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3D TUMOR MODELS WITH DEFINED CELLULAR AND PHYSICO-CHEMICAL COMPONENTS: IMPACT OF RECAPITULATIVE TUMOR MICROENVIRONMENT ON DISEASE PROGRESSION

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The high attrition rates observed in cancer drug discovery (up to 95% of failure of drugs tested in phase I trials) have raised the awareness of the scientific and industrial communities towards the need for more predictive pre-clinical models. These models should be more representative of the disease and consequently help to eliminate at pre-clinical stages drug candidates that lack efficacy or safety. Tumor microenvironment is composed by a network of fibroblasts, endothelial cells, immune-competent cells within the extracellular matrix (ECM). Interactions between these components are critical for tumor initiation, proliferation, migration and metastasis. The design of in vitro cancer cell models that recapitulate the tumor microenvironment and 3D architecture provides higher physiological relevance as they more closely resemble the *in vivo* cellular context.

We have established methodologies for scalable generation of 3D cancer cell models in stirred-tank culture systems, and applied these to a large panel of tumor cell lines from different pathologies, including breast, colon, hepatic and lung tumor cell lines. Large numbers of spheroids were obtained per culture (typically 1000-1500 spheroids/mL) with representative characteristics of native tumors, such as morphology, proliferation and hypoxia gradients, in a cell-line dependent mode.

With the aim of increasing the relevance of spheroids as tumor cell models, several aspects of tumor microenvironment were incorporated, such as the presence of stromal cells (fibroblasts and monocytes) and specific physico-chemical parameters, namely oxygen levels. Heterotypic 3D breast and Non-Small Cell Lung Carcinoma (NSCLC) cancer models, based on co-cultures of tumor cells with stromal cells were established by using an alginate matrix to provide physical support to cells. Tumor spheroids were microencapsulated alone or with fibroblasts and monocytes, thus allowing the establishment of an epithelial tumor compartment and a stromal compartment of increasing complexity. Cultures were performed in stirred-tank vessels for 15 days with continuous monitoring. In both breast and lung tumor models, the presence of fibroblasts was associated with secretion of pro-inflammatory cytokines and accumulation of collagen in the microcapsules. Long-term culture (up to 15 days) resulted in phenotypic alterations in co-cultured breast tumor spheroids, such as loss of cell polarity, reduced cell-cell adhesions, collective cell migration and increased angiogenic potential. In contrast, the effects of fibroblasts were not as significant in NSCLC co-cultures using H1650, H1437 and H157 cell lines suggesting that the effect of tumor-stroma cross-talk is cell line dependent. Moreover, these models have also been shown as feasible tools for drug screening by assessing the effect of chemotherapeutic and specific inhibitors compounds on mono- and co-cultures.

In conclusion, we have developed scalable, robust and versatile methodologies for the generation and culture of 3D cancer models, enabling long-term *in vitro* recapitulation of tumor-stroma crosstalk, via reconstruction of key aspects of the tumor microenvironment, allowing continuous monitoring of disease progression events *in vitro*. In addition, it is easily transferable to industry for feeding high-throughput systems or miniaturized bioreactors used in drug development, target validation and target identification.

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