Filter-well Technology for Advanced Three-dimensional Cell Culture: Perspectives for Respiratory Research

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Summary — Cell culture has long been a valuable tool for studying cell behaviour. Classical plastic substrates are two-dimensional, and usually promote cellular proliferation and inhibit differentiation. Understanding cell behaviour within complex multicellular tissues requires the systematic study of cells within the context of specific model microenvironments. A model system must mimic, to a certain degree, the in vivo situation, but, at the same time, can significantly reduce its complexity. There is increasing agreement that moving up to the third dimension provides a more physiologically-relevant and predictive model system. Moreover, many cellular processes (morphogenesis, organogenesis and pathogenesis) have been confirmed to occur exclusively when cells are ordered in a three-dimensional (3-D) manner. In order to achieve the desired in vivo phenotype, researchers can use microporous membranes for improved in vitro cell culture experiments. In the present review, we discuss the applications of filter-well technology for the advanced 3-D cell culture of human pulmonary cells.

Key words: bronchial epithelium, cell culture, extracellular matrix, in vitro, lung, microporous membrane, toxicology.

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Introduction

The practice of cell and tissue culture, as a tool to determine various biochemical and biological mechanisms, has largely depended on the use of methods in which cells are grown on two-dimensional (2-D) surfaces (e.g. micro-well plates, glass slides, tissue culture flasks and Petri dishes), because of the ease, convenience and high level of cell viability achieved with 2-D culture. However, the flat surface of traditional cell culture plastics represents a poor topographical approximation of the more-complex, three-dimensional (3-D) architecture of the extracellular matrix (ECM) demonstrated by in vivo tissue architecture. Natural ECMS are highly-hydrated networks, composed of insoluble hydrated macromolecules (e.g. collagen, laminin and fibronectin), soluble macromolecules (such as growth factors, chemokines and cytokines), and proteins on the surfaces of neighbouring cells (1). A cell’s ability to generate a basal phenotype and respond to perturbations in its environment is a coordinated response to the molecular interactions with these ECM effectors. Indeed, many physiological (e.g. morphogenesis and organogenesis) and pathological (e.g. tumour growth) cellular processes have been demonstrated to occur exclusively when cells are organised in a 3-D fashion (2).

The conventional 2-D cell culture systems have improved the understanding of basic cell biology, but their disadvantages are a consequence of forcing epithelial cells to feed and excrete from only the top or apical surface. This is in direct contrast to the in vivo 3-D environment typically observed for epithelial cells, such as airway lung tissues, where the apical (top) surface is exposed to air while the baso-lateral (bottom) surface is embedded in a complex 3-D matrix, i.e. there is an air–liquid interface (ALI; Figure 1). 2-D substrates are considerably limited in emulating complex 3-D microenvironments, because of the lack of structural architecture and finite material selections. Furthermore, inhabiting a 2-D rigid substrate requires a dramatic adaptation by surviving cells, because of the lack of the unique ECM environment and forced feeding and excretion from only the apical surface of each cell type. For these reasons, 2-D culture substrates are likely to misrepresent real situations, by forcing cells to adjust to artificial, flat and rigid surfaces (3). The reason for such reactions is that, in the human body, nearly all tissue cells reside in an ECM consisting of a complex 3-D fibrous meshwork with a wide distribution of fibres and gaps that provide complex biochemical and physical signals. The ECM not only facilitates attachments between cells, the basement membrane (BM) and the surrounding matrix, but it also alters the transport of oxygen, hormones and nutrients, the removal of waste products, and the migration of other cell types (1).