PEG-fibrinogen Hydrogel Microspheres Support 3D Tumorigenic Growth of MCF7 Breast Cancer Cells

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Statement of Purpose: The tumor microenvironment is known to play an influential role in the malignant progression of breast cancer. However, current 2D monolayer culture and 3D spheroid models are not able to capture key features of the complex tumor extracellular matrix (ECM), severely limiting their ability to provide clinically-relevant data. In order to address this challenge, we have developed a novel 3D in vitro model, "tumor microspheres", which is formed through the encapsulation of cancer cells within poly(ethylene glycol)-fibrinogen (PEG-Fb) hydrogel microspheres. Tumor microspheres support long-term 3D culture of the cancer cells. The tumor microsphere model can be potentially used for the investigation of specific tumorigenic mechanisms and provide a platform for drug-testing applications for a range of different cancer cell types.

Methods: MCF7 breast cells were encapsulated within PEG-Fb hydrogel microspheres using a dual-phase, water-in-oil emulsion technique. Resulting tumor microspheres were maintained in culture for 28 days. For comparison, MCF7 cells were also spontaneously aggregated to form tumor spheroids, the current gold standard for 3D cancer cell culture, using the standard hanging droplet method. The tumor spheroids (TS) and the tumor microspheres (TM) were analyzed for size and circularity. Live/dead™ staining was performed to quantify cell viability and total cell number over time. Ultrastructural differences in cells grown in TS and TM were visualized through SEM imaging. 3D tumorigenic morphology was assessed through confocal microscopy and quantitative image analysis. Finally, the potential of the technique to be used for a wide range of cancer cell types, including breast (SK-BR-3, MDA-MB-231), prostate (PC-3, PC-3-metastatic) and colon (HT29) cancers was demonstrated.

Results: A novel fabrication technique was developed for the generation of PEG-Fb hydrogel microspheres. MCF7 cells were successfully encapsulated within the microspheres and maintained in culture for 28 days. Size analysis showed that TM with 20×10^6 cells/ml had the highest proportion of microspheres in the desired range of 100-300 μm, which was significantly larger than TS, and the lowest variability in size. TM also had significantly higher circularity with less variation than TS. Cells in TM displayed lower cell-cell junction lengths and greater disorganization as compared to cells in TS. Analysis of 3D morphology revealed lower nuclear and cellular area, higher nuclear-to-cytoplasmic ratio and nuclear volume density, loss in apico-basal polarity and reduced cell-cell junction length in TM as compared to TS, indicating increased tumorigenic phenotype in the tumor microspheres. Finally, other cancer cell types were also cultured within PEG-Fb microspheres through 14 days, with different cell lines showing different growth and phenotypic characteristics.

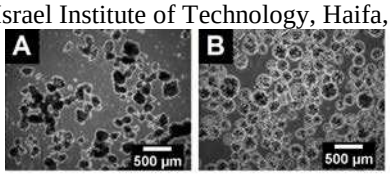

Fig. 1: (A) Tumor spheroids (TS) and (B) tumor microspheres (TM) on day 28 of culture.

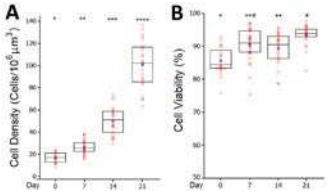

Fig. 2: (A) Cell proliferation and (B) cell viability in tumor microspheres over time.

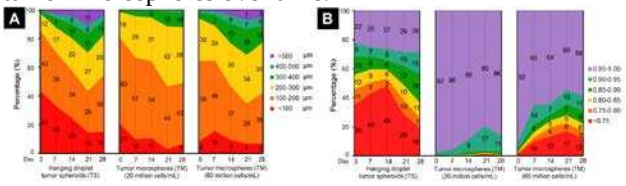

Fig. 3: (A) Size ranges and (B) circularity ranges of tumor spheroids and tumor microspheres through 28 days

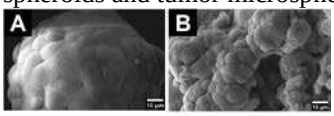

Fig. 4: Differences in ultrastructure of (A) TS and (B) TM

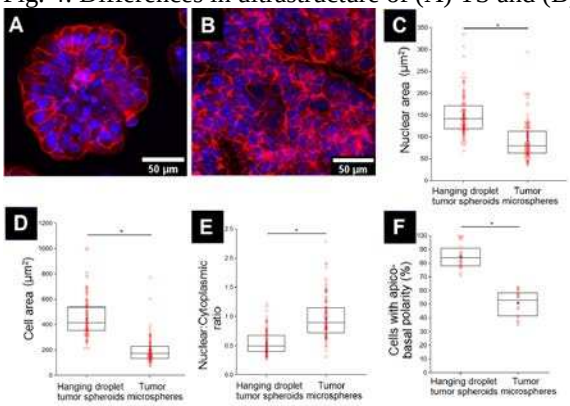

Fig. 5: 3D morphology of (A) TS and (B) TM, DAPI: nuclei, Phalloidin: F-actin (C-F) Morphological differences between TS and TM

Conclusions: MCF7 cells in tumor microspheres (TM) displayed higher tumorigenic phenotype as compared to those cultured as standard tumor spheroids, thereby demonstrating the influence and importance of the surrounding PEG-Fb biomaterial in the 3D culture of cancer cells. The tumor microsphere model can be further used for the study of cancer cell interactions with the microenvironment and in drug-testing applications.

References: Seliktar et al., Biomaterials 2005; 26(15): 2467-2477.