

# Three-Dimensional Culture Model of Tumoral Cells



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## Abstract

The mammospheres are a multicellular tumor spheroid model. These are a widely used culture system to mimic the three-dimensionality of tumors. In this manuscript, we narrate the development of a model using a murine mammary adenocarcinoma, which we used to study tumor physiology and therapeutic applications. We test three techniques published in previous works. The best condition was used V-shaped 96-well plates with an agarose monolayer, with this we were obtained compact and robust spheroids. After that, we studied the interaction between tumoral cells and mesenchymal stem cells (MSC) derived to human umbilical cord in three dimensional culture. Additionally, for evaluate spheroid's structure by optical microscopy we performed histological staining of the mammospheres with hematoxylin-eosin. Moreover, we made immunohistochemistry staining to evaluate cell proliferation and VEGF expression index, with Brdu and VEGF, respectively. We found that the presence of MSC could modify the mammospheres structure. We can conclude that mammospheres can be performed with more than one cell type similarly to what happens in a *in vivo* grafted tumour and this can be used to study the tumour physiology and therapeutic agents on solid tumors without the use of laboratory animals.

**Keywords:** Mammospheres; Murine mammary adenocarcinoma; Mesenchymal stem cells

**Abbreviations:** Brdu: Bromodeoxyuridine, MSC: Mesenchymal Stem Cells, VEGF: Vascular Endothelial Growth Factor

## Introduction

*in vitro* Solid tumors are formed by a mass of cancer cells in direct contact with stromal cells (e.g., fibroblasts, myofibroblasts, endothelial cells, pericytes, adipocytes and immune cells) surrounded by a complex extracellular matrix (CEM). The interaction of these cell populations influence the tumor growth, the resistance to death, the invasiveness and metastasis. In addition, the tumor growth and development needs adequate tissue perfusion to maintain a high metabolic rate and prevent hypoxic zones [1-3].

The study in solid tumors traditionally is developed in two ways:

- I. Model *in vitro*, a monolayer culture, which is maintained in a controlled environment to study the effect produced by modify a variable on cultured cells. The problem with this method is that does not allow us to understand the behaviour *in vivo*, because it does not have of the interaction with the cells of the CEM.
- II. Model *in vivo*, working with laboratory's animals, it has a complex microenvironmental and can be manipulated, but we can't control all cellular conditions. Further, this model is

more expensive and time-consuming than the first, and can require a large number of animals [4].

In our cell culture laboratory, we have a cell line called TN60, a murine mammary adenocarcinoma. These tumor cells (TC) are undifferentiated and have fast growth [2]. With these cells, we created the spheroids or three-dimensional cultures. These were called mammospheres and allow us to maintain good control of the system and, simultaneously, generate spheroid structures that resemble a solid tumor grafted into the animal [5,6].

How do you generate the mammospheres? We need to grow the TC on non-adherent or poor adherent surface to promote the interaction between cells and obtain cellular aggregates, and avoid a conventional monolayer culture. We work with liquid overlay technique (LOT) because it is simple and economical. We used V-shaped 96-wells plates and non-adherent culture plates, using agarose as the non-adherent substrate. [4,6-9]. The TC were incubated in DMEM medium supplemented with 10% FBS at 37 °C with 5% CO<sub>2</sub> for a week.

Cell aggregates began to grow 24 hours after incubation at 37 °C, under the conditions with agarose-coated, on 96-wells plates

and non-adherent culture plates. These spheroid structures form a necrotic and hypoxic core, surrounded by a layer of actively proliferating cells, similar to that found in tumors grafted into mice. We observed compact structures, which are quite resistant to mechanical dissociation, i.e. by replacement of the culture medium, and in the processes of fixation and paraffin embedding. When we compare the cellular morphology of TC from the mammospheres or from the tumors, we can see that they are very similar.

In the last years, mesenchymal stem cells (MSCs) have been demonstrated to play important roles in tumor pathogenesis and, for this reason, are the subject of intense investigation. We developed mammospheres with the co-culture of TC and hUC-MSC to analyse the interaction and investigate specific mechanisms existing among both kind of cells [10-12]. The hUC-MSC would be mimicking the extracellular matrix. We chose to work with culturing in V-shaped 96-wells plates with a monolayer of 1% agarose on each well. We co-cultured 10.000 TC and different concentrations of hUC-MSC, 1.000 and 5.000 cells, with culture medium appropriate. These were incubated under the same conditions mentioned before. The co-cultured of TC and hUC-MSC was successful, because it was obtained cell grow as three-dimensional culture. We saw by optical microscopy that these spheroids had a structure more compact and rounder than mammospheres only composed of TC.

Spheroids have been manipulated to obtain histological samples, they have been fixed, processed and analysed by optical microscopy high-quality samples. Then, we have performed immunostaining with selected antibodies to determine the index of DNA synthesis of TC (with bromodeoxyuridine, Brdu, a marker of S phase cell cycle) and the VEGF expression (with VEGF clone VG1 monoclonal of mice, a marker of neovascularization) [2,13,14].

### Conclusion

Based on the results, we can conclude that the mammospheres culture under the condition of V-shaped 96-well plates with 1% agarose are the most representative of tumoral model *in vivo* because these spheroids form a necrotic and hypoxic core, surrounded by a layer of actively proliferating cells, similar to that found in tumors grafted into mice. The mammospheres can be performed with more than one cell type. This point is very interesting because we can include microenvironment tumoral cells, to analyse effects or interaction between them and TC, mimicking to *in vivo* model and far away the monolayer culture.

Understanding the mechanisms through which TC, hUC-MSC and the tumoral microenvironment are interacting, it

could reveal a new target for cancer treatment and control, such as antiangiogenic therapy, using this three-dimensional culture technique, which is cheaper, require less work-time and without the use of laboratory animals. The development of three-dimensional culture will contribute to study the tumor physiology and test effects of various therapeutic agents on solid tumors.

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