Cost-effective Techniques for 3D Culture of Heterotypic Tumor Models

Mohammad A. Akbarsha[1,2], Perumalsamy Balaji[3,4], Sellathamby Shanmugaapriya[4], Lauri Paasonen[5]

1. National College (Autonomous), Tiruchirappalli 620001, India
2. Mahatma Gandhi – Doerenkemp Center for Alternatives, Bharathidasan University, Tiruchirappalli 620024, India
3. National Center for Alternatives to Animal Experiments, Bharathidasan University, Tiruchirappalli 620024, India
4. Department of Biomedical Science, Bharathidasan University, Tiruchirappalli 620024, India
5. GrowDex® Biomedicals, UPM Tukholmankatu 8, Biomedicum, Helsinki, Finland

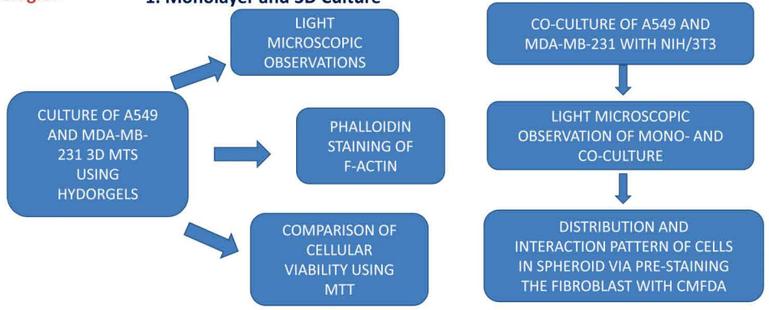
Introduction

- Culture of cells in 3D-format, more specifically as spheroids, would show structural similarities to tumors *in vivo* and thus they are useful as relevant models for tumor biology as well as toxicology
- Also, culture of fibroblast cells together with epithelial cells *in vitro* can not only provide a complex tumor model but also give deeper insights into cellular interactions and the phenotypes involved in tumor progression and / or toxic responses.
- One of the main requirements in generating such complex 3D spheroid models is the scaffold which supplies nutrients and mimics extracellular matrix (ECM) conditions. Invariably, the scaffold material is expensive.
- Thus, efforts are in progress to develop novel and particularly cost-effective scaffold materials for the culture of cells into spheroids

Objectives of the Study

- To develop nanofibrillar GrowDex and Egg White isolated from Hen's (*Gallus gallus domesticus*) egg as the scaffold to generate 3D multicellular tumor spheroids (MTS) of A549 and MDA-MB-231 cancer cells
- To develop heterotypic tumor spheroids by co-culturing epithelial cell types with NIH/3T3 fibroblast in GrowDex scaffold

Methodologies



Light Microscpic Observation

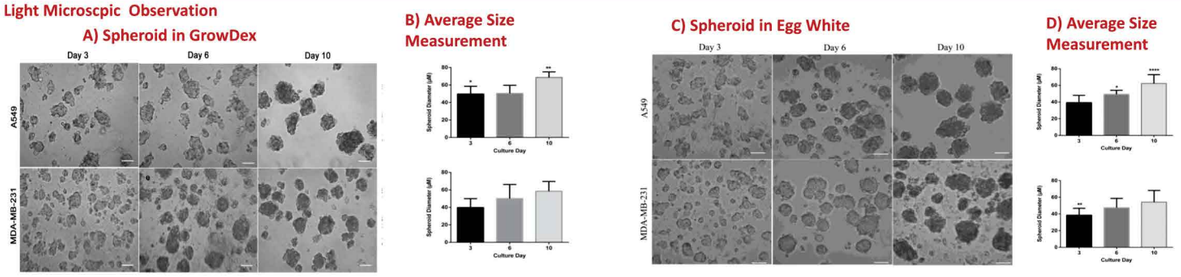
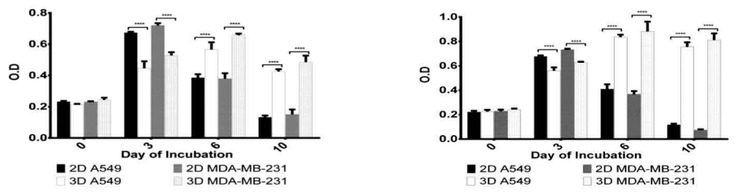


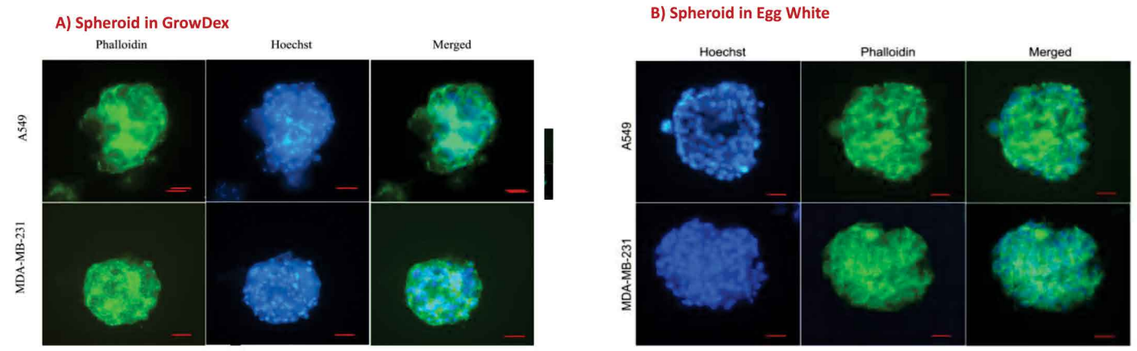
Figure A & C: The cells (A549 and MDA-MB-231) are generally polygonal or cuboidal in shape; but when cultured in GrowDex and egg white they formed into spheroids. The spheroids of A549 appeared less organized and grape-like whereas those of MDA-MB-231 appeared spherical and mass-type.
Figure B & D: The average diameter of the spheroids, assessed over 10 days of culture, revealed that diameter of the spheroids increased with increasing periods of incubation.

Comparison of Cellular Viability Using MTT



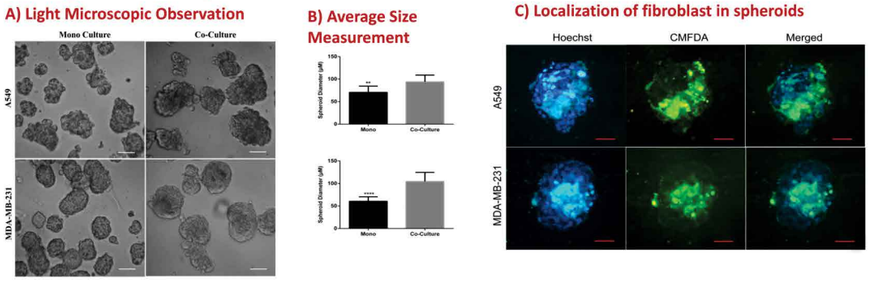
- The results indicated that GrowDex / egg white is not toxic to A549 and MDA-MB-231 cells; rather, it facilitates cell proliferation.
- The proliferation rate of cells in 2D culture was initially high, until day 3 and, thereafter, it decreased. But in 3D culture the proliferation rate increased gradually and the cell viability was also maintained, despite the cells having been continuously cultured for 10 days.

Phalloidin Staining of F-Actin



- Figure A&B: Fluorescence detection revealed that F-actin filaments were densely aggregated in the cortex and at the intercellular junctions in both the spheroids, A549 and MDA-MB-231. Further it substantiated that A549 spheroid appeared as grape-like whereas MDA-MB-231 spheroids appeared mass-type as revealed in light microscopic observation.

Heterotypic Co-culture using GrowDex



- **Figure A & B:** After 5 days of co-culture, light microscopic observation revealed that size of co-cultured A549 and MDA-MB-231 spheroids were increased 1.33- and 1.72- folds, respectively, when compared with their respective monoculture spheroids Interestingly a small dark core was observed in both the co-cultured spheroids, which was not seen in monoculture spheroids.
- **Figure C:** After 3 days of co-culture, it was clearly seen that the fibroblast cells fluoresced bright green, whereas all the cells were counterstained blue using Hoechst. The result further indicated that the fibroblast cells were mostly accumulated in the core region whereas fewer cells were located on the periphery

Summary and Conclusion

•The study concludes that the 3D spheroids of A549 and MDA-MB-231 cells, each separately, and each in combination with NIH/3T3 fibroblasts, were successfully developed using NFC GrowDex as well as egg white hydrogels.
•The nano-structure of these materials provides mechanical space (e.g., viscoelasticity) which is relevant to the ECM; supports the growth of cells; and helps to form spheroids without any additional ECM components.
•These kinds of materials can be used to produce complex *in vivo*-like co-culture models that incorporate multiple cell types as discussed elsewhere.

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