Polymeric Composite Tissue Test System Development for Breast Cancer Research

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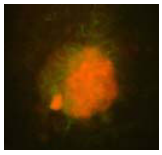
Statement of Purpose: Biological behavior of carcinoma cells is regulated not only by cell signalling, but also by effects of the surrounding extracellular matrix (ECM) and stromal cells. In this figure, human estrogen-positive breast carcinomous cells, MCF-7, are prompted by cytokines secreted from adipocytes to form a dome shaped structure and nest above adipocytes in a 2-D culture. ECM proteins, growth factors, stiffness of ECM, and other recognized tissue microenvironmental cues are crucial in the regulation of tumourigenic phenotypes *in vivo*. Thus, we established a 3-D co-culture system that is manufactured particularly for breast cancer research for the testing of the effects of new chemotherapies. The primary advantage 3D cultures have over 2D monolayers is their well defined geometry and compositions, making it possible to relate structure to physical function and to enable theoretical analyses.



Methods: Mouse bone marrow stromal cells in the amount of 10^7 were cultured on Solohill beads (Sigma-Aldrich) in a stir flask at 20 rpm. After 5 days of cultivation, 0.1mL of Solohill beads was removed to each well of a 12-well plate. Two kinds of gelation mixtures were prepared in this study. The first gelation mixture was composed of 730μL of 1.1% agarose solution (Sigma-Aldrich) and 270μL of type I collagen solution (Inamed Biomaterials). The second mixture was composed of 1mL of type I collagen solution, 318μL of 4.7% of gelatin (Sigma-Aldrich) in culture medium, 162μL of 10X PBS and 40μL of NaOH. The gelation mixture was used to disperse a pellet of MCF-7 cells; the cell suspension was adjusted to 10^6 cells per milliliter. A 1mL volume of cell suspension was distributed to each well. The mixture of beads and cell suspension was swirled gently and incubated for 1hr. A 2mL volume of culture medium was added to each gel. Photographs were taken daily and a LIVE/DEAD assay was conducted after 14 days of cultivation.

Results: Over the course of studying the effects of microenvironment on MCF-7 cells, we observed that the aggregation of MCF-7 cells was altered by the composition of hydrogel. MCF-7 cells immediately formed spheroids (Fig 1(a)) within the agarose-based composites. MCF-7 cells cultured in collagen-based composites, however, spread within the matrix and formed cell colonies (Fig 1(d)). After 11 days, MCF-7 cells aggregated to form spheroid-like structures (Fig 1 (f)) in collagen-based composites. LIVE/DEAD assays were conducted to examine the viability of MCF-7 cells within both composites on Day 14 (Figure 2). The results showed that MCF-7 cells were still viable within both composites, and that the aggregation in collagen-based composites was not due to necrosis.

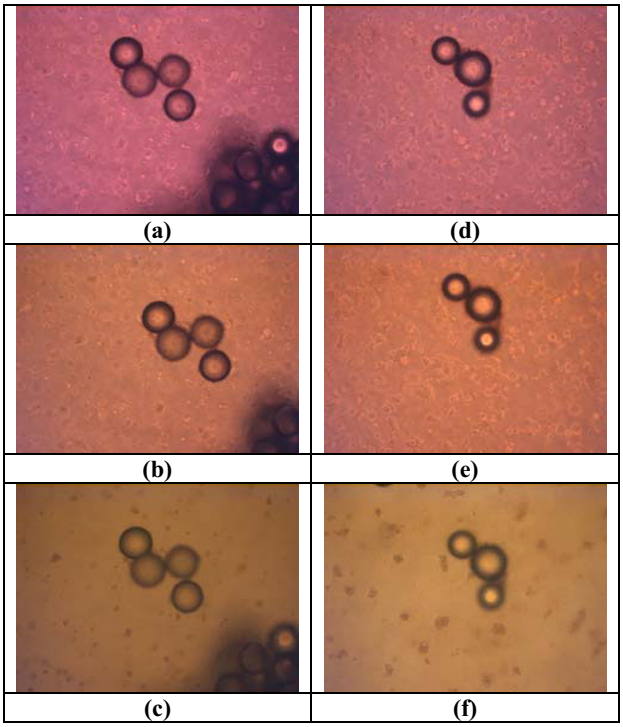


Figure 1 Cells in agarose-bead composite at (a) Day 1, (b) Day 4, (c) Day 11 and in collagen-bead composite at (d) Day 1, (e) Day 4, (f) Day 11. All pictures were taken at a total magnification of 100X.

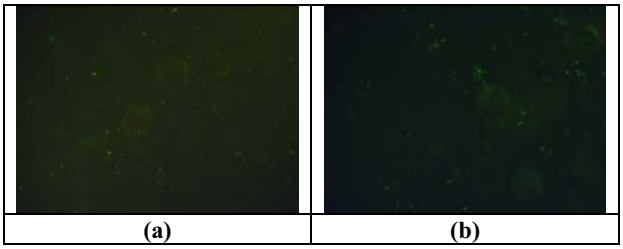


Figure 2 LIVE/DEAD of MCF-7s in (a) agarose and (b) collagen on Day 14. Green fluorescence indicates the live cells. Pictures were taken at a total magnification of 100X.

Conclusions:

Unlike the traditional 2-D culture systems, the polymeric bead-gel composite system provides various environmental cues, such as the stiffness of matrix and the growth factors from stromal cells, to alter the morphology of MCF-7 cells. The understanding of the formation of cell spheroids or colonies within matrices may assist in the understanding of tumorigenesis and metastasis *in vivo*.

Acknowledgements

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