# Engineered Polystyrene Scaffolds For *In Vitro* Three-Dimensional Disease Models

Bergenstock, M.K.[1], Lau, W.[1], Sun, W.[2], Liu, Q[1].
[1] 3D Biotek, LLC, 675 US Highway One, North Brunswick, NJ 08902, USA
[2] Mechanical Engineering, Drexel University, PA 19104, USA

**Statement of Purpose:** Emerging technologies in tissue engineering can now be used to create diseased tissue or organ models for therapeutic, drug screening, and disease biology studies. There is much focus on developing *in vitro* disease models as current two-dimensional (2D) *in vitro* systems do not mimic *in vivo* environments and ultimately fall short in predicting *in vivo* cell behavior. Major shortcomings of 2D cell systems are altered cell morphology and an inaccurate representation of the dynamic 3D cellular environment experienced by cells *in vivo*. To this end, we have engineered a novel transparent 3D polystyrene (PS) scaffold to solve this problem. We hypothesize that these PS scaffolds can be used to recreate a superior 3D *in vitro* model for pathogenesis research and drug discovery.

**Methods:** Porous PS scaffolds were engineered using 3D Biotek's Precision Micro-fabrication Technology (Fig. 1A-C). Uniquely, fiber diameter is controlled by nozzle diameter while spacing between fibers is controlled by a motion control system. The struts of each layer are oriented 90ºC relative to the struts of the layer immediate below. Before use, scaffolds are tissue culture surface treated and γ-radiation sterilized. This study implemented 96-well compatible 3D Insert™-PS scaffolds, 5 mm in diameter (Fig. 1D), with a configuration of 150 µm fiber diameter and 200 µm pore size (PS1520).

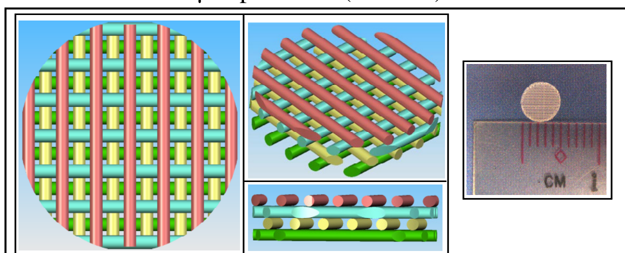


Figure 1. Four-layer structural design of PS scaffolds. Offset fibers allow each of the scaffold's four distinct layers to become visible when viewing with an inverted light microscope.

Human epithelial MCF-7 cells, HepG2 hepatocarcinoma cells, and normal HEKn cells were seeded in 2D, onto treated tissue culture plates (TCP) and in 3D (20 µl), into the porous PS scaffolds, according to 3D Biotek's static seeding protocol. Cells were seeded at concentrations of $1 \times 10^4$ cells/96-2D well/96-3D PS scaffold.

**Results:** Cell growth and morphology on 2D TCPs and on 3D Insert™-PS were monitored using an inverted light and fluorescent microscope. Compared with cells cultured in 2D (Fig. 2A), which grew in a characteristic monolayer, cells grown on 3D PS scaffolds (Fig. B-C) formed aggregates and rounded cell structures within the scaffold's 3D environment. Results revealed that MCF-7, HepG2, and HEKn cells grown in 3D Insert™-PS scaffolds have superior cell metabolic activity compared with these cells grown on 2D TCPs (Fig. 3A).

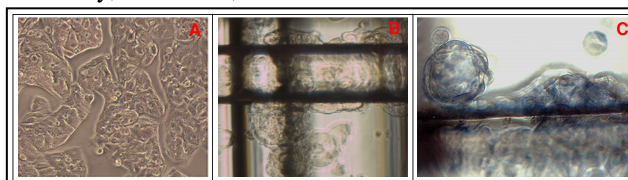


Figure 2. MCF-7 2D and 3D cell morphology. 100X (A-B), 200X (C).

Furthermore, after treatment with chemotherapeutic agents, such as tamoxifen (MCF-7) and methotrexate (HepG-2), the cytotoxic response was lower in cells cultured in 3D compared with those on 2D TCPs during the entire time-course (Fig. 3B).

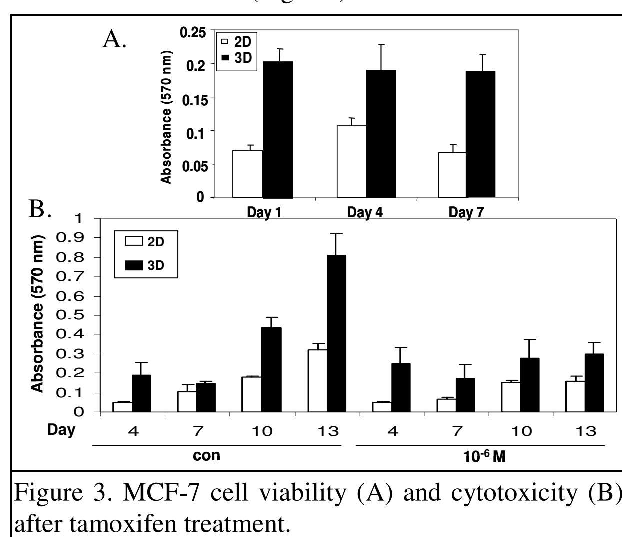


Figure 3. MCF-7 cell viability (A) and cytotoxicity (B) after tamoxifen treatment.

**Conclusions:** This study demonstrates that novel 3D Insert™-PS scaffolds can be used to create superior *in vitro* 3D tissue and disease models. Cells cultured on these PS scaffolds exhibit a unique morphology that is not found in 2D monolayer culture. Furthermore, MCF-7, HepG2, and HEKn cells exhibit greater proliferation, cell viability, and an increased resistance to cytotoxic effects of drugs compared with cells cultured on traditional 2D TCPs. Moreover, using 3D Insert™-PS scaffolds in pathogenesis studies can more effectively recreate an *in vivo* microenvironment and imitate a cell or tissue's true physiological response. We have successfully engineered a biologically relevant *in vitro* model system that can improve drug discovery success rates to combat rising rates of cancer and other diseases. This precise and reproducible *in vitro* model will accelerate the drug discovery process and significantly reduce development costs.

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