# Effects of Exemestane on Two-Dimensional and Three-Dimensional T47D Breast Cancer Cell Cultures

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## Abstract

These studies examined the T47D human ductal breast epithelial tumor cell line in two-dimensional (2D) and three-dimensional (3D) c

## Introduction

Two-dimensional cell culturing on flat plastic surfaces is the current standard technique for cell culturing, but presents many limitations when executing cellular research. Three-dimensional cell culturing in a medium that allows cells to produce floating cultures provides a platform which better emulates *in vivo* environments. This system can be provided by culturing the cells in a collagen matrix, which mimics the extracellular matrix and is therefore closer to natural cellular conditions. These conditions give researchers the ability to study patterns of gene expression and other biological activities that better mimic the natural environment in living organisms.[1]

The T47D human ductal breast epithelial tumor cell line expresses nuclear estrogen receptors, which are required by the cell to activate certain genes essential for cell growth and replication.[2] Estrogens are a class of sex hormones consisting of estradiol, estriol, and estrone. These hormones have the ability to cross cell membranes, giving them the capability to diffuse directly into the nucleus. Once estrogens enter the nucleus, they bind to the substrate-binding domain of the estrogen receptor causing the formation of a receptor dimer. The DNA-binding domain of the receptor then binds to specific sites on DNA and up- or down-regulates gene expression depending on the role of the transcription factor binding site.[3]

Aromatase is a cytochrome P450 enzyme responsible for the aromatization of estrogens from androgens. It is localized in the endoplasmic reticulum and consists of

This reaction causes aromatase to denature and degrade due to exemestane analogous structure to the natural substrate androstenedione.[6] Exemestane is produced under the trade name AROMASIN® by Pfizer Inc.

The purpose of this research was to compare the structure, the growth, and the inhibition of T47D cells in two-dimensional and three-dimensional cell cultures

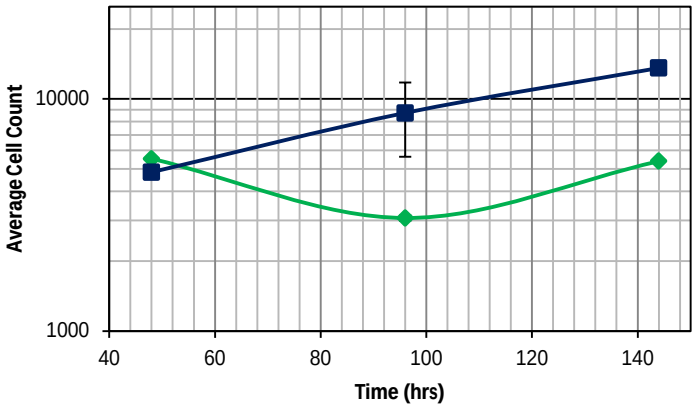
## Materials and Methods

**T47D cell culturing**: Two-dimensional cultures were grown as monolayers on flat plastic surfaces. Three-dimensional cultures were grown as floating cultures in 24-well plates by utilizing 1 ml of suspended cells in growth medium and 0.5 ml of 10% collagen gel per well. The 10% collagen gel was produced by mixing type A gelatin (from porcine skin) in deionized water. The mixture was then autoclaved at 121 °C at 15 psi for 15 minutes to ensure sterility. The collagen was then aliquoted into 24-well plates, wrapped with Parafilm, and stored at 4 (. Both cultures utilized Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12) with 10% fetal bovine serum (FBS), 1 mM sodium pyruvate, 2 mM L-glutamine, 100 U penicillin, and 0.1 mg/ml streptomycin growth medium.

**Microscopy**: An inverted microscope was utilized to view and photograph cells at 40x, 100x, and 200x. Photos of the cells were obtained with an OptixCam Summit Series: OCS-1.3 MP Microscope Camera utilizing OptixCam OCview version 7.1 software on a laptop computer. ImageJ program was utilized to create three-dimensional surface plots from stacks of photos at slightly different focal planes.

**Growth curve**: T47D cells were cultured in a 24-well plate, which contained collagen gel and non-collagen wells. After 48, 96, and 144 hours of incubation, cells were removed from the wells and a Coulter Z1 particle counter was utilized to quantitate the cells present.

**Exemestane Dose Response Curve**: T47D cells were cultured in either 48- or 24-well plates. Once the cells had grown to 50% confluence, the media


Average Cell Count
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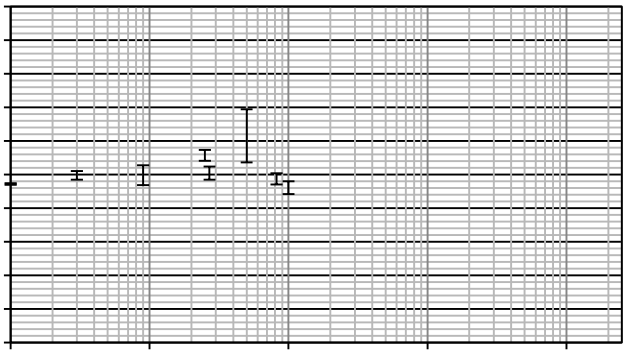

6. Geisler J, King N, Anker G, Ornati G, Di Salle E, Lønning PE, Dowsett M. “In vivo inhibition of aromatization by exemestane, a novel irreversible aromatase inhibitor, in postmenopausal breast5(e)-