Review

"Bridging the gap between cell culture and live tissue"

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Abstract

Traditional in vitro two-dimensional (2-D) culture systems only partly imitate the physiological and biochemical features of cells in their original tissue. In vivo, in organs and tissues, cells are surrounded by a three-dimensional (3-D) organization of supporting matrix and neighbouring cells, and a gradient of chemical and mechanical signals. Furthermore, the presence of blood flow and mechanical movement provides a dynamic environment (Jong et al., 2011). In contrast, traditional in vitro culture, carried out on 2-D plastic or glass substrates, typically provides a static environment, which, however is the base of the present understanding of many biological processes, tissue homeostasis as well as disease.
It is clear that this is not an exact representation of what is happening in vivo and the microenvironment provided by in vitro cell culture models are significantly different and can cause deviations in cell response and behaviour from those distinctive of in vivo tissues.
In order to translate the present basic knowledge in cell control, cell repair and regeneration from the laboratory bench to the clinical application, we need a better understanding of the cell and tissue interactions. This implies a detailed comprehension of the natural tissue environment, with its organization and local signals, in order to more closely mimic what happens in vivo, developing more physiological models for efficient in vitro systems. In particular, it is imperative to understand the role of the environmental cues which can be mainly divided into those of a chemical and mechanical nature.

1 Introduction

Traditional in vitro two-dimensional (2-D) culture systems only partly imitate the physiological and biochemical features of cells in their original tissue. In vivo, in organs and tissues, cells are surrounded by a three-dimensional (3-D) organization of supporting matrix and neighbouring cells, and a gradient of chemical and mechanical signals. Furthermore, the presence of blood flow and mechanical movement provides a dynamic environment (Jong et al., 2011). In contrast, traditional in vitro culture, carried out on 2-D plastic or glass substrates, typically provides a static environment, which, however is the base of the present understanding of many biological processes, tissue homeostasis as well as disease.

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2 Environmental cues of chemical nature

2.1 Exosomes and Non-coding RNA

Exosomes are secreted vesicles that include membrane particles, microvesicles, ectosomes, exosome-like vesicles or apoptotic bodies (Ostrowski et al., 2010), often found in physiological fluids such as normal urine (Pisitkun et al., 2004), plasma (Caby et al., 2005) and bronchial lavage fluid (Admyre et al., 2003). They are secreted by several types of cell, such as dendritic cells (Zitvogel et al., 1998), mast cells (Raposo et al., 1997), T cells (Peters et al., 1989), platelets (Heijnen et al, 1999), Schwann cells (Fevrier and Raposo, 2004), tumor cells (Andre et al., 2001), endometrial cells (Greening et al., 2016) and embryos (Wydooghe et al., 2015). They contain evolutionarily conserved set of proteins but also have unique tissue/cell type-specific proteins that reflect their cellular source (Mathivanan et al., 2010).

Although still controversial, they are believed to be important for intercellular communication. They are presently being investigated for their role as markers for disease and a possible use for diagnostic purpose has been postulated. Furthermore, they represent a very promising biochemical cue in cell to cell communication and tissue engineering. in neuronal and embryo-maternal communication, inter-embryo communication and pathogenesis.

2.2 Chemical gradient and macromolecular crowding

Gradients of distinct molecules play key roles in a variety of processes, affecting the commitment of cells as well as their role in vivo (Keenan and Folch, 2008) and a main need is to create in vitro methods that allow cells to be exposed to chemical gradients that may be tuned, quantified and controlled in such a way to mimic the gradients that are present in vivo (Benny et al., 2016).

It is obvious that a gradient across 3-D space more closely recreates the in vivo milieu and may be more advantageous to help elucidate biological processes are modulated by biomolecular gradients.

An alternative strategy is based on the principles of macromolecular crowding (MMC), a biophysical phenomenon that directs the intra- and extra-cellular milieu in multicellular organisms and increases thermodynamic activities and biological processes (Zimmerman and Harrison, 1987). MMC uses the principles of volume occupancy, where macromolecules take volumes larger than their 'real' volume owing to their high hydrodynamic radius, thereby reducing the space for other macromolecules belonging to the same system (Ellis, 2001; Lareu et al., 2007).

Several reports have recently shown the impact of these aspects. For instance, the addition of neutral or negatively charged molecules to the culture media was shown to increase collagen type I deposition in in vitro culture of human fibroblasts (Lareu et al., 2007). This has been explained with the observation that in vivo cells are entrapped in highly crowded extracellular space, where the conversion of the de novo synthesised procollagen to collagen takes place rapidly, whereas in the diluted culture environment the conversion of procollagen to collagen is very slow (Lareu et al., 2007). Although the full applicability of this approach is under evaluation, a promising application of MMC for cell based therapies is addressed to the combination with cell sheet technology (L'Heureux et al., 2007; Peck et al., 2012) to accelerate the production of cell sheets rich in extracellular matrix (English et al., 2012).

2.3 Epigenetic modifications

Cell differentiation processes are regulated by the expression of different sets of genes responsible for a distinct phenotype, under the control of regulatory mechanisms that include DNA methylation and histone modifications.

All these differences in gene expression drive development and differentiation, leading to the acquisition of epigenetic marks and the fixation of a specific fate that has been considered stable and potentially irreversible until recently. However, following the pioneering work carried out by Jones (Taylor and Jones, 1979), in the last years, many groups reported that it is possible to directly interact with cell fate definition through the use of epigenetic modifiers (Pennarossa et al., 2013; Brevini et al., 2014; Chandrakantan et al., 2016; Manzoni et al., 2016) (Figure 1). These studies are particularly intriguing, since they allow a better understanding of epigenetic restriction, and better clarify the mechanisms leading to the acquisition of a mature somatic phenotype.

Figure 1: Fibroblasts obtained from biopsies cultured on gelatin coated culture dishes (left) and exposed to epigenetic modification (right). Striking changes in morphology are evident and are accompanied by a decrease in the global DNA methylation.
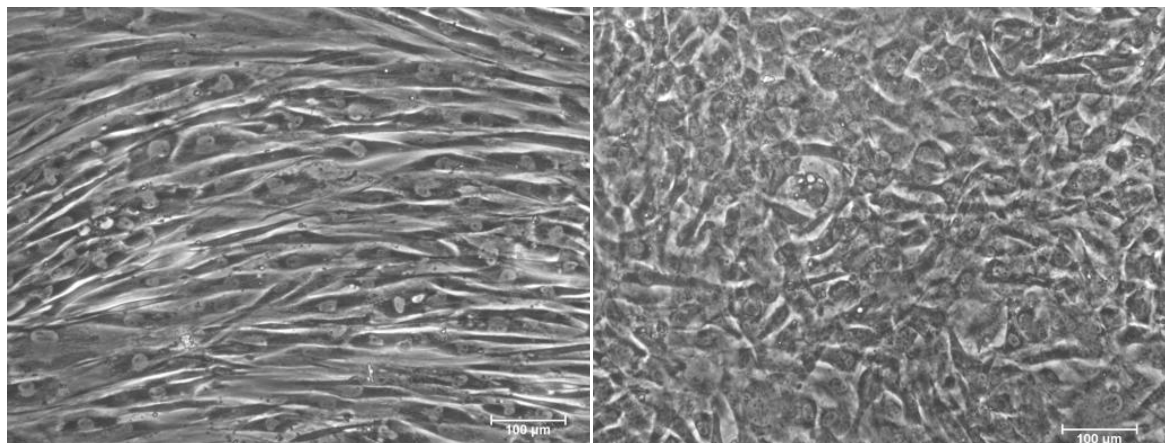
3 Environmental cues of mechanical nature

In vivo, cells are surrounded by a complex 3-D organization of neighbouring cells and ECM, which interact to provide chemical as well as mechanical stimuli. Integrin and surrounding matrix are not only in charge of ensuring physical attachment, but also convey chemical and mechanical signals from the outer environment (Janmey and McCulloch, 2007), suggesting that the dimension where cells are grown is a key point for the determination of cell fate. Until recently, cell culture has been performed in monolayers, that, although providing useful biological information, lack the ability to reproduce the morphology and 3-D architecture of the original tissue. The benefits of 3-D cell cultures are widely appreciated and more cell-based technologies are now becoming available that enable researchers to preserve the native 3-D structure of cells in vitro, offering many advantages over conventional monolayer culture. First of all, the mechanisms that underline the process of tissue formation, such as migration, proliferation, adhesion, differentiation and apoptosis can be better investigated. In addition, the 3-D environment enables cells to form cell-cell and cell-matrix interaction that may otherwise be precluded in monolayer culture (Baker and Chen, 2012). Deconstructing the elements contributing to the 3-D microenvironment and the associated processes will aid us in better understanding the underlying mechanisms that guide cell growth in vivo.

3.1 Technologies for 3-D cell culture

The concept of 3-D cell culture is not new and has been around for over a century. What has changed more recently is the ease of access to new innovative technologies on the market that enable researchers to more readily practice 3-D cell culture. These can generally be categorized into three areas, namely aggregate/spheroid methods; hydrogels/extracellular matrix technologies; and solid scaffold products. There is no single solution, each approach

has strengths and weaknesses, and researchers must select the technology most appropriate for their needs to address the scientific questions that they are interested in.

Aggregate-based technologies promote bringing cells together to create 3-D tissue-like masses or spheroids, often called 'micro-tissues' by exploiting the biophysical properties acting on the media in which they are grown. Cells grown as 3-D aggregates secrete their own extra-cellular matrix (ECM) and self organise creating multiple cell-cell interactions. Different methods have been developed to generate this type of culture for routine use. The traditional hanging drop approach involves the culture of cells in a drop of medium suspended from the lid of a culture dish. These suspension cultures are adequate for cells that can proliferate in a non-adhesive environment where aggregation is favoured. An alternative strategy involves using attachment-resistant surfaces such as coatings with hydrophilic polymers (Jo and Park, 2000) or micro-patterned surfaces (Yoshii et al., 2011). Both methods inhibit cell adherence and forces cells to float in the medium, stimulating them to coalesce and form spheroids. Regardless of how cell aggregates are formed, these methods make it possible to scale down experiments and work in smaller volumes and are therefore amenable for higher throughput applications. Co-culture of different cells types is also possible, establishing signal rich environments to study the effect of paracrine signalling in real tissue (Torisawa et al., 2009).

Hydrogels differ from solid scaffolds in terms of the strength of physical support. Hydrogels are loose scaffolds consisting of cross-linked natural or synthetic materials within an aqueous environment for cell encapsulation. These highly absorbent matrices are better suited to modelling soft tissues basic of their tissue-like flexibility and viscoelasticity (Tibbit and Anseth, 2009). Hydrogels can be derived from a variety of sources that in turn affect their compatibility and properties. For example, animal-derived hydrogels mainly use collagen, which is the most abundant protein in the ECM. Matrigel® is an example popular commercially available hydrogel composed of tumour extract derived from mouse sarcoma cells. It is known to contain growth factors, a rich protein mix including collagen IV, laminin and entactin and other undefined constituents (Vukicevic et al., 1992). Matrigel® can promote cellular functions that would otherwise be unseen by providing a 3-D microenvironment and the necessary endogenous factors (Benton et al., 2014). The use of defined synthetic hydrogels overcomes some of the issues of animal derived materials such as batch variation and unknown constituents.

Solid scaffolds were originally devised for applications in transplantation, however, there has been a growing interest to introduce scaffolds for routine use in 3-D cell culture. The materials used in the fabrication process are important in shaping the purpose of scaffold-based technology. Components of the native ECM including collagen, fibrin, and hyaluronic acid (HA) (Gerecht et al., 2007) have been used effectively to create 3-D matrices to support cell growth. These constituents have the benefit of being biocompatible and possessing readily available adhesion sites that can increase the complexity of the tissue. Despite being beneficial in the context of tissue engineering, working with biological materials in the laboratory can affect consistency. A partial solution has been to use biodegradable polymers such as poly(glycolic acid), poly(lactic acid) and their co-polymers poly(lactic-co-glycolic acid) (Mikos et al. 1993). This is not ideal because their degradation results in the release of unwanted by-products that can alter cell behaviour. The added variability coupled with short shelf life and problematic storage make biodegradable materials unsuitable for standard use in 3-D cell culture. In light of these shortcomings, synthetic scaffolds with defined composition

have risen as a more consistent alternative. Inert and non-degradable materials such as synthetic polymers can be carefully tweaked to capture the cellular niche, creating scaffolds suitable for cell culture (Bokari et al. 2007; Knight et al. 2011). The lack of biological activity and natural cell adhesion sites can be overcome by coating these substrates with ECM proteins such as laminin and fibronectin (Knight and Przyborski, 2014). Despite providing physical support in the form of 3-D spaces where cells can proliferate, these voids have poor mass transfer since these cultures are static systems. For these reasons, scaffolds are usually engineered as thin membranes (e.g. 200μm) that permit sufficient exchange of nutrients and waste products. This in turn enriches the physiological accuracy of these models allowing researchers to study in vivo phenomena in a controlled in vitro setting.

Advances in technology have led to new opportunities for growing cells in culture and the creation of 3-D tissue-like constructs. This is primarily as a consequence of inter-disciplinary research between cell biology and the biophysical sciences, introducing new materials and methods of manufacture to create platforms tailored to support 3-D cell growth in vitro. The success of these technologies will depend on their adoption, validation and application. The creation of tissue-like constructs in a reliable and reproducible manner according to clearly defined protocols is essential. Moreover, in-depth characterisation of the anatomy and physiology in vitro models alongside their native counterparts will be critical to convincing the scientific community and encourage users to adopt these new approaches (Figure 2).

Figure 2: This figure showcases the potential of 3-D cell culture technology and how it can be used to create tissue-like models. Histological images reveal the cellular structure of real human skin (left) and a human skin equivalent (right). The full thickness of the epidermis is shown resting on the underlying dermis. The model is built on the Alvetex® platform that consists of a porous polystyrene scaffold in which human dermal fibroblasts are seeded. These cells produce exogenous collagens to form the dermal compartment. Human keratinocytes are then seeded onto the surface of the dermal model which is subsequently raised to air-liquid interface where they differentiate, stratify and form a mature epidermis. The layers of cells in the 3-D culture model (right) replicate those in the real tissue (left), including the formation of the skin barrier and the surface stratum corneum.

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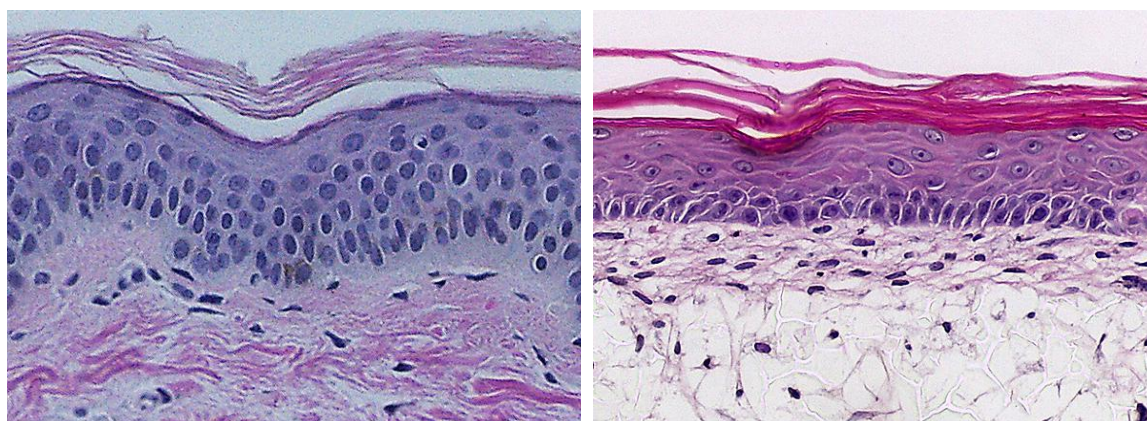
There is no doubt that 3-D cell culture is a rapidly growing and important field of science and requires interdisciplinary research to be innovative and develop. These advances will continue to enable biomedical researchers to recreate the structure and function of human tissues in the lab for research, screening and safety assessment. This technology combined with human stem cell science will open new opportunities for tissue engineering in the lab where renewable sources of human cells can be generated to create robust and reproducible 3-D models of human tissues. Furthermore, to reproduce the conditions in vivo requires many other factors such as oxygen control, perfusion, growth factors, cytokines, hormones, mechanical stiffness, etc. Today we are applying technology to improve current practice, to make incremental advances over existing models, to enable greater insight into biological processes. As technology advances, we will further improve our cell culture models, edging closer to in vivo conditions, but researchers must always remember that it is a model they are studying in the lab and not real tissue. 3-D cell culture takes a big step towards achieving the goal of recreating the growth conditions cells experience in vivo.

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