Production of Cancer Tissue-Engineered Microspheres for High-throughput Screening

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There is a need for new in vitro systems that enable pharmaceutical companies to collect more physiologically-relevant information on drug response in a low-cost and high-throughput manner. For this purpose, three-dimensional (3D) spheroidal models have been established as more effective than two-dimensional models. Current commercial techniques, however, rely heavily on self-aggregation of dissociated cells and are unable to replicate key features of the native tumor microenvironment, particularly due to a lack of control over extracellular matrix components and heterogeneity in shape, size, and aggregate forming tendencies. In this study, we overcome these challenges by coupling tissue engineering toolsets with microfluidics technologies to create engineered cancer microspheres.

Specifically, we employ biosynthetic hydrogels composed of conjugated poly(ethylene glycol) (PEG) and fibrinogen protein (PEG-Fb) to create engineered breast and colorectal cancer tissue microspheres for 3D culture, tumorigenic characterization, and examination of potential for high-throughput screening (HTS). MCF7 and MDA-MB-231 cell lines were used to create breast cancer microspheres and the HT29 cell line and cells from a stage II patient-derived xenograft (PDX) were encapsulated to produce colorectal cancer (CRC) microspheres.

Using our previously developed microfluidic system, highly uniform cancer microspheres (intra-batch coefficient of variation (CV) ≤ 5%, inter-batch CV < 2%) with high cell densities (>20×10^6 cells/ml) were produced rapidly, which is critical for use in drug testing. Encapsulated cells maintained high viability and displayed cell type-specific differences in morphology, proliferation, metabolic activity, ultrastructure, and overall microsphere size distribution and bulk stiffness. For PDX CRC microspheres, the percentage of human (70%) and CRC (30%) cells was maintained over time and similar to the original PDX tumor, and the mechanical stiffness also exhibited a similar order of magnitude (10^3 Pa) to the original tumor.

The cancer microsphere system was shown to be compatible with an automated liquid handling system for administration of drug compounds; MDA-MB-231 microspheres were distributed in 384 well plates and treated with staurosporine (1 μM) and doxorubicin (10 μM). Expected responses were quantified using CellTiter-Glo® 3D, demonstrating initial applicability to HTS drug discovery. PDX CRC microspheres treated with Fluorouracil (5FU) (10 to 500 μM) and displayed a decreasing trend in metabolic activity with increasing drug concentration. Providing more physiologically relevant tumor microenvironment in a high-throughput and low-cost manner, the PF hydrogel-based cancer microspheres could potentially improve the translational success of drug candidates by providing more accurate in vitro prediction of in vivo drug efficacy.