Novel Modular Plasticware for Three-Dimensional (3-D) Cell Culture

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Statement of Purpose: The paradigm shift from two-dimensional (2-D) to 3-D cell culture techniques[1] has continued its rapid growth (Fig. 1). In otherwise similar experimental conditions, a shift to 3-D from monolayer cell culture affects cell function and behavior greatly including nuclear structure, signal transduction and gene expression. 3-D culture conditions are associated with development into tissue-like structures, more similar to those formed in living organisms and better able to reproduce in vivo-like responses[2]. However, the majority of related research and commercial activity has centered around novel 3-D scaffolds utilized in 2-D plasticware. A novel 3-D plasticware design was investigated for its ability to accommodate the spectrum of 3-D matrix materials and configurations, long term cell viability, usefulness for imaging analysis, and suitability for co-culture of multiple cell types.

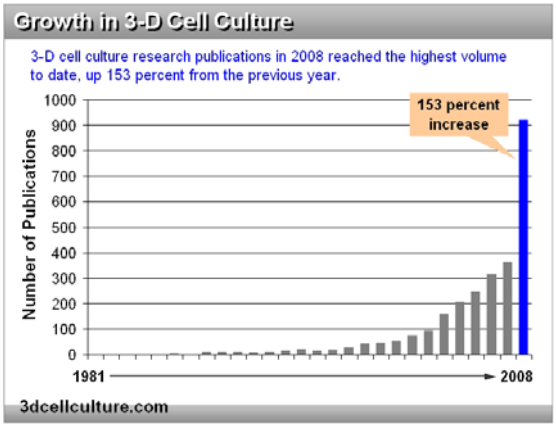

Figure 1: Dramatic increase in publications featuring cells cultured in 3-D

Methods: Custom 3-D modular plasticware was initially fabricated from polycarbonate and later injection molded from tissue culture polystyrene. Two modules were coupled to form the functional 2-chamber unit. Solid gasket sheets or semi-permeable membranes (to achieve single cell type or co-culture experiments respectively) between modular units were utilized to achieve leak-free culture. Plasticware and associated membranes and gaskets were sterilized by ethylene oxide. A variety of scaffold materials were tested over the course of multiple experiments, including PLL beads (mean diameter 800 μm), degradable porous polymer/ceramic composites, and crosslinked alginate hydrogel prepared both as beads (mean diameter 1-2 mm) as well as *in situ* in the 3-D chamber. Multiple cell types were utilized over the course of the trials, including murine 3T3 fibroblasts and D1 adult bone marrow stromal cells (both from ATCC, Manassas, VA), the latter of which was concurrently differentiated down both the osteogenic and chondrogenic pathways. Cells were maintained using a perfusion flow circuit (Figure 2). Experiments were conducted for various durations to a maximum of 28 days. Analysis methods included assessment of cellular activity via glucose consumption and lactic acid production, AlamarBlue™ Cell Viability assays, LIVE/DEAD® Viability/Cytotoxicity staining, and fluorescence microscopy utilizing a Zeiss LSM510 Laser Confocal Microscope.

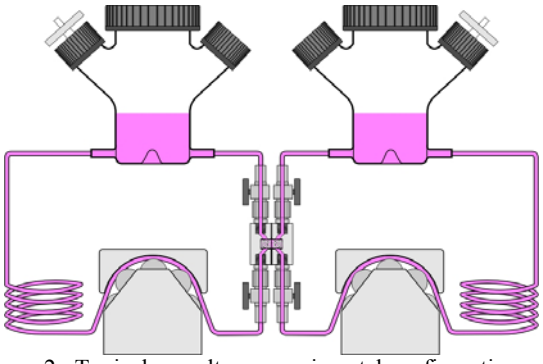

Figure 2: Typical co-culture experimental configuration with coupled modular 3-D cell culture chambers and parallel flow circuits permitting phenotype-specific supplemented medium.

Results: The plasticware successfully accommodated the various forms (solid, hydrogel, beads) of 3-D scaffolds of various materials as desired for cell type selection. The various cell-scaffold combinations tested were shown to exhibit excellent viability via AlamarBlue™, glucose consumption and lactic acid production to 28 days. Special attention was paid to viability at the interior of the cell-scaffold constructs, a significant concern for 3-D cell culture, which was demonstrated using 3-D confocal microscopy (Figure 3).

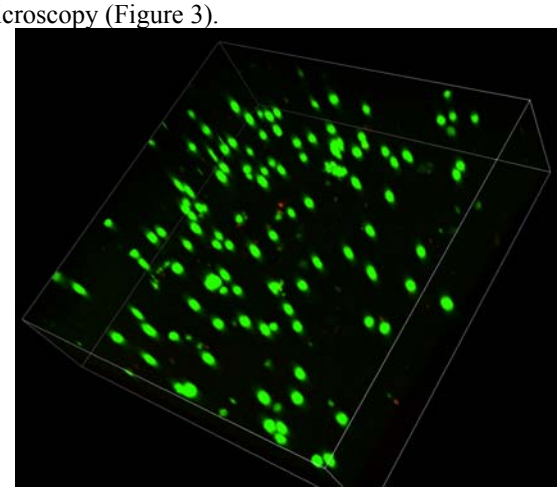

Figure 3: 3-D confocal LIVE/DEAD® analysis of 3T3 fibroblasts showing high viability at scaffold interior

Conclusions: The novel modular plasticware design was demonstrated to be a potentially universal platform for 3-D cell culture, adaptable to each scaffold material and cell type as well as segregated co-culture experiments.

References: [1]Prestwich GD. J Cell Biochem 2007 (101:1370–1383). [2]Mazzoleni G. Genes Nutr 2009 (4:13–22).