Cultrex® 3-D Culture Spheroid Assays Recreate Tumor Physiology

Current *in vitro* methods are inaccurate for predicting cancer cell response because they lack *in vivo* tumor physiology. Multicellular tumor spheroids (MCTS) exhibit structural and functional properties of tumors, so they provide more predictive models for assessing tumor response. Cultrex 3-D Spheroid Assays provide reproducible and quantitative 96 well formats for generating MCTS and evaluating their physiological functions. The Cultrex 3-D Spheroid Proliferation/Viability Assays are available in colorimetric or fluorometric configurations for analysis of *in vitro* tumor growth and survival. The Cultrex 3-D Spheroid BME Cell Invasion Assay recapitulates early stages of metastasis by embedding spheroids in an extracellular matrix hydrogel and evaluating invasion of cells out of the spheroid and into the surrounding matrix. The Cultrex 3-D Spheroid Assays provide more physiological systems for evaluating tumor cell behavior.

To improve existing cancer models and decrease the time and cost associated with drug development, there is an immediate need for *in vitro*, cell-based assays that incorporate tissue-specific structure and function. Many of the *in vitro* assays currently in use do not accurately represent the disease of interest because they lack either a reproducible format and/or physiological context for evaluating tumor cells *in vitro*. The most commonly used *in vitro* method for drug screening and pathway analysis entails culturing cancer cells on rigid, tissue culture treated plastic surfaces where the cells adhere non-specifically, growing as a monolayer (Figure 1A), and as a result, these cells lose both structural and functional properties associated with tumors *in vivo*. Cancer cells may be suspended in a physiological environment composed of extracellular matrix (ECM) hydrogels to generate a disperse population of small invading structures (Figure 1B), representative of metastatic seeding events; however, these structures lack uniformity and do not develop physiological characteristics of maturing tumors [1]. By controlling cell adhesion properties and promoting cellular aggregation, cells spontaneously assemble into spheroids (Figure 1C) that exhibit both structural and functional properties of tumors [2]. These multicellular tumor spheroids (MCTS) may then be embedded in an ECM hydrogel to evaluate their invasive potential (Figure 1D). Thus, MCTS and ECM may be used in concert to better predict the physiological tumor response, providing a solution to the current conundrum observed in basic research and drug development.

The use of MCTS as tumor models is not a new concept; in fact, cancer researchers have been utilizing spheroid cultures for over 40 years [3, 4]. However, many cancer models were previously thought to be incompatible in MCTS cultures since they do not spontaneously assemble into compact spheroids. Recent studies indicate that the cells need the appropriate ECM to modulate this process [5]. Trevigen has developed an optimized system, providing the necessary reagents to evaluate tumor forming cells using this method. Simply harvest cells, resuspend in spheroid formation ECM, and then culture in a 96 well spheroid formation plate. Spheroids generally form in 48 to 72 hours. Cell number determines spheroid size, and the size is important for creating physiological gradients (Figures 1F through 1H). For most tumor models, a spheroid diameter between 400 – 500 µm is recommended. This is sufficient to establish physiological gradients for nutrients, oxygen, pH, and catabolites due to limitations in diffusion through multicellular layers [1, 6, 7]. Another effect of these gradients is the establishment of heterogeneous cell populations with necrotic cells in the core, quiescent cells in the deeper layers, and proliferating cells on the spheroid surface (Figure 1H). Once assembled, these MCTS exhibit structural and functional properties for cancer cells *in vivo* and provide a physiologically predictive model of an avascular tumor.
The Cultrex 3-D Spheroid Proliferation/Viability Assays can be readily utilized as a tool to evaluate tumor growth and survival. Since each well produces one spheroid, researchers have complete control over spheroid size with virtually no well to well variability (Figure 2A). Once formed, these multicellular tumor cell aggregates can be treated with pharmacological compounds to evaluate the effect on tumor spheroid growth (Figure 2B); alternatively, specific genes or pathways may be altered to evaluate its effect on expansion of the in vitro tumor [8]. The growing spheroid can be monitored in real-time and label-free using image analysis software to measure spheroid area. At the same time, cell number may be quantitated at an end point using biochemical cell viability reagents and a multiwell plate reader. The colorimetric kit utilizes dimethyl thiazolyl diphenyl tetrazolium salt (MTT), while the fluorometric kit uses Resazurin (Figure 2B). Both substrates are reduced by metabolically active cells, in part by the action of dehydrogenase enzymes; however, MTT is converted from a yellow tetrazolium to a purple formazan for colorimetric assessment [9], while Resazurin is metabolized to Resorufin for fluorescence analysis [10]. Both methods are useful for determining changes in cell numbers for the MCTS model.

The Cultrex 3-D Spheroid BME Invasion Assay provides a system to evaluate processes and modulators of cancer cell dissemination from the tumor, modeling early metastatic events. Using the same ECM-modulated process for spheroid formation, an invasion matrix comprised of basement membrane proteins and collagen I is applied to the tumor spheroid, fully embedding it [5]. The invasion matrix forms a hydrogel network on which the cells can invade out of the tumor spheroid and into the surrounding matrix in a time-dependent manner which can be quantitated by measuring the increase in spheroid size as a consequence of invasion area (Figure 3A). Again, the impact of pharmacological compounds (Figure 3B) and genetic manipulation can be evaluated to determine the functional impact on this process.

In conclusion, there is an urgent need for in vitro tumor models that provide both a physiological context and reproducible format for assessing tumor cell functions. Whether screening anti-cancer compounds or performing pathway analysis, the Cultrex 3-D Spheroid Proliferation/Viability Assays and the Cultrex 3-D Spheroid BME Invasion Assay provide easy, quantitative, high-throughput tools for cancer research.

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Figure 1. The evolution of *in vitro* cancer cell culture models. Cell culture of MDA-MB-231 cells in: A) 2-D tissue culture treated plastic, B) 3-D Basement Membrane Extract (BME) hydrogel, C) Low adhesion spheroid culture in the presence of extracellular matrix, and D) Spheroid culture is embedded in and invades into a surrounding hydrogel. MDA-MB-231 cells were seeded at: E) 137 cells/well, F) 412 cells/well, G) 1,235 cells/well, H) or 3,704 cells/well. Increasing the cell number resulted in an increase in spheroid size and created concentric, heterogeneous cell populations. The outer cell layers are viable by calcein staining (green) while the cells within the core are dead by ethidium bromide (red). Scale bar = 500 µm.
Figure 2. 3-D culture proliferation of MDA-MB-231 breast cancer spheroids. A) Increasing the seeding density of MDA-MB-231 spheroids (cells/well) yields an increasing the metabolism of non-fluorescent Resazurin to fluorescent Resorufin, allowing quantitation of cell number by fluorescence intensity. B) Dose-response of MDA-MB-231 spheroids (3,000 cells/well) to Etoposide after a 96 hour treatment period.
Figure 3. 3-D culture cell invasion of MDA-MB-231 breast cancer spheroids. A) MDA-MB-231 cells invade out of the spheroid (3,000 cells/well) and into the surrounding matrix in a time-dependent manner over a 96 hour period. B) Sulforaphane inhibits spheroid invasion (area) of MDA-MB-231 spheroids (3,000 cells/well) in a dose-dependent manner after a 96 hour invasion and treatment period. Scale bar = 500 µm.
References