# Three-dimensional cell culture: the missing link in drug discovery

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**Cells, grown as monolayers (2D models), are routinely used as initial model systems for evaluating the effectiveness and safety of libraries of molecules with potential as therapeutic drugs. While this initial screening precedes preclinical animal studies before advancing to human clinical trials, cultured cells frequently determine the initial, yet crucial, 'stop/go' decisions on the progressing of the development of a drug. Growing cells as three-dimensional (3D) models more analogous to their existence *in vivo*, for example, akin to a tumour, and possibly co-cultured with other cells and cellular components that naturally occur in their microenvironment may be more clinically relevant. Here, in the context of anti-cancer drug screening, we review 2D and 3D culture approaches, consider the strengths and relevance of each method.**

## The need for more efficient and cost-effective preclinical screening of anti-cancer drugs

The design and development of all new drugs, for the most part, follow a similar trend of progression. Typically a potentially druggable target is identified and, often with the aid of *in silico* modelling, lead compounds are designed, developed and optimised to act on this target. Preclinical testing is then performed with compound libraries to establish which members of the library exhibit efficacy towards the target in question. Preclinical testing involves both *in vitro* analyses in appropriate cell line models, as well as *in vivo* studies in relevant animal models. These are performed to determine toxicity, in addition to pharmacokinetic and pharmacological characteristics testing to investigate absorption, distribution, metabolism and excretion properties; fundamental in determining the basic safety and potential usefulness of the drug. Finally, clinical testing of suitable compounds, through various stages of clinical trials, is performed in humans; an essential step to evaluate the ultimate usefulness of the drug.

While there has been an increase in the number of potential anti-cancer agents being advanced for development over the past 10 years [1], the number of these products that progress successfully throughout clinical development is low, at approximately 10% [2]. Lack of clinical efficacy and/or unacceptable toxicity are two of the main causes of drug failures during development [3,4]. Because of the high costs (typically ~1 billion US dollars) in getting new drugs approved and the fact that many oncology drugs fail during clinical testing, especially during phase III – the most expensive phase of clinical development [5,6], it is imperative that compounds that are potentially ineffective or have an unacceptable toxicity profile are dismissed as early in the evaluation process as possible. This would preferably be before clinical trials and, ideally, even before animal testing has begun. Failing early in the developmental stages enables the cost of failed molecules to remain relatively low, that is, the further into the development process a compound fails, understandably the more money a company has invested and so stands to lose [7]. It is, therefore, necessary to improve *in vitro* cell-based testing methods for a more informed prediction of drug candidate efficacy and safety, and thereby sieve out poorly functioning compounds while prioritising promising candidates [6].

The ability of an *in vitro* assay to produce reliable biomedically relevant information is essential in drug development; therefore, it is necessary that the cells used in this testing mimic the phenotype of cells within the target tissue [7,8]. 2D cell culture (i.e. monolayer culture) is conventionally used in *in vitro* drug candidate testing; however, limitations of 2D culture suggest that an alternative method should be considered. These limitations include the lack of cell–cell and cell–extracellular matrix (ECM) signalling that

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 1359-6446/06/$ - see front matter  http://dx.doi.org/10.1016/j.drudis.2012.10.003

occurs in the 3D (three-dimensional, multi-cellular spheroids) *in vivo* environment where such signals are essential to cell differentiation, proliferation and a range of cellular functions [9,10]. For example, integrins, which are cell surface receptors, anchor cells to the ECM and are also involved in the cells' interpretation of biochemical cues from their local environment [11,12]. It is probable, therefore, that 3D cellular assays would be more analogous to – and so predictive of – *in vivo* events compared to more simplified 2D cultures in which essential signalling pathways may have been lost or, at least, compromised [13]. The use of 3D *in vitro* systems in drug research and development has, therefore, been suggested as a potential link to bridge the gap between monolayer cultures and animal model studies [6,14].

This article reviews both 2D and 3D cell culture. The 3D procedures discussed include methods that modify cell culture surfaces and thereby promote 3D culture formation by preventing cells from attaching to their surface; the hanging drop method which supports cellular growth in suspension; rotary systems that encourage cells to adhere to each other to form 3D spheroids; 3D scaffolds and matrices, which provide extracellular support and allow 3D cell growth; and microfluidic systems that support 3D cell culture. While the same methods are relevant regardless of the drug types being assessed, a comprehensive review of all applications of 2D and 3D cultures is not possible; so in the context of our own research here we focus on model systems for screening anticancer drugs; particularly in the context of breast cancer. Throughout this review we highlight the differences between each culture method. We propose the necessity to incorporate 3D cell methods into drug development for human therapy, based on their ability to mimic tissue-like structures more effectively than 2D cell culture. Advantages and disadvantages of various methods for 3D culture are subsequently summarised in Table 1.

## A brief history of cell culture in 2D

The process of cell culturing was developed, in 1907, by Harrison while investigating the origin of nerve fibres [15]. Specifically, explanted pre-differentiated neural tissue from frog embryos was placed in a drop of lymph hanging from a sterile cover-slip, kept sealed and in a moist chamber. This method allowed tissue growth and differentiation to be continually observed [16]; demonstrating a means by which cells of interest could be maintained outside the body of origin and observed over time.

Substantial improvements have been made on the 2D cell culture technique initially developed by Harrison. Containers used for culturing have been developed which enable cells to be fed with ease and that allow more space for cell growth. Additionally, the traditional use of blood plasma as the only source of nutrition for the growing cells changed to the use of synthetic medium. There are many advantages including the fact that batches of synthetic medium can be made reproducibly; do not contain antigens which can cause allergic reactions; and are relatively cheap to produce. Antibiotics and anti-fungal agents have been developed that are suitable for cell cultures and thus help to prevent bacteria and fungi from infecting cultures. While these additives are not a substitute for good cell culture practice, they can be useful for maintenance of infection-free cells, if contamination is envisaged to be a problem.

Cells are typically grown as a monolayer on a flat surface, most commonly in culture flasks or sometimes in Petri-dishes with medium as a source of nutrition and at body temperature (37°C). Medium is often supplemented with bovine serum and L-glutamine to aid cell growth. When reaching confluency, cells are sub-cultured so as to avoid complications from senescence or nutrient-exhaustion from medium. To sub-culture, cells are cleaved from the bottom of their culture dish (with trypsin and/or EDTA) and a quantity

**TABLE 1**

**Proposed advantages and disadvantages of different 3D cell culture methods**

| *Method type* | Advantages | Disadvantages |
|---|---|---|
| **Forced-floating** | • Relatively simple<br>• Inexpensive<br>• Suitable for high-throughput testing<br>• Spheroids produced are easily accessible | • Variability in cell size and shape if not as fixed cell no./well<br>• DIY plate-coating is relatively labour intensive |
| **Hanging drop** | • Inexpensive if using standard 96-well plate<br>• Homogenous spheroids suitable for high-throughput testing<br>• Spheroids produced are easily accessible | • More expensive if using specialised plates<br>• Labour intensive if preparing plates in-house<br>• Small culture volume makes medium exchange, without disturbing cells, difficult (proposed easier handling with commercially available formats) |
| **Agitation-based approaches** | • Simple to culture cells<br>• Large-scale production relatively easily achievable<br>• Motion of culture assists nutrient transport<br>• Spheroids produced are easily accessible | • Specialised equipment required<br>• No control over cell no./size of spheroid (can be overcome by additional culture step; see 'Forced-floating methods')<br>• Time consuming for HTS due to extra step required for homogenous spheroids<br>• Cells possibly exposed to shear force in spinner flasks (may be problematic for sensitive cells) |
| **Matrices and scaffolds** | • Provide 3D support that mimics *in vivo*<br>• Some incorporate growth factors | • Can be expensive for large-scale production<br>• Can have difficulty in retrieving cells following 3D culture formation |
| **Microfluidic cell culture platforms** | • Described as suitable for high-throughput testing | • Specialised equipment required adding expense<br>• Further analysis of 3D cultures produced may be difficult |