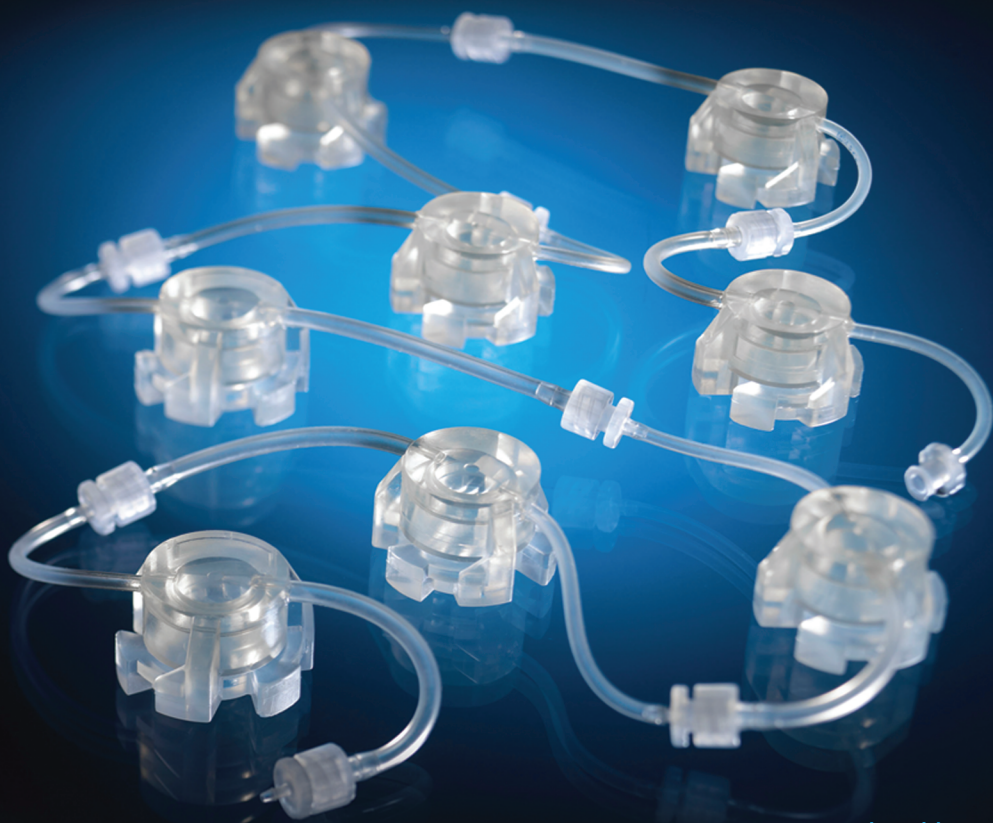


# CELLULAR METHODS AND PROTOCOLS IN VITRO TESTING



edited by  
John W. Haycock  
Arti Ahluwalia  
John M. Wilkinson



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# Preface

The laboratory culture of eukaryotic cells has now been routine practice for over four decades, underpinning a multitude of biological and medical applications—from cancer studies to drug discovery, from toxicology to stem and developmental cell biology. Irrespective of the applications, the tools and techniques that are used to grow an adherent cell in vitro outside a living organism comprise of the cell culture medium, the tissue culture incubator and a substrate, most commonly made from “tissue culture polystyrene” or glass. The substrate, in almost all instances, takes the form of a flat two-dimensional (2D) surface. Although a very large number of biological studies have been performed using 2D in vitro cell culture, one might be forgiven for questioning the relevance of such studies when interpreting the information in light of in vivo models, which consider the relevance of multi-cellular tissues and organs, a blood supply, an immune system, plus endocrine and neuronal signals.

To overcome the limitations of 2D models, a number of 3D in vitro models are being developed for a range of tissues and organs, which take account of the spatial organisation and arrangement of the cells therein. The development of all such models has a common objective, which is to establish scientifically robust platforms for enabling the improved interpretation of data beyond what simple 2D cell cultures provide. A more ambitious objective is to approach the physiological relevance of an in vivo model—and furthermore, in so doing to **Replace, Reduce, or Refine** the necessity of the in vivo model itself, especially those based on animal studies. Thus, the development of 3D cell culture in vitro models directly addresses the 3Rs principle, first introduced by Russell and Burch in 1959 in their book *The Principles of Humane Experimental Technique*.

For this to become a possibility, it is necessary to establish a growth environment that mimics the native tissue structure as closely as possible. Thus, 3D culture models typically combine materials science, cell biology, and bioreactor design. The integration of these approaches is particularly important, given the practical and applied directions of such work. For example, the promise of regenerative medicine to replace body parts suffering acute injury or degeneration associated with aging is frequently reported in the popular media. However, the routine uses of such therapeutic treatment are presently few and far between. To accelerate progress, accurate and relevant 3D culture models must be developed, as they will be essential for the development of such technologies.

3D cell culture models generally include the study of whole animals and organotypic explant cultures (including embryos), cell spheroids, microcarrier cultures, and tissue-engineered constructs. So while not all 3D cell culture models require a scaffold, their use has seen a rapid increase in recent years. This is in large part due to advances in biomaterial science and fabrication methods for 3D printing, for example, micro-stereolithography of polymers for creating scaffolds with micrometer resolution, or similarly electrospinning, micro-extrusion, micro-injection moulding and ink jet printing.

The potential to use human primary cells or stem cells is highly relevant when developing 3D *in vitro* cultures, not only for considering the 3Rs as an alternative approach but also for understanding the fundamental processes of cellular differentiation. This becomes particularly important when considering methods for therapeutic intervention. The ability to isolate cells with the capacity to renew, mitotically divide, and differentiate into a diverse range of cell types is of fundamental importance—and yet many studies on stem cells still use a 2D environment. New evidence is now emerging on the relevance of the 3D environment and the ability of cells to “sense” their 3D environment including the extracellular matrix stiffness, reported to determine stem cell differentiation along a particular lineage. This logically extends, though, to the culture environment and the ability to supply nutrients in the culture medium to a growing niche of metabolically active cells while simultaneously removing the waste products for maintaining survival. Considerable evidence also exists on the development

and need for communication of different cell types in 3D culture, where paracrine signalling is responsible for the differentiation and development of cell types in co-culture to form a nascent functioning tissue, for example, keratinocytes and fibroblasts for 3D skin models or neuronal and glial cells for 3D nerve models. Thus, the rapid development of bioreactor systems for allowing controlled flow, the spatial organization of cell-scaffold constructs, as well as organ crosstalk for this purpose is gaining momentum.

*Cellular in vitro Testing: Methods and Protocols* reports on a wide range of methods for the applications of 3D in vitro cell culture models, either for the purposes of in vitro testing or in the long-term development of forming tissues for therapeutic purposes. The scope and contents of the book have arisen from selected presentations given at the Annual *Quasi-Vivo*® User Group Meetings, and the style of each chapter is based on a descriptive protocol style, such that readers will be able to reproduce individual methods in the laboratory step-by-step. The book starts with a review chapter, which gives an overview of methods for connected culture experiments using perfused flow chambers. Thereafter, there are nine chapters which cover key areas in human airway inhalation toxicity, quantifying xenobiotic metabolizing enzymes in hepatocytes, cardiac patches, risk assessment of nanoparticles, the development of nanosensor scaffolds, a paracrine human skin model for irritancy detection, drug testing using intestinal mucosa, a human bronchial model and the use of a porous scaffold for simple and routine 3D culture.

We hope this book serves as a useful methods and protocols manual for laboratory scientists who need to develop the underpinning scientific basis and technical details for 3D in vitro cell cultures. We are personally indebted to all of the international experts who have kindly contributed chapters and taken great care and time in preparing their contributions for this book.

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**Arti Ahluwalia**  
**John M. Wilkinson**  
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