Developments in three-dimensional cell culture technology aimed at improving the accuracy of in vitro analyses

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Abstract

Drug discovery programmes require accurate in vitro systems for drug screening and testing. Traditional cell culture makes use of 2D (two-dimensional) surfaces for ex vivo cell growth. In such environments, cells are forced to adopt unnatural characteristics, including aberrant flattened morphologies. Therefore there is a strong demand for new cell culture platforms which allow cells to grow and respond to their environment in a more realistic manner. The development of 3D (three-dimensional) alternative substrates for in vitro cell growth has received much attention, and it is widely acknowledged that 3D cell growth is likely to more accurately reflect the in vivo tissue environments from which cultured cells are derived. 3D cell growth techniques promise numerous advantages over 2D culture, including enhanced proliferation and differentiation of stem cells. The present review focuses on the development of scaffold technologies for 3D cell culture.

Demand for 3D (three-dimensional) cell culture technology

The availability of accurate informative in vitro assays is an increasingly important challenge facing the pharmaceutical industry. Rising cost-to-delivery ratios and the poor predictive value of existing in vitro tests places great emphasis on the development of more realistic cell culture models. Since costs mount as pharmaceuticals near market, efficient and realistic in vitro research is of huge potential value, enabling informed strategic decisions to be made earlier rather than later.

Cell-based in vitro assays are a key component of drug discovery research. Cultured mammalian cells are important tools for providing predictions of drug activity, metabolism and toxicity in vivo. Human stem cells are particularly valuable in this context since they promise to provide a potentially limitless supply of a wide range of human cell types for use in drug testing. However, traditional cell culture environments are far removed from real-life tissues. In vivo, cells are supported by a complex 3D ECM (extracellular matrix), which facilitates cell–cell communication via direct contact and through the secretion of a plethora of cytokines and trophic factors. In contrast, cells grown in culture are generally confined in 2D (two-dimensional) monolayers without many of the physical and chemical cues which underlie their identity and function in vivo. These factors are particularly important when considering the potential for directed differentiation of stem cells in vitro.

In vitro, cells can behave very differently depending on the growth substrate employed. Conventional tissue culture is carried out on 2D surfaces without scope for cells to adopt natural morphologies or to communicate efficiently with their neighbours. This 2D confinement is far removed from the aforementioned 3D complexities of living tissue. Engineering the cell culture microenvironment to create growth conditions that more accurately mimic the in vivo behaviour of cells is an essential step for improving predictive accuracy during pharmaceutical development [1]. It has been shown using alternative cell culture applications that the growth and function of cells as multicellular 3D structures is significantly different to their growth as conventional 2D monolayer cultures [2]. The design of 3D culture systems for drug development is an important part of this process. Findings show that refinement of the in vitro environment significantly influences the way in which cells respond to small molecules [3].

Considerable effort has therefore been made to engineer materials for 3D in vitro cell culture in the belief that provision of a 3D spatial environment will overcome some of the restrictions associated with 2D culture. Progress in this area is beginning to bridge the gap between traditional cell culture and living tissue environments. The present article briefly reviews some currently available technologies for in vitro 3D cell culture, and we include reference to a recently developed technology designed to enable routine 3D cell culture.

Current technologies for in vitro 3D cell growth

Although there are a variety of technologies available that enable 3D cell growth, most of these have been developed...
In addition, biodegradation during an elimination of these products due to degradation, leading to life-threatening issues, and improper storage of such biopolymers can lead to limited utility for routine biodegradability, however, is not necessarily an attractive feature. This has been shown that such materials benefit the repair of damage in the early stages and drug responses. Granted that this technology does not require a degree of 3D cell growth, however, growing cells as individual spherical masses is not necessarily suitable for all requirements, as the distribution of cells throughout the material is not entirely even, and there are issues about mass transfer given the thickness of the scaffold under static growth conditions. Furthermore, it is not clear whether cells will respond differently to the alginate substrate compared with conventional 2D plasticware, which is familiar and has been used for many years.

An alternative and one of the early most successful approaches has been the culture of cells on biodegradable polymers such as poly(glycolic acid), poly(lactic acid) and their copolymers poly(lactic-co-glycolic acid) [6]. Previous work has shown that such materials benefit the repair of articular cartilage during implantation and encourage tissue regeneration [7]. Their degradation over time paves the way for replacement by viable functioning cells and aids the integration of co-transplanted cells with host tissues. Biodegradability, however, is not necessarily an attractive feature for routine in vitro cell culture, where there are shelf-life issues, and improper storage of such biopolymers can render such products useless due to degradation, leading to changes in their properties, quality and variation of results. In addition, biodegradation during an in vitro experiment introduces another variable that may influence how cells behave and is therefore not necessarily a desirable feature.

Hydrogels are a common form of material that has successfully been used to support ex vivo 3D cell growth for a variety of systems, such as bone, cartilage and nervous tissues [8–12]. Hydrogels comprise a cross-linked natural base material such as agarose, collagen, fibrin or hyaluronic acid with a high water content. They can be engineered to support preferential cell growth and function. Hydrogels in essence trap cells in an artificial ECM environment and may be modified to incorporate biologically active molecules such as laminin [12] and altered physical parameters such as charge [13]. There are several commercially available hydrogel-based products, including Extracell™ (Glycosan Biosystems), a chemically defined hyaluronan-based substrate. Injectable hydrogels have proven to be successful for tissue repair [8]; however, their use for routine 3D cell culture is restricted by various practical issues, including expense, shelf-life, gel preparation, storage and consistency.

The development of inert non-degradable scaffolds made from synthetic polymers overcomes several of the limitations that other technologies experience. These structures consist of pores or voids into which cells can grow and which are joined by interconnecting holes. Methods of fabrication for porous materials include emulsion templating [14,15], leachable particles [16] and gas foaming technology [17]. Gas-in-liquid foam templating has been used as a method to create porous scaffolds for cell culture applications [18]. However, gas bubbles can coalesce, leading to a broad range of scaffold porosities and it is therefore difficult to control the consistency of the material and consequently the reproducibility of the cultures grown therein. Electrospinning is also a well-established technique giving rise to electrospin synthetic fibres woven into mats designed to support 3D cell growth [19]. However, the consistency and porosity of electrospun materials is difficult to control. Ultra-Web™ (Corning) was developed as a commercial polyanide electrospin nanofibre mat for cell culture. Cells grow as monolayers on the roughened topography created by the nanoscale Ultra-Web™ fibre mat, rather than within the physical lattice of the material.

Technology for routine 3D cell culture

A number of these fabrication technologies have been applied to the manufacture of porous polystyrene-based scaffolds. Polystyrene is an attractive medium as a scaffold to support 3D cell culture because it is chemically inert, stable and, most importantly, consistent and directly comparable with conventional 2D tissue culture plasticware. The vast majority of in vitro cell culture experiments and data generated from them over the last few decades have been conducted on polystyrene surfaces in one form or another. There is no doubt that the transition to 3D cell culture models is a major step change; however, the development of polystyrene-based scaffolds will ease the impact that this has, given that the polystyrene substrate remains the same albeit it has changed from a 2D into a 3D geometry. Polystyrene scaffolds are also advantageous given that they are generally simple and inexpensive to mass produce, and they are designed as a consumable product with a long shelf-life. These attributes make polystyrene-based substrates well suited for routine 3D cell culture.

Emulsion templating enables the controlled manufacture of porous polystyrene that can subsequently be tailored to support 3D cell culture [14,15]. Alvetex™ (Reinnervate) is a substrate was engineered to be 3D cell culture friendly such as polystyrene-based hydrogels, polystyrene surfaces, alginate, and traditional 2D plasticware.
new product that utilizes this technology and produces a polystyrene-based scaffold that has a relatively uniform structure. The scaffold is formed by polymerization in a biphasic emulsion, consisting of an aqueous and a non-aqueous monomer/surfactant phase, termed HIPE (high internal phase emulsion) [20]. The resulting polymer (poly-HIPE) product consists of a templated porous network of voids, linked by interconnecting pores (Figure 1A). These structures in the final poly-HIPE material are highly controllable through manipulation of key manufacturing parameters [14,15]. Alvetex™ has been developed as a solution for routine 3D cell culture and is designed for incorporation into existing cell culture products, such as welled plates or well inserts (Figure 1B). The scaffold has been engineered into a thin 200-μm-thick membrane to address the issue of mass transfer, enabling cells to enter the material and allowing for sufficient mass exchange of gases, nutrients and waste products during static culture. The polystyrene that forms the scaffold is cross-linked, which gives the material improved structural stability and strength even when presented as a thin membrane. Alvetex™ is compatible with standard cell culture plasma treatment, γ sterilization methods and, if required, can be coated using standard cell culture reagents such as collagen or fibronectin.

For routine 3D cell culture, the development of any new technology must consider issues such as cost, ease of use, application and reproducibility, especially when the application is for drug discovery. A technology that is expensive, difficult to use or is inconsistent in some manner will not satisfy these demands and will fail to be accepted by the scientific community. Importantly, any such technology requires vigorous exemplification and validation as evidence
of its ability to support true 3D cell culture over a range of alternative cell types. Although Alvetex™ is developed as a generic solution for 3D cell culture, it has also been promoted on the basis of demonstrating its application in several key areas of research. For example, cultured liver cells are a valuable tool for the in vitro study of drug metabolism and toxicity [21]. Liver-derived cell lines represent a convenient model for liver toxicology studies, although commonly available lines often display poor metabolic responses when subjected to drug treatments. However, responses by such cells are significantly enhanced when cultured in 3D using Alvetex™ [22,23]. Similarly, pilot data reveal that primary hepatocytes have enhanced cell viability and superior capacity to metabolize hepatotoxic drugs when cultured in the polyHIPE scaffold compared with 2D plasticware. Culture systems able to contribute enhanced metabolic activity and/or more realistic resistance/sensitivity in response to specific drugs would be of potential value to the pharmaceutical industry, enabling more accurate toxicological assays.

Another essential element required is a reliable source of cultured cells. Developments in stem cell technology are focused on the differentiation of functional tissues from human stem cells, and this potentially represents a valuable opportunity for a renewable source of functional hepatocytes [24,25]. Expansion of stem cell populations and control of stem cell differentiation towards the formation of specific functional cell types will rely on the introduction of new methods and technologies. This will no doubt require the need for more sophisticated cell culture models including systems for 3D cell growth. Examples of human-derived hepatocytes and pluripotent stem cells grown in 3D culture are shown in Figure 2.

**Conclusions**

Culture technology has, in general, changed little over the last few decades. The development of new technologies in the physical sciences has, however, provided innovative solutions to improving current practice, and the growth and performance of cultured cells in 3D. The investment of time towards developing and validating such in vitro models is likely to have a significant impact on the success and overall efficiency of pharmaceutical development in the future.

**References**


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