

# 3D Tumor Spheroid Generation Using a Droplet Microfluidic Device

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The use of three-dimensional (3D) spheroids has received significant attention in recent years because cells behave differently in 2D environments when compared to 3D environments including changes in growth rates, cellular phenotype, and their response to drugs. While several approaches exist to generate 3D spheroids, they are often limited by large volume requirements, inconsistencies in the size of the spheroids, and heterogeneity of cellular distribution in the spheroid. Microfluidic devices can generate 3D cell cultures from single progenitor cells, which will dramatically reduce the heterogeneity commonly associated with spheroids generated from multiple cells. The goal of this work was to generate a microfluidic device to generate, isolate, and interrogate 3D spheroids. The novelty of this work is the incorporation of a thiol-acrylate (TA) hydrogel serving as a scaffold for 3D spheroid formation in the device. The first step was to develop a microfluidic droplet trapping array capable of isolating and containing discrete hydrogel droplets containing a small number of cancer cells that will eventually form into 3D spheroids. Preliminary studies fabricated and tested the microfluidic droplet trapping array concurrently with identifying an optimal protocol to incorporate the hydrogel scaffold. Several failed attempts were observed with respect to device fabrication and hydrogel incorporation before a final design was identified. We were able to run a successful experiment using a 70-micron trapping array with the flow rates 150  $\mu\text{L/hr}$  for oil and 90  $\mu\text{L/hr}$  for the hydrogel that created droplets and trapped MCF-7 breast cancer cells inside the device.