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Development in Inexpensive Method for Three Dimensional (3D)- An Approach for Cancer Studies

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Abstract: ***Cell culture is an imperative tool for biological research. Two-dimensional cell culture has been used traditionally, but growing cells on ECM plastic surfaces does not precisely model the in vivo condition. As compared with two-dimensional studies, the three-dimensional (3D) cell culture allows biological cells to interact with their surroundings medium in all three dimensions. Three-dimensional (3D) cell culture models are becoming progressively widespread in existing cancer research anddrug resistance studies. 3D cell culture compensates in providing more physiologically relevant information and most predictive data for in vivo trials. The modernizations and development in 3D culture systems for cellular response to different anticancer drugs.***

Keywords: cell culture; three-dimensional cell culture; *in vivo,* ImageJ

1. Introduction

Cancer remains one of the leadingdestroyers in the world. Every year, 7.5 million patients succumb to it. Besides loss of human lifespan, in that location is a strangulating economic burden associated with this disease for every patient, residential district and nation.With the increasing pollution, indiscriminate use of pesticides and the ever increasing dominance of Pharmaceutical industries to prescribe drugs, the number of cancer patients and the related economic burden is expected to grow, unless some really different and effective schemes are developed.

Cancer studies depend on in-vivo models, but these have allied probability and ethical concerns (Knight *et al*., 2007 andWells *et al.,* 2011]. These faces have been overcome by using in vitro models that permit a controlled approach in looking into cancer cell biology. The enormous study of such models involves culturing cells on conventional two-dimensional (2D) plastic-ware in which cells acclimatize to the flat polystyrene substrate, flatten and grow as a monolayer. This does not mimic the environment that cells experience in vivo. The sound structure of cultured cells as monolayers is not realistic; cell-to-cell contact is defined and the microenvironment generated by the cells, via extracellular matrix (ECM) [Yamada, *et al.,* 2007].

Three-dimensional (3D) in vitro models are becoming a popular alternative to a bridge and bring down the gap between 2D culture assays and animal examples. 3D models represent a more practical approach enabling cells to retain their native 3D morphology, form communications with adjacent cells and create more complex structures. 3D model offers the ideal environment for cells to arise and function in 3D creating a more representative tissue model.

In our experiments we demonstrate the development of breast cancer cell line, MCF-7.A 3D model is produced, that closely resemble the structure of tumor tissues grown in vivo and the viability of these cells equivalent to conventional 2D cultures is measured. The data prove that when MCF 7 cells are cultured in 96 well plates and subjected to different discourses in both 2D and3D forms, comparatively better in vivo responses were observed in 3D culture.

2. Material and Method

The cell lines used in this study was breast cancer cell line MCF-7. This cell line was purchased from National Center for Cell Sciences (NCCS), Pune and maintained in MEM medium supplemented with 10% fetal bovine serum, 1% antibiotics and 5 % CO_2 respectively, at 37^0C in humidified air.

- Tissue culture plates
- Hemocytometer
- Phase-contrast microscope
- Camera
- CO_2incubator
- CO_2 supply
- Camera
- ImageJ analysis software

a) Experimental Design for 3D Model:

Coating of cell culture plate

1) Cell culture plates were first overlaid with 0.3% autoclaved Agarose containing plain MEM medium. (Pour 50-100 µL Agarose in each well).
2) After the medium has solidified, place 10,000 cells/ well with gentle rocking in Orbital Shaker for 3 hours in aseptic condition, this will allow the cells to get uniformly organized.
3) The plate was then placed in a CO_2 chamber with 5 % carbon dioxide for the next 24 hours for proper organization.
4) All 3D cell cultures were maintained for next 3 days and media was changed each day.
5) The samples were then imaged every day.

b) Analysis of Developmental stages of 3D model with ImageJ Software

1) Open ImageJ Software
2) Select File
3) Import images which were capture at different interval
4) Select rectangular selections and apply to images
5) Select analyze and select plot profile
6) The following result will display

3. Result

a) Developmental stages of 3D model

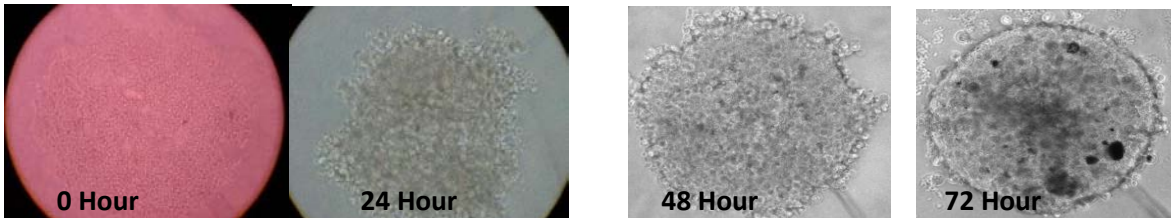

Figure 1: Developmental stages of 3D model (Images Resolution: Lens 20X and camera zoom 5X)

3D cell model development, shows that the cell get aggregated and they form periphery aggregation which was not found in 2D cell culture. The cell was developed upto 72 hours and that shows different morphological arrangement at different time interval (Figure 1).

b) Analysis of Developmental stages of 3D model with ImageJ Software

The images acquired after every day can be further examined by using computing software ImageJ. Displays a two-dimensional graph of the intensities of pixels along a line or rectangular selection. The X-axis represents distance along the line and the Y-axis is the pixel intensity.

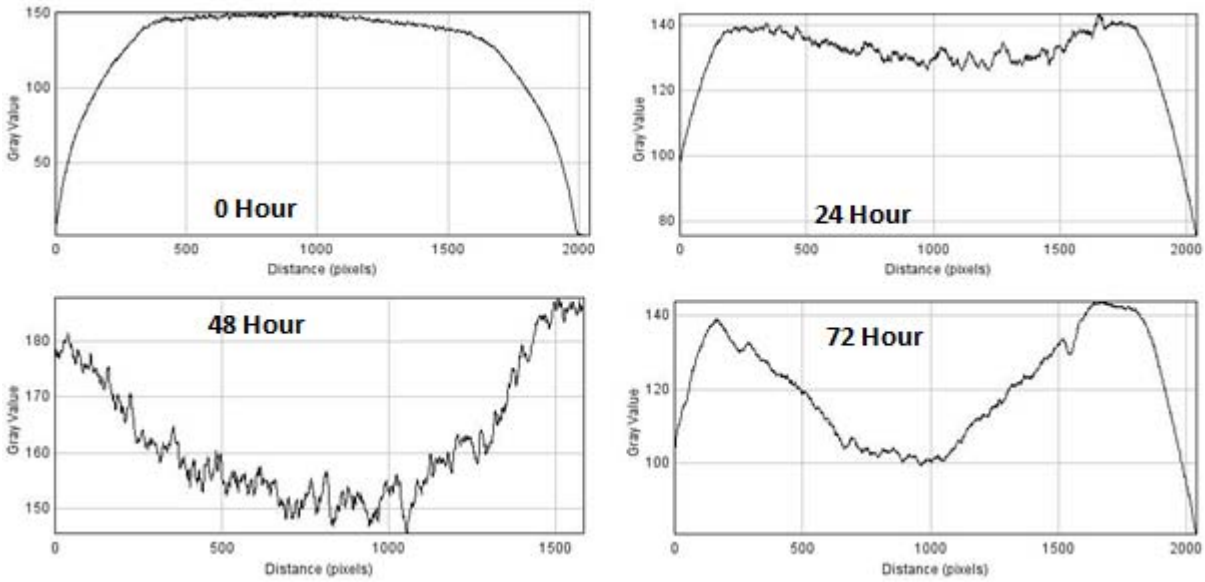

Figure 2: Plot profile by ImageJ software

From plot profile at 0 hours the cells were assemble at the centre of the well, but they are not attached one another. Gradually the time increasing at 24 hours the cells are grown and form clusters. At 48 hours they were started form peripheral margin, but at 72 hour scenario has been seen different, it was completely the margin were found. This will indicated that the cell are closely associated with each other and form Tumor like structure.

From the plot profile their gray values and distance in pixels shows the development 3D model of cancer cells (Figure 2)

4. Discussion and Conclusion

3D model study demonstrates that human cancer cells provides a realistic method that is a step closer to replicating the in vivo-like conditions of tumor growth. As also the results of analysis of developmental stages of 3D model with ImageJ Software supports our view that these 3D cultures could generate valuable data concerning the development of 3D model. If compared to conventional 2D method, this 3D approach provides new insights to study the effectiveness and mode of action of upcoming cancer studies.

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