Journal of Cellular Biochemistry 51:249–251 (1993)

VIEWPOINT

Advances in Cellular Construction

The discovery of each new biological tool invariably leads to new and greater insights and understanding of intracellular and molecular interactions. Few of us have ever considered gravity as a variable or the impact of gravity on cellular functions. It has only been in the last few decades that we have made the intellectual transition from a generalized model of a cell being a bag of water to our current understanding of a complex cytoskeleton with constituent cellular processes and adaptive capacities. Gravitational adaptive processes have not been considered for several centuries due to a lack of tools to examine hypotheses.

The National Aeronautics and Space Administration (NASA), through its efforts to simulate a microgravity environment, discovered several direct and indirect effects of gravity upon cellular function, structure, and organization [Goodwin et al., 1992, 1993]. Solid body rotation, as seen in the rotating wall vessel (RWV), randomizes the gravitational vector while supporting cellular co-location in three-dimensional space in a low shear, high mass transfer environment [Tsao et al., 1992]. In general, culture systems address a specific parameter (for example, shear) at the expense of mass transfer of nutrients and metabolic wastes, three-dimensionality, and/or co-location of dissimilar cell types. The RWV is the first reactor designed to simultaneously integrate co-location, shear, mass transfer, and three-dimensional growth without sacrificing any one parameter.

Based upon RWV findings, the generally accepted 5 dyne/cm^2 damage threshold [Cherry and Kwon, 1990] appears excessive and has impeded technical advances in tissue development [Tsao et al., 1992]. On a macro scale, this may intuitively be a reasonable conclusion to draw [Merchuk, 1991]; however, in retrospect, any shear damage integrated over several months imposes specific selection pressures on expanding mixed cell populations [Croughan et al., 1987]. In the context of economies of scale, any application of metabolic energy toward cellular repair results in a loss of metabolic resources for differentiation and replication. Consequently, there has been an inability to co-culture cells with dissimilar shear sensitivities and accompanying divergent metabolic demands, e.g., mix tumors and normal tissues.

The development of normal tissues is of paramount importance to all of the biological and medical sciences. Understanding the interactions of tissues, their growth and development, and the mechanisms by which aberrancies occur form the foundation for improved quality of life. Despite the great medical utility for cultured human tissues and the numerous decades that have been devoted to tissue culture, for the most part, success has been extremely limited. These limits center around a lack of understanding of the basics involved in cell-to-cell interactions, structural support and its influence on architecture, and the importance and roles of various growth factors. The common denominator impeding cell culture progress is the lack of a supportive culture system. For normal human tissue to develop, there are three requirements:

1. Quiescent co-location of dissimilar cell types for synergistic cell-to-cell interactions that lead to the exchange of growth factors and to establishing cell-to-cell linkages.
2. Mass transfer rates that accommodate molecular scaffolding, which will facilitate mechanical stability and the consequential histological changes.
3. A micro-environment that includes various growth factors required for development and maturation.

The RWV enables all three events in a single environment. Whereas gas sparging provides excellent mass transfer of oxygen, the tradeoff is high shear forces and cellular damage at the gas/air interface [Lee et al., 1992]. Alternately, a hollow fiber reactor is characterized by excellent mass transfer rates and low shear; however, it lacks a spatial environment for three-dimensional growth of tissues, and mass transfer disrupts important local autocrine and paracrine

Received September 25, 1992; accepted October 15, 1992.

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milieus [Prewett et al., 1993; Schwarz et al., 1992]. The impeller-stirred bioreactor achieves a balance between shear forces, mass transfer of nutrients and removal of metabolic wastes, and three-dimensional growth [Cherry and Huller, 1992; Glacken et al., 1983]. However, balance occurs at the extremes of each variable. These deficiencies are well characterized and include high shear at impeller surfaces, high shear gradients at the liquid-to-wall interface, and disruptive eddies. The net accumulation of destructive shear forces inherent in impeller devices has precluded their use in tissue production. However, impeller-stirred bioreactors have become the mainstay for shear resistant suspension cell protein production.

In general, tissue research has utilized three conventional technologies. T-flasks allow for the layering of cells to obtain multicellular microscopic tissue-like architectures leading to numerous important insights into structure and function. Methyl cellulose and similar gel matrices have improved our ability to culture microscopic, three-dimensional tissues by augmenting the media viscosity, thereby permitting three-dimensional growth [Rutzky et al., 1979]. This technology imposes size restrictions due to limitations of diffusional mass transfer. Commercial interests have extended the three-dimensional concept by suspending a scaffolding in media and facilitating three-dimensional growth from biosynthetic materials. Improvement in de novo matrix production on biomaterials has led to sheets of maturing tissue and has paved the way for tissue transplantation. Although severely limited in structural integrity, these advances have rejuvenated the tissue culture field. Other systems of encapsulation, concentric cages, induced fluid dynamic streamlines, and synthetic growth matrices all address a specific niche at the expense of other requirements.

For the first time, we have a well-characterized quiescent cell culture environment (RWV) that alleviates shear without obvious mass transfer tradeoffs [Shi et al., 1992; Cherry and Papoutsakis, 1988]. The environment encourages colocation of cells, production of matrix, and tissue differentiation, and appears to have a reduced dependency on supplemental growth factors. Although gravity limits the growth of delicate tissues to 2–6 mm in diameter, resultant tissues are high-fidelity and free from confounding physiological and biological processes. Use of a defined culture medium removes most of the signal-to-noise ratio associated with serum proteins and physiological adaptation. The process paves the way for viral investigations and the use of various therapeutics to prevent introduction, growth, and release of virus.

Tissue models developed in the RWV are high-fidelity regardless of the tissue source: normal human primaries or cancerous. Resultant models have been utilized in oncogene product research and Phase I clinical trials, while normal tissues are being sought for transplantation and viral studies. Although this technology has focused on human applications, it is assumed that animal models and insect tissue for recombinant protein production will do equally well.

Until now, unit gravity has constrained us to low-fidelity tissue models and disruptive culture environments. With the newly acquired tools and models, we will further our understanding of cellular growth function and differentiation under the complex conditions associated with matrix formation and cell-to-cell interactions.

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