A Novel *In Vitro* Three-Dimensional Breast Cancer Model Using Polystyrene Scaffolds

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Abstract

Tissue engineering can be used to create a diseased tissue or organ model for therapeutic, drug screening and disease biology studies. There is much focus on developing *in vitro* tumor models as current two-dimensional (2D) *in vitro* systems do not mimic *in vivo* tumor environments. We hypothesize that transparent three-dimensional (3D) polystyrene (PS) scaffolds can be used to create a superior 3D *in vitro* model for tumor studies and drug discovery. In this study, we cultured MCF-7 breast cancer cells on both 2D PS-tissue culture plates (TCP) and 3D PS scaffolds (3D Insert™-PS). We compared 2D versus 3D MCF-7 cell growth and the susceptibility of these cancer cells to anticancer drugs. MCF-7 cells cultured on 3D PS scaffolds formed cell aggregates that could be found along scaffold fibers and within pores, whereas cells cultured on 2D PS-TCPs grew in a characteristic flat monolayer. Alamar blue and MTT assays demonstrate that MCF-7 cells cultured on 3D scaffolds have significantly enhanced cell proliferation and metabolic activity at all time points during a 14 day time-course when compared with cells cultured on 2D PS-TCPs. After tamoxifen treatment, cytotoxic response was significantly lower at days 4 and 7 in MCF-7 cells cultured on 3D scaffolds compared with cells cultured in 2D. These data demonstrate that cancer cells cultured on 3D PS scaffolds are more robust and resistant to anticancer drug treatments than cells cultured on traditional 2D TCPs. Therefore, 3D PS scaffolds offer a superior 3D cell culture environment for drug toxicity and cancer research.

Materials and Methods

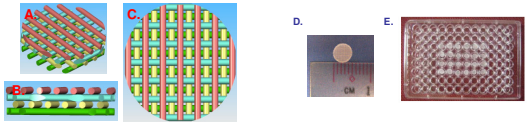
Cells

MCF-7 cells were purchased from ATCC (Manassas, VA) and MCF-7:WS8 cells were a generous gift from V. C. Jordan. MCF-7 cells were cultured in Minimum Essential Medium (Gibco) containing 10% fetal bovine serum (FBS), 2 mM L-glutamine, 10 mM NEAA, 1 mM sodium pyruvate, 100 IU/ml Pen-Strep, and 0.01 mg/ml bovine insulin (Sigma Aldrich). MCF-7:WS8 cells were maintained in ***Yellow Media***: phenol red-free RPMI (Gibco) containing 10% 4X Dextran Coated Charcoal-Stripped FBS (Gibco), 1 mM sodium pyruvate, 10 mM Non-Essential amino acids, 100 IU/ml Pen-Strep. Twenty-four hours before 17β-Estradiol (Sigma) and ICI 182,780 (FUL) (Tocris) treatments, media was changed to ***Red Media***: RPMI (Gibco) containing 10% FBS 1 mM sodium pyruvate, 10 mM Non-Essential amino acids, 100 IU/ml Pen-Strep. MCF-7 and MCF-7:WS8 cultures were maintained in 5% CO_2 at 37ºC.

Polystyrene (PS) Scaffold Microfabrication (3D Insert™-PS)

Porous polystyrene scaffolds **(Figure 1)** were engineered using 3D Biotek's Precision 3D Micro-fabrication Technology. **Figure 1A-C** represents the four-layer structural design of 3D Insert™-PS scaffolds. Offset fibers make each of the PS scaffold's four distinct layers visible when viewing with an inverted light microscope. Uniquely, fiber diameter is controlled by nozzle diameter and spacing between fibers is controlled by a motion control system. This study utilized 96-well compatible PS scaffolds, 5.0 mm in diameter **(D)**, with 150 µm fiber diameter and 200 µm pore size configuration (PS1520) that fit into a standard 96-well tissue culture plate **(E)**. The total cell growth area of a 96-well 3D Insert™-PS1520 is 1.06 cm^2 compared with 0.32 cm^2 of total cell growth area in a 96-well 2D TCP. Before use, PS scaffolds are plasma treated and terminally sterilized by γ-radiation.

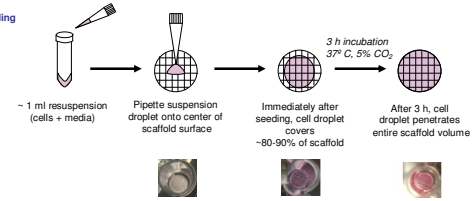
Figure 1. 3D 96 well PS scaffold



Cell Seeding and Culture

2D and 3D cell culture was carried out in treated and non-treated tissue culture plates, respectively. Cells were resuspended in 1 ml growth media before using $1x10^4$ cells/well in 200 µl (2D) and $1x10^4$ cells/scaffold in 20 µl (3D). To seed each scaffold, 20 µl of the cell suspension was slowly pipetted onto the top surface of each 3D Insert™-PS. To ensure high seeding efficiency, the cell suspension droplet was not allowed to contact the sides of the wells. After a 3 h incubation in 5% CO_2 at 37ºC, 180 µl of media was added to the 3D wells. After 24 h, scaffolds were gently transferred into new wells of a non-treated tissue culture plate, and fresh media was added. Media was changed every 48 h in 2D and 3D cultures until use **(Figure 2)**.

Figure 2. Cell Seeding



Cell Imaging

MCF-7 cell growth and morphology on 2D TCPs and on 3D Insert™-PS was monitored using an inverted light microscope.

Cell Proliferation

Alamar blue (Invitrogen) and 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assays (Cayman Chemical) were performed according to manufacturer's instructions with MCF-7 cells in 2D TCPs and on 3D Insert™-PS on the indicated days. DNA assays were performed on Day 7 with MCF-7:WS8 cells in 2D TCPs and on 3D Insert™-PS. Twenty-four hours before E2 (1 nM) and FUL (1 µM) treatments, all MCF-7:WS8 cell culture media was changed to ***red media***.

Cytotoxicity Assays

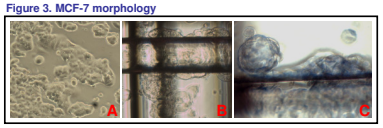
Tamoxifen (TAM) (Sigma, H7904) treatment began 24 h after 2D and 3D cell seeding. Cytotoxicity was measured at pre-determined days during the TAM treatment time-course by MTT and Lactase Dehydrogenase Activity assays (LDH) (Cayman Chemical) according to manufacturer's indications.

Results

Cell Morphology

MCF-7 cells growing in 2D TCPs and on 3D PS scaffolds were imaged using an inverted light microscope **(Figure 3)**. Compared to cells cultured in 2D **(A)**, which grew in a characteristic monolayer, cells grown on 3D PS scaffolds **(B-C)** formed aggregates **(B)** and rounded cell structures **(C)** within the scaffold's 3D environment. 100X **(A-B)**, 200X **(C)**.

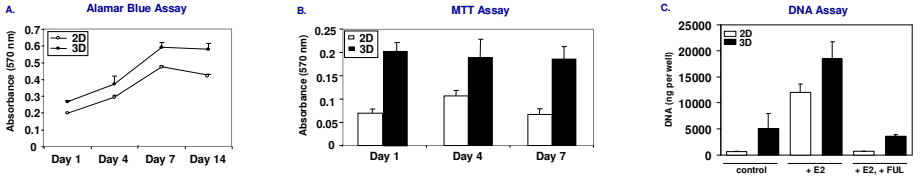
Figure 3. MCF-7 morphology



Cell Viability

MCF-7 cells were seeded at concentrations of $1x10^4$ cells/96-2D well/96-3D PS scaffold. At various time-points, cell proliferation assays demonstrated that MCF-7 cultured on 3D Insert™-PS scaffolds are viable **(Figure 4)**. At all time points during the proliferation study, MCF-7 cells cultured on 3D scaffolds have increased proliferation **(A)** and enhanced metabolic activity **(B)** as compared with MCF-7 cells cultured in 2D TCPs. Furthermore, MCF-7 cells cultured on 3D scaffolds have increased proliferation after 7 days of E2 and E2 + FUL combination treatments, compared with MCF-7 cells cultured in 2D TCPs **(C)**.

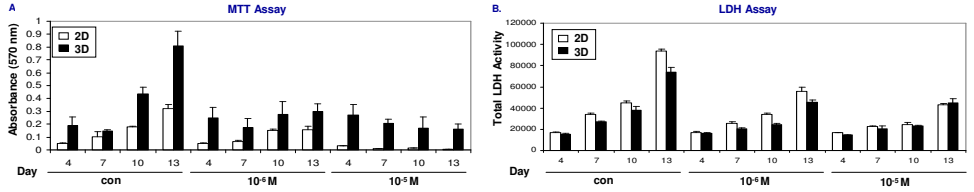
Figure 4. MCF-7 viability



Cytotoxicity

Twenty-four hours after initial cell seeding at concentrations of $1x10^4$ cells/96-2D well/96-3D PS scaffold, MCF-7 media was changed to contain 10^{-6} M and 10^{-5} M tamoxifen. Tamoxifen, a chemotherapeutic agent, was used to compare the cytotoxic response of MCF-7 cells cultured in 2D TCPs with MCF-7 cells cultured on 3D PS scaffolds **(Figure 5)**. Fresh media, containing 10^{-6} M and 10^{-5} M tamoxifen was added every 48 h until assay. MTT **(A)** and LDH **(B)** assays were performed at various days throughout the tamoxifen treatment time-course. In response to tamoxifen treatment, 3D cell cultures show less cytotoxicity when compared with 2D cell cultures. After 10^{-5} M tamoxifen treatment, MCF-7 cells cultured on 3D PS scaffolds have comparable LDH activities **(B)** but greater metabolic activity **(A)** compared with MCF-7 cells cultured in 2D TCPs. This suggests that tamoxifen may significantly inhibit MCF-7 growth in 2D while only slightly retarding MCF-7 growth on 3D PS scaffolds, resulting in greater 3D proliferation and therefore more LDH production in these cultures.

Figure 5. MCF-7 cytotoxicity



Conclusion

This study demonstrates that novel 3D Insert™-PS scaffolds can be used to create superior *in vitro* tumor models. Cells cultured on these PS scaffolds exhibit a unique morphology as well as differentiate into 3D structures that are not formed in 2D monolayer cultures. Furthermore, MCF-7 cells cultured on 3D Insert™-PS have greater proliferation, cell viability, and an increased resistance to cytotoxic effects than MCF-7 cells on 2D TCPs. Taken together, these data suggest that using 3D Insert™-PS scaffolds in pathogenesis studies may more effectively recreate an *in vivo* microenvironment and imitate a tumor's true physiological response. In conclusion, 3D Insert™-PS provides a model system for a better understanding of cancer cell organization, proliferation, and the evaluation of different anticancer drugs.

Acknowledgements

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