

An Overview on Tissue Engineered Models for Cancer Study

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Received: 15 November 2017/ Accepted: 2 January 2018

Abstract:

There is increasing recognition that three-dimensional (3D) tissue culture technologies have many uses within the biomedical sciences beyond the scope of regenerative medicine. Three-dimensional models show clearer results than other two-dimensional models for testing anticancer drugs. Advances in tissue engineering have traditionally led to the design of scaffold- or matrix-based culture systems that can better reflect the biological, physical and biochemical environment of the natural extracellular matrix. Since developing new therapies for cancer is closely related to the understanding of the basic principles that are applied in tissue engineering models. In this study, we introduce some tissue engineered models of cancer.

Key words: Tissue engineering, Cancer, 3D models

Introduction

Tissue engineering is taken account as a newly appearing biomedical technology, which assists and enhances the repair and regeneration of inadequate and injured tissues. It utilizes the principles from the areas of materials science, cell biology, transplantation, and engineering in an attempt to treat or substitute injured tissues. Tissue engineering and development of complex tissues or organs, Like heart, muscle, kidney, liver, and lung, are still a distant milestone in twenty-first century(1). The major regenerative tissue engineering approach includes transplantation of cells onto scaffolds. The scaffold tries to mimic the function of the natural extracellular matrix, providing a template for the

growth of target tissues. Scaffolds should have sufficient architecture and strength to serve their intended function(2). Tissue engineering is viewed as a new and exciting method which has the possibility to produce tissues and organs *de novo*.(3) One of the most serious diseases in the United States and around the world is Cancer. There is no absolute cure for cancers. Developing new and efficient therapies for cancer is closely related to understanding and mimicking tumor and cancer *in vitro* to study all aspects and nature of it, its signaling pathways, drug effects and so more(4). By means of tissue engineering some 3D models can be produce that mimic tumor and cancers like the body. Here we

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introduce present 3 models of cancer developed by using tissue engineering principles(5).

Conventional *in vitro* cancer models

In vitro testing of anticancer drugs typically involves growing cancer cell lines in monolayers on tissue culture plastic and utilizing high-throughput screening procedures to assess drug efficacy. This part of cancer drug testing started in the late 1980s when the National Cancer Institute constituted a panel of 60 unique cancer cell lines for use in screening antineoplastic drug candidates. In addition, the distributed adoption of bioinformatics profiling has yielded insignificant information about cancer biology, led to the discovery of antineoplastic drugs with novel mechanisms of action, and assisted to delineate resistance mechanisms for several cancer kinds(6). In spite of these advances, the monolayer culture has stayed a poor predictor of whether a given drug will finally yield clinical benefit(7). Through multiple passages, the immortalization process results in choosing tumor cells that quickly proliferate and that have an advanced ability to survive on tissue culture plastic(8). These cells, therefore, may not be representative of the tumor as a whole and are especially susceptible to therapies that target quickly dividing cells(9). Perhaps the most dramatic alterations happen, however, because of the removal of the cells from their native microenvironment, comprising the extracellular matrix (ECM), soluble signals, 3D architecture, stromal cells, and irregular microvasculature that surround the tumor cells *in vivo*(10).

Animal models

In order to replicate the biological complexness of human tumor growth in a desirable way, it is

suggested to study human cancer within a model of animal. This provides tumor cells with many of the 3D micro-environmental cues that are missing during monolayer growth(11). The most ordinary mouse model utilized in drug testing for solid tumors is the subcutaneous human tumor xenograft(12). In this model, human tumor cells are implanted just beneath the skin of immunodeficient mice, and a few days or weeks are permitted for primary tumor growth before drug candidates are executed.³¹ Throughout the cure regimens, which often contain several weeks, tumor size can be monitored easily through physical caliper measurements; the tumors can then be subjected to a more thorough histologic and molecular analysis once animals are euthanized. Because the 3D tumor micro-environment is of murine origin rather than human, subtle but fundamental alterations persist that impact tumor cell behavior, and these are exacerbated by the artificial subcutaneous tumor localization(11). Advanced animal models have since been developed to overcome many of these limitations; orthotopic models involve tissue-specific rather than subcutaneous xenograft implantation,(13) and autochthonous tumor models involve spontaneous murine tumor progression through methods like genetic manipulation and carcinogen exposure(14). Even though these models often provide more biologically relevant data, the complexness and hardness of the experiments often precludes their widespread use, they are not currently accessible for all cancer kinds, and they still have shortcomings associated with the foreign mouse physiology(15).

3D Tumor Models

Human tumor spheroids

By far, the most widely employed 3D model is the human tumor spheroid, a small, tightly bound cellular aggregate that tends to form when changed cells are maintained under non-adherent conditions. These cellular aggregates can range in size from 20 μ m all the way up to 1mm in diameter, depending on the cell kind and growth conditions. Spheroids have been widely utilized in cancer research because the 3D architecture and extensive cell-cell contacts provided by spheroid growth appear to better mimic the *in vivo* cellular environment than two-dimensional (2D) monolayer cultures(16). Spheroids also indicate many of the biological properties of solid tumors, containing cell morphology, growth kinetics, gene expression, and drug response(17). Spheroid formation can be induced by a diversity of various techniques. The methods that was utilized earlier in cancer biology, and a method still widely employed today, is spinner flask culture, where fluid turbulence hinder attachment and advance cellular aggregation(18). A easier method for spheroid production involves liquid overlay of a cell suspension over a non-adherent surface, like agar-coated plates(19). Even though this technique avoids the shearing problems and equipment requirements related with spinner flask culture, the non-existence of fluid flow results in more pronounced diffusional gradients within the spheroid(20). Moreover, the spheroids made through liquid overlay are more different in size and number, although this differences can be ignored by culturing individual spheroids within 96-well plates(21). To optimize experiments to assure more concordant spheroid size and composition, recent adaptations involve spheroid formation within hanging drops and microfluidic chips, but these methods also need specialized equipment and extra handling steps that often

outweigh any relative benefits(22-23). The enhanced chemo resistance in spheroids compared to monolayer cultures may be described in part by enhanced cell-cell contact, 3D cellular architecture, and increased deposition of tumor-derived ECM within the spheroid, a lower overall cell proliferation rate, or a collection of these factors. Another inherent problem with spheroid use for drug testing is the non-existence of explained endpoints and the absence of a correct means for testing cell viability in 3D culture. As drug testing transitions from monolayer culture to 3D culture, many have simply utilized the same cell proliferation or cell viability assays to assess drug response(24). However, these assays are often not precise and truth in 3D culture because of the need for assay reagents to diffuse into and out of the cell mass. These assays can also be invalid because of differences in the cells' metabolic and proliferative rates throughout the spheroid(25).

Gel embedded models

Because spheroid culture only succeeds in mimicking a small part of the *in vivo* microenvironment, more developed 3D cancer models have been progressed utilizing biological gels as a substrate for spheroid growth. The growth of cancer cells in a 3D scaffold is a work that has been used for several years, an enhancing recognition that 3D culture exerts an impact upon the cancer phenotype has resulted in renewed interest. The earliest efforts to grow cancer cells within 3D substrates utilized small explants of cancer tissue within a natural gel. Because of its ubiquitous nature and relative ease of isolation, collagen was considered as one of the earliest biomaterials to be widely employed for 3D cell culture(26). Traditionally, collagen gel embedding involved the encapsulation of small tumor explants

(1–2mm) within a collagen matrix that permitted the explants to keep their viability and cellular architecture *ex vivo*. This method was utilized to culture a wide variety of cancer kinds in a way that preserved the native tissue architecture and cell viability over several months(27). Collagen gel culture has also been widely utilized to grow dissociated cancer cell suspensions. When encapsulated within a 3D gel, cancer cells will often aggregate into 3D spherical structures. Many researchers demonstrated these malignant aggregates to be spheroids; however, unlike traditional spheroids, which are characterized by their absence of adhesion to a surface, cells within collagen experience both cell–cell and cell–matrix attachments that can influence their behavior(28). Other natural polymeric gels have been utilized for cancer cell cultures. Alginate is a natural polymer derived from brown seaweed that gels in the presence of calcium ions and has been utilized as a scaffold for encapsulation of different kinds of cells(29). The major advantage related to alginate is that gelation can be fulfilled at room temperature after adding the cells to the polymer. A method for 3D culture of carcinoma cell lines within calcium alginate pellets was developed in the early 1980s and was later adapted for the growth of microscopic colorectal cancer explants. In both cases, the cancer cells could be maintained in the system for over a month and indicated cell growth rates and tissue organization similar to those of *in vivo* cancers(30). Other widely employed gel material that results in spheroid formation of embedded cancer cells in Matrigel (BD Biosciences, San Jose, CA). Matrigel is a basement membrane extract derived from the Engelbreth-Holm-Swarm mouse sarcoma that includes a diverse array of components, containing collagen type IV,

laminin, and other ECM molecules, as well as different soluble signals, such as cytokines and growth factors(31). This material is widely utilized to study both normal and malignant cells owing to its ability to advance cell differentiation, migration, and recreation of an *in vivo* cellular architecture and it has been examined as a substrate for 3D tissue growth. Several kinds of normal epithelial cells will form hollow, polarized, acinar structures when cultured within Matrigel, whereas corresponding metastatic epithelial cells will form solid, disorganized spheroids(32).

Advanced tissue-engineered models

In recent years, as a result of advances in the tissue engineering and biomaterials fields, a few numbers of researchers have moved beyond simple gel culture systems and have started utilizing more tunable and mechanically superior polymer matrices as substrates for 3D cancer growth to provide a more well-defined architecture for tumor cell growth. For instance in a research investigated the growth of a breast cancer cell line, MCF-7, on porous chitosan scaffolds. Chitosan is a natural polymer derived from the deacetylation of chitin, and its surface properties can be altered by varying its degree of deacetylation(33–34). In their first study, porous chitosan scaffolds were formed with varying degrees of deacetylation, and these scaffolds were examined for their ability to advance breast cancer cell attachment and growth. These investigators understood that the cancer cells had optimal attachment and proliferation on scaffolds with a high degree of deacetylation and that the cell metabolic activity under these conditions was similar to that seen in 2D cultures(34). The response of these cancer cells to varying doses of tamoxifen, a drug commonly utilized in breast cancer treatment, was

then investigated, and these responses were compared with those seen in monolayer cultures. A much lower level of cell death and a 10-fold increase in drug resistance were observed compared to the monolayer cultures, revealing that 3D growth in this system conferred therapeutic resistance to the cells. Even though the authors discounted the influences of restricted drug diffusion into the construct on this enhanced resistance, this phenomenon was not directly examined(33). In a study that conducted later, the same group utilized this cancer growth system for monitoring of cancer therapeutics(35). Cancer cells within PLA (Poly lactic acid) micro particles were exposed to three cancer drugs commonly utilized to treat breast cancer (doxorubicin, paclitaxel, and tamoxifen) and the drug responses were compared to those seen in monolayer cultures. As in studies utilizing chitosan scaffolds (33-34) a large increase in drug resistance was present, ranging from 12 to 23 times that seen in monolayers. However, Labhasetwar and coworkers also investigated drug accumulation in the cells in an attempt to investigate the impact of drug availability on this enhanced resistance(35). A study on 3D cancer cell growth within solid scaffolds investigated the angiogenic properties of cancer cells in a 3D environment. Mooney and colleagues also used a porous PLGA (poly lactide-co-glycolic acid) scaffold, this time to culture an oral cancer cell line. Larger scaffolds were employed (8.5-mm-diameter disk) than in the studies that were explained above, and scaffolds were fabricated with a gas-foaming=particulate-leaching method (36). After seeding cells onto the scaffolds, the cell morphology and proliferation, hypoxia, secretion of proangiogenic factors, and drug response were investigated. The impacts of cancer growth in the 2D monolayer

culture, in the 3D Matrigel culture, and in mice after implantation of cell-seeded PLGA scaffolds were compared. Cells grown *ex vivo* within these PLGA constructs resembled those grown *in vivo* both histologically and in terms of the overall growth kinetics. Hypoxia levels observed within the constructs were similar to those often seen within tumors, as was the increase in the release of proangiogenic factors related to this rise in hypoxia. Interestingly, the mixture of angiogenic factors released was not similar to that released from cells grown in the monolayer controls under artificially hypoxic conditions, recommending that the 3D architecture within the construct effects how the cells respond to hypoxia. The investigation of the drug response of the cancer cells within these PLGA constructs involved only one compound, a phosphatidylinositol-3 kinase inhibitor, and enhanced drug resistance of the cells within this construct was found compared to that seen in the monolayer controls(36).

Tissue engineered *in vivo* cancer xenograft models

Application of micro-environmental conditions in *vivo* tissue engineered models by utilizing biomimetic platforms could improve the understanding their impact on the growth of cancer and metastatic spread is known that factors, like alterations in interactions between cells and ECM, angiogenesis, development of hypoxia, or signaling of cells that reside within spatially distinct and tissue-specific niches, increase tumor aggressiveness, but these inexplicit conditions are often only partially designated and indicated by currently applied *in vivo* model systems.(37). An essential observation is that, because the angiogenic characteristics of the tumor cells are dramatically changed in the 3D culture

micro-environment and resemble more closely those of *in vivo* tumors, preculture in three dimensions could induce greater malignant potential. Moreover, tumors generated with the 3D-cultured cells are more resistant to cytotoxic drugs *in vivo*, which better replicates the clinical situation and thus provides a desirable model for drug testing(36).

Microfluidic models

Microfluidic technology holds great promise for the creation of advanced cell culture models. Microfluidic systems represent a new kind of cell culture vessel that expands our ability to control the local cellular microenvironment. Microfluidic systems enable patterning of molecules and cells as well as both passive and active cell handling and environmental control. Temporal and spatial control on the micrometer scale (0.1–100 μm) has been used in fundamental studies from the subcellular to the organismal level, for instance in studies of cell division axis orientation and geometric influence on cell survival (38-40). Two important aspects of microfluidic interfacing are automation and ease of handling, both of which must be considered when designing microfluidic systems(41). Many research devices have been made from poly (dimethylsiloxane) PDMS, and some interfacing strategies have relied on its elastic properties. However, the material properties of PDMS may be problematic for cell culture models. Because several molecules involved in cell microenvironments are small and hydrophobic, it is necessary to characterize the effect of PDMS absorption of such molecules upon cell culture in microfluidic devices. Although glass is very common for chemical analysis devices, the cost is too high for disposable devices. Therefore,

thermoplastic materials are the most desirable for microfluidic cell culture devices(42).

Progress in microfluidic technology has enabled the development of *in vitro* assays that facilitate the study of cellular behavior under tightly controlled microenvironments with high spatiotemporal resolution. Hence, modeling the tumor micro environment by integrating the interactions among multiple cell types with biochemical and biophysical factors is a very attractive target for microfluidics toward this end, a number of microfluidic designs have been developed to study growth-factor gradients in cancer cell migration, tumor-stromal cell interactions, and tumor-endothelial cell interactions (43-45).

The reduced volume in microfluidic 3D systems facilitates the system's capability as a high throughput screening (HTS) tool to investigate numerous micro environmental components influencing tumor development and progression. It allows screening of a broader array of micro environmental parameters and increases our understanding of how the interplay among these parameters regulates cancer development and progression(46). The development of micro-fabrication techniques such as microcontact printing and soft lithography further enhanced microfluidic capabilities, that is, better control over topology and surface chemistry,[18] hence paving the way for more sophisticated mechanistic investigations of tumor biology(47). The ultimate advancement in the applicability of microfluidics to oncology stems from the use of 3D scaffolds to more fully recapitulate the native tumor niche and incorporate the effects of matrix composition, structure and mechanical properties on tumor progression(48). In addition to the use of microfluidic platforms to study tumor migration and drug

response, another research focus has been the development of microfluidic tumor models for the study of tumor vascularization and anti-angiogenic therapy(49).

Organoids

Organoids are 3D *in vitro* culture systems derived from self-organizing stem cells. They can recapitulate the *in vivo* architecture, functionality, and genetic signature of original tissues. Surgery, chemotherapy, and radiation therapy have long been employed to treat cancer; however, most regimens have been unsuccessful in eradicating cancer cells completely. One of the main reasons for this is inter and intra-patient tumor heterogeneity. Cancer cells display heterogeneity in terms of their distinct mutational and epigenetic profiles, morphological and phenotypic differences, gene expression, metabolism, proliferation rates, and metastatic potential(50). To understand cancer biology including tumor heterogeneity, and to translate this knowledge into clinical applications, preclinical cancer models are required(51). Cancer cell lines and animal models have long been used for cancer modeling. Cancer cell lines accumulate multiple additional mutations over time in culture conditions. While patient-derived tumor xenografting (PDX) recapitulates the original tumor more closely than do cancer cell lines or animal models, there are several challenges to using PDX. PDX is labor-intensive, time-consuming and expensive, and engraftment efficiencies differ among tumors, making it difficult to use for high-throughput screening. Thus, there are strong needs for a better model system for studying cancer. One of the attractions of the Organoids culture system rests in the fact that similar culture techniques can be used for normal tissue and neoplastic tissue: patient

biopsies and resections can be developed into Organoids which mimic the original cancer tissue. Tumor Organoids (tumoroids) can be generated very efficiently from tumor tissue and easily propagated under defined conditions. Therefore, tumor Organoids can bridge the gap between 2D cancer cell lines and animal-based model systems, including PDX(52). Tumoroids recapitulate patient-specific histological features and physiological phenotypes. In addition, transplantation of patient-derived Organoids can form lesions resembling an original tumor, suggesting that tumoroids can be used for drug screening. Clonally derived Organoids from human pancreatic tumor cells may prove to be a valuable tool to predict patient-specific sensitivities and clinical outcomes upon novel therapeutic drug treatment for pancreatic ductal adenocarcinoma (PDAC) in a similar fashion(53). A catalog of patient-derived prostate cancer cells from biopsy specimens and circulating tumor cells could be developed for long-term cultures of prostate cancer Organoids. These Organoids recapitulated the essential characteristics and molecular diversity of prostate cancer subtypes, making them a facile model system for pharmacological studies(54).

Organoids derived from normal tissue can also model cancer. Using gene manipulation technologies in Organoids, tumor-related genes can be studied in an isogenic (identical genotype) background. In one study, the expression of mutant KRAS and TP53 was induced in pancreatic Organoids derived from human PSCs by using a lentivirus expression system. Transplantation of these Organoids into the mammary fat pads of NOD (non-obese diabetic) SCID (severe combined immunodeficiency) mice showed abnormal ductal architecture and nuclear

morphology, consistent with neoplastic transformation(55).

Conclusion

Tissue engineered models of cancer prepare some advantages that conventional 2D *in vitro* or animal models lacked. These 3 models mimic many aspects of natural tumors such as micro environmental components, architecture and signaling. Use and improve of Tissue engineered models of cancer can lead to better understanding, developing efficient drug and cure of cancer.

Conflict of interest

The authors declare any conflict of interest.

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