

Novel fabrication methods for tissue-engineered spheroid models of human breast cancer to mimic native tumor microenvironment

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Statement of Purpose: The tumor microenvironment of breast cancer plays an influential role in the progression of the disease, including angiogenesis and metastasis. Current established two-dimensional (2D) models cannot accurately replicate the complexities involved in the breast tumor physiology. Hence three-dimensional (3D) tissue-engineered models, employing the use of biomimetic materials, have become more reliably predictive of the native tumor microenvironment. In addition, various fabrication methods have been developed for these models that can accurately incorporate key features of the native stroma in 3D *in vitro* culture. This study developed two novel fabrication methods for the creation of tissue-engineered 3D breast cancer models, using biomimetic PEG-based scaffolds. These techniques enable the creation of spheroids spanning a wide range of sizes and facilitate cellular interactions with the surrounding biomimetic scaffold. The 3D biomimetic engineered constructs so obtained, can closely replicate key microenvironmental characteristics of native tumors (including the formation of a necrotic core of cells surrounded by viable, proliferative cell layers). Finally, the bio-chemical and mechanical properties of the engineered microenvironments can be tuned to match that of native tumors.

Methods: To create micron-scale tumor constructs, a water-in-oil, dual-photoinitiator emulsion system was used to encapsulate human breast cancer MCF-7 cells within poly(ethylene glycol)-fibrinogen (PEG-Fb) hydrogel microspheres. The resulting ‘tumor microspheres’ were in the micron-scale size-range. Encapsulated cancer cells were maintained in 3D culture for 28 days and temporal changes in spheroid characteristics were quantified.

To encapsulate cancer cells within larger PEG-based hydrogel millibeads, a novel technique was developed. In this method, cancer cells were suspended in PEG-based hydrogel precursor and small droplets of this cell laden precursor were suspended on the surface of an oil layer where the droplets floated due to surface tension differences. The droplets were photocrosslinked under visible light exposure to form “tumor millibeads” in the millimeter-scale size-range and maintained in 3D culture for 7 days.

Live/dead assay on tumor microspheres and tumor millibeads was conducted to assess cell viability. SEM analysis and 3D morphological analysis (by fluorescence staining and visualization through confocal microscopy) of both models was done to evaluate distribution of cells within the 3D constructs.

Results: Tumor microspheres obtained in the first method were of size range 100-500 μm in average diameter. Encapsulated cells proliferated leading to increased cell

density and the formation of dense colonies within the tumor microspheres. The microspheres also showed the presence of a necrotic core of cells surrounded by viable proliferative layers on the outer edge, thereby mimicking *in vivo* and native tumor tissue.

Next, we aimed to fabricate larger, more uniform sized and physiologically relevant tumor constructs to better model native tumor size. Tumor millibeads, formed using the second method, were larger and more uniform in size and circularity (1-3 mm in average diameter). Cells encapsulated within tumor millibeads were uniformly distributed and were able to be maintained in 3D culture long-term. The tumor millibeads also displayed a necrotic region in the core surrounded by viable, proliferative layers at the periphery, thereby mimicking the heterogeneity of large, native tumors.

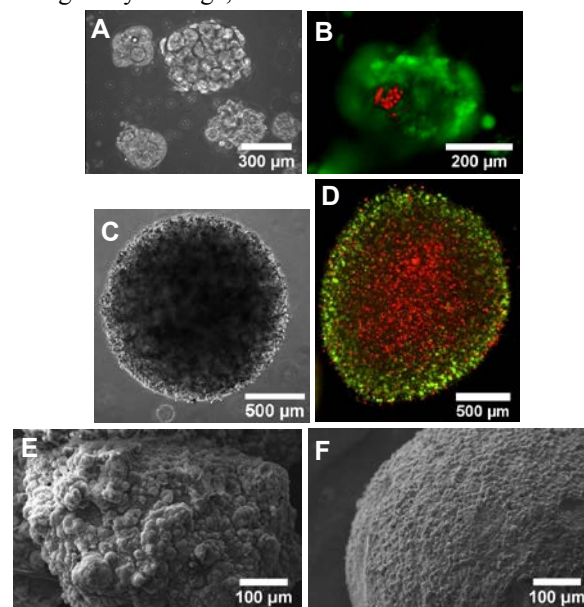


Fig. 1: Tumor microspheres (A, C, E) and tumor millibeads (B, D, F) as visualized through phase contrast (A, B), live/dead staining (C, D) showing the presence of necrotic core surrounded by viable cell layers and SEM imaging (E, F)

Conclusions: Overall, this study establishes two novel fabrication methods for obtaining 3D tissue-engineered breast tumor tissue. Tumor constructs fabricated using these methods are able to replicate key features of the tumor microenvironment and can be tuned to match the mechanical and bio-chemical properties of the native tumor microenvironment. In the future, the tumor microspheres and tumor millibeads can be used for the investigation of different tumorigenic phenomena and high-throughput testing of various anti-cancer drugs

References & Acknowledgements: Seliktar et al., *Biomaterials* 2005; 26(15): 2467–2477.

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