical restraints [28][64].

The commercial application of human tissue-based models is further limited due to the collection and maintenance of the tissues and access to clinical metadata. Precision-cut tumor slices have the potential to represent the native tumor complexity and heterogeneity, allowing to konw the cells in their microenvironment [61]. However, post-processing of slices is critical and restricted to a specific laboratory/system, which causes difficulties in reproducibility [61]. For 3D in vitro models to be integrated into existing workflows, they must be low-cost, rapid, and robust in terms of translation into clinical context [28]. However, it is not easy to standardize the use of these models, since their production requires multidisciplinary approaches that are expensive too [28].

It is possible to incorporate further complexity into the available cancer models, resulting in difficulties in throughput and interpretation [49]. To improve 3D models and R&D productivity, the cost, the throughput ability, and the overall convenience of the model should be weighed against the predictive value of the model. Should it favors one side over the other or look for a balance? A balance should be the answer, but the cost can be overlooked for the precision models for those who can afford them. For this reason, the models showing promising results or potential should be further tested, characterized, and validated academically and industrially.

The incorporation of 3D models into the drug development pipeline has the potential to deliver more translatable data to the clinic and reduce the number of animals used [53]. AstraZeneca and Genentech published a comprehensive one with hepatic spheroids that supports their value for hepatotoxicity risk assessment in drug discovery [65].

Although these models are potentially more expensive than 2D culture, depending on the procedures and equipment used, and have lower throughput, they can be of major importance if used in the target validation phase, increasing target confidence [53].

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