Abstract

During their lifetime, 1 in 8 American women will develop breast cancer, and an urgent need exists for targeted, safe therapeutics, particularly for refractory disease. Regulatory agencies are currently seeking improved preclinical oncology models to overcome hurdles to efficient drug development, including bridging the gap between the use of 2D cell lines and 3D animal models. To more accurately model initiation and progression of breast cancer and to more efficiently define targeted therapeutics, the breast microenvironment must be considered. Breast stroma, composed of fibroblasts, endothelial cells, and adipocytes, plays a key role in the process of carcinogenesis and metastasis. These cell types secrete extracellular matrix, growth factors, and hormones that affect how therapeutic agents access and target cancer cells. We have used Organovo’s NovoGen BioprintingTM Platform to develop a model of human breast cancer in which breast cancer lesions were surrounded by a physiologically-relevant stromal milieu consisting of adipose (differentiated from mesenchymal stem cells), mammary fibroblasts, and endothelial cells. This system has several advantages over current screening tools, including the ability to simultaneously measure the effects of small molecules on cancer cells as well as different cell types in the breast microenvironment. Histomorphological analyses of bioprinted neotissues demonstrated that they were stable and viable for at least 14 days in culture and characterized by clear compartmentalization of adipose, stromal, and epithelial components. During the bioprinting process, robust microvascular networks were created using endothelial cells as a component of the 3D tissue design. Mammary neotissues were fabricated directly into multi-well plates and used to establish biological response profiles to signal mediators including estradiol, progestin, prolactin, and HGF as well as the standard chemotherapeutic agents cisplatin, paclitaxel, and tamoxifen. These cell types secrete extracellular matrix and spontaneously organize into relevant tissue microarchitectures in the absence of exogenous scaffold material.

The NovoGen MMX™ Bioprinter

Figure 1. The Novogen MMX™ Bioprinter deposits multiple types of cell aggregates in spatially-defined patterns. Following bioprinting, the cells secrete extracellular matrix and spontaneously organize into relevant tissue microarchitectures in the absence of exogenous scaffold material.

Figure 2. Left, schematic of bioprinted neotissues. A nodule of human breast cancer cells is surrounded by a stromal compartment composed of endothelial cells, fibroblasts, and adipocytes. Right, bioprinted neotissues immediately after printing. Cancer cells are labeled with blue dye.

Histologic Analysis of Bioprinted Neotissues

Figure 3. Histology of bioprinted neotissues. Tissues were analyzed to determine tissue architecture and relative positions of cell types. In panels C-E, breast cancer cells were labeled with CellTracker Green CMFDA (Invitrogen, Carlsbad, CA).

Conclusions

1. Bioprinted tissues retain compartmentalized structures with interaction between stromal and cancer cells.
2. Following bioprinting of the stromal compartment, formation of endothelial networks and differentiation of adipocytes were observed.
3. Isolated 2D cancer cells were more susceptible to tamoxifen-induced toxicity than cells incorporated into 3D bioprinted constructs when treated with the same dose of tamoxifen for the same duration.

Safe Harbor Statement

Any statements contained in this report and presentation that are not statements of historical fact are forward-looking statements that are subject to a number of risks and uncertainties, including but not limited to those described in the Company’s filings with the SEC including its report on Form 10-Q filed November 8, 2013 and its transition report on Form 10-KT filed with the SEC on May 24, 2013 and our other filings with the Securities and Exchange Commission. You should not place undue reliance on these forward-looking statements, which speak only as of the date of the Current Report. These forward-looking statements should be considered with caution. We do not undertake any obligation to update any of the forward-looking statements to reflect actual results, later events or circumstances or to reflect the occurrence of unanticipated events.