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Research review paper

Three-dimensional perfused cell culture

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ABSTRACT

Compelling evidence suggests the limitation and shortcomings of the current and well established cell culture method using multi-well plates, flasks and Petri dishes. These are particularly important when cell functions are sensitive to the local microenvironment, cell–cell and cell–extracellular matrix interactions. There is a clear need for advanced cell culture systems which mimic *in vivo* and more physiological conditions. This review summarises and analyses recent progress in three dimensional (3D) cell culture with perfusion as the next generation cell culture tools, while excluding engineered tissue culture where three dimensional scaffold has to be used for structural support and perfusion for overcoming mass transfer control. Apart from research activities in academic community, product development in industry is also included in this review.

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1. Introduction

Our current understanding on biological processes is largely based on studies of homogenous populations of cells cultured on flat-surfaces using multiwall plates, cell culture flasks and Petri dishes, referred as the two-dimensional (2D) static cell culture or on animal model systems. Typical 2D culture involves growing a single or mixed cell types of fresh isolated functional cells from human tissues/organs and preclinical species or immortalized cell lines on flat plastic or glass substrates. Culture media are changed frequently to provide fresh nutrients and remove the metabolic waste. However tissues and organs in the body are three dimensional (3D) structures and are continuously perfused by the blood circulation network. It is generally recognised that there is a

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significant difference in cell behaviour and functions between a flat layer of cells sitting in a medium bath and a complex, three-dimensional tissue fed by blood circulation found in the body. Actually tissue-specific architecture, mechanical and biochemical cues and cell–cell interaction are lost under the simplified conditions in 2D culture (Bissell et al., 2003; Cukierman et al., 2001, 2002; Nelson and Bissell, 2006). On the other side, species specific behaviours cannot be captured by the time-consuming and costly animal models, although many important cellular behaviours can be revealed in a physiological and systematic manner. For example, idiosyncratic human toxicity, biological relevance to humans, human tumours, immunogenicity, and response from therapeutic antibodies are considered unpredictable from animal data (Dixit and Boelsterli, 2007; Kuperwasser et al., 2004).

Three-dimensional (3D) cell cultures mimic to a certain degree the *in vivo* situation by allowing cell aggregation to form tissue spheroids or embedding cells on or in a defined scaffold that mimics the extracellular matrix (ECM) of structural proteins and other biological molecules found in real, living tissues. They can possess, by design, key desired features and functions but with significantly reduced complexity (Lutolf and Hubbell, 2005). In 3D culture, the physiological cell–cell and cell–matrix interactions regulate proliferation and differentiation in both space and time, and hence tissue function and homoeostasis could be maintained, as proved by cell biology and proteomic studies (Friedl and Brèocker, 2000; Schmeichel and Bissell, 2003). 3D culture models can be used to study human tissue physiology and pathophysiology *in vitro*.

To introduce external control of the physiochemical environment in culture and to eliminate the fluctuation and uncertainty in microenvironment (*e.g.*, the pH, oxygen, glucose, and growth factors) around the cultured cells, perfusion can be used where cell culture medium with well defined and known compositions, is driven through the cultured cells. Such perfusion, mimicking the blood circulation in the human body, can control physiological chemostatic conditions, create gradients of oxygen, growth factors and other biochemical signals and maintain cell–cell communications and cell–extracellular matrix (ECM) interaction (Griffith and Swartz, 2006; Pampaloni et al., 2007; Yamada and Cukierman, 2007). Moreover, adding perfusion element into 3D cell culture offers the hydrodynamic effect in (i) effectively reducing diffusional limitation in promoting convective mass transfer, and (ii) controllable shear possibly to stimulate cell functions.

Three dimensional perfused cell culture bridges the gap between conventional 2D cell culture and animal models. In combination with human stem cell technology, various human tissue models can potentially be created in laboratories for the study of human tissue physiology, pathology, and for the testing drug efficacy and toxicity, chemical toxicity and safety of consumer products and ingredients.

In this review, the current progress on the technologies for 3D perfused culture is reviewed, focusing its use as a research tool for cell study, but tissue engineering applications are mostly excluded intentionally. The importance on 3D structure, perfusion and combination of 3D and perfused culture on development of *in vitro* physiological relevance models for a wide variety of systems will be addressed separately. The applications of 3D perfusion culture in drug toxicity testing and stem cell research are also discussed.

2. The importance of 3D culture

2.1. Microenvironment cues in vivo

Cells within a tissue interact with neighbouring cells and with the ECM through biochemical and mechanical cues. Cell–cell and cell–ECM interactions establish a 3D communication network that maintains the specificity and homeostasis of the tissue. The tissue architecture and geometric property, and mechanical stress and fluid flow direct cell morphogenesis and functions. The possible micro-environmental cues that might influence the growth and differentiation status of most cell types are grouped as chemical, physical and spatiotemporal cues demonstrated in Fig. 1 (adapted from Bottaro et al., 2002; Lai et al., 2011; Lutolf and Hubbell, 2005). Chemical cues comprise of soluble factors including growth & survival factors, cytokines, morphogenetic proteins, metalloproteinase and death ligands, small molecule agonists, steroid hormones, peptides, and ions (Bottaro et al., 2002). Chemical cues are the easiest factors to manipulate independently to control cell growth and differentiation by supplementing them in the culture medium. Physical cues are provided through adhesive and structural ECM components (*e.g.* fibronectin, vitronectin, laminin, tenascins, collagen, fibrillin) and proteoglycans (PGs, *e.g.* aggrecan, biglycan, perlecan) as well as glycosaminoglycans (GAGs, *e.g.* hyaluronic acid, chondroitin sulphate, heparin sulphate). Consequently matrix property (*e.g.* matrix fibre strength, degree of crosslinking, pore size, and matrix degradability) and matrix force (*e.g.* contraction and swelling) are governed by the compositions and coordination of ECM. For instance collagen fibres resist tensional forces and compaction by cells while PGs resist compressive force by controlling tissue hydration (Griffith and Swartz, 2006). Mechanical and environmental stresses include matrix stiffness, dynamic or static mechanical forces, shear forces, gradients of chemical cues and pH and oxygen tension. This may result in changes in cell shape and motility, and regulate the morphogenesis of cellular structures (Discher et al., 2009; Fischer et al., 2009; Soucy and Romer, 2009). Cell–cell interactions are regulated by cellular adhesive proteins and their ligands which help regulate cell migration, proliferation, apoptosis and differentiation (Bornstein and Sage, 2002).

The concept of 3D cell culture has been developing from initial introduction of the spatial context to recent attempting on a more comprehensive meaning of the third dimension. Many examinations reveal that cues generated from the third dimension are highly interconnected with the other cues. For example, cellular responses to ECM signals were found a context-dependent: ECM signals presented in 3D differ from those presented in 2D (Green and Yamada, 2007). For instance, focal adhesion formation during cell–ECM adhesion is mechanistically different in naturally derived hydrogels when compared to protein-coated tissue culture polystyrene substrates (Cukierman et al., 2001; Li et al., 2011a, 2011b). These context dependent changes are due to cell–substrate mechanical interactions, involving local forces generated by the cell during spreading (Balaban et al., 2001) and primary (tissue) cell sensing of the synthetic substrate stiffness, or local variations of ECM elasticity *in vivo* (Discher et al., 2005). Dimensionality has currently become a statement for describing potential differences between conventional 2D monolayer culture, 3D cell culture systems and the physiological situations *in vivo*.

2.2. 3D vs 2D cell culture

Culturing cells, either single or multiple types of cells, in 2D monolayer formats is well established and convenient to set up with good viability of the cells. Cells are seeded onto a cell culture plastic or glass surface within multiwall plates, flasks, or Petri dishes, and adhere to the surface. Culture medium containing desired nutrients and simulating factors, or tested chemicals, is added to bathe the cells. Cells consume the nutrients and produce metabolic waste. Hence the cell culture medium needs to be changed regularly. When the proliferating cells spread and cover most of the surface, they have to be harvested and reseeded with reduced cell density, a process called passaging. The cell culture equipment (CO2 incubator) and consumables (multiwall plates, flasks, Petri dishes) are readily available in variety. Equipment, even automated ones, for biological assays of functions of cells cultured in this way has been developed, for example, based on

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