

# ADVANCED FUNCTIONAL MATERIALS

## Supporting Information

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High Throughput, Polymeric Aqueous Two-Phase Printing of  
Tumor Spheroids

*Ehsan Atefi, Stephanie Lemmo, Darcy Fyffe, Gary D. Luker,  
and Hossein Tavana\**

((Supporting Information can be included here using this template))

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**Supporting Information**

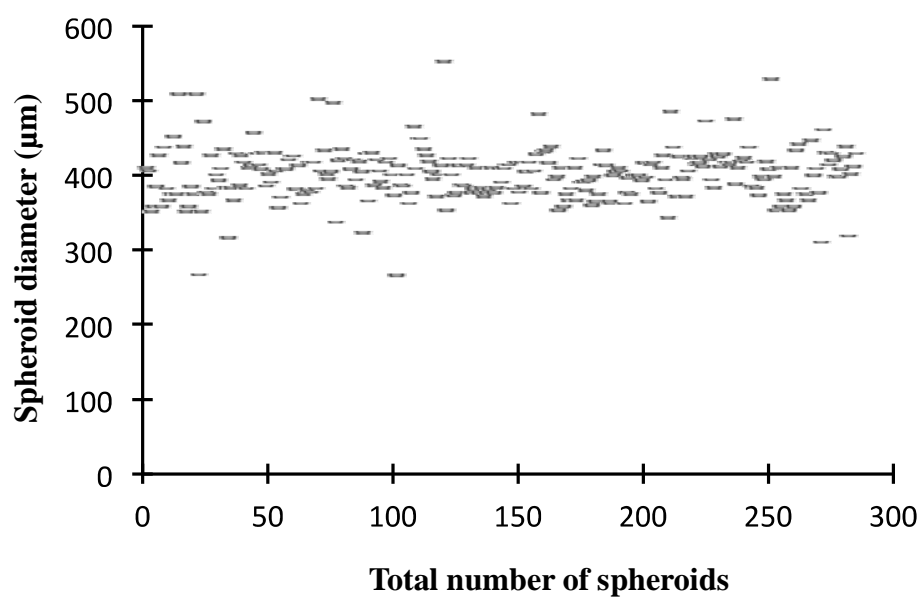
**((High Throughput, Polymeric Aqueous Two-Phase Printing of Tumor Spheroids))**

*((Ehsan Atefi<sup>1</sup>, Stephanie Lemmo<sup>1</sup>, Darcy Fyffe<sup>1</sup>, Gary D. Luker<sup>2-4</sup>, Hossein Tavana<sup>1\*</sup>)*

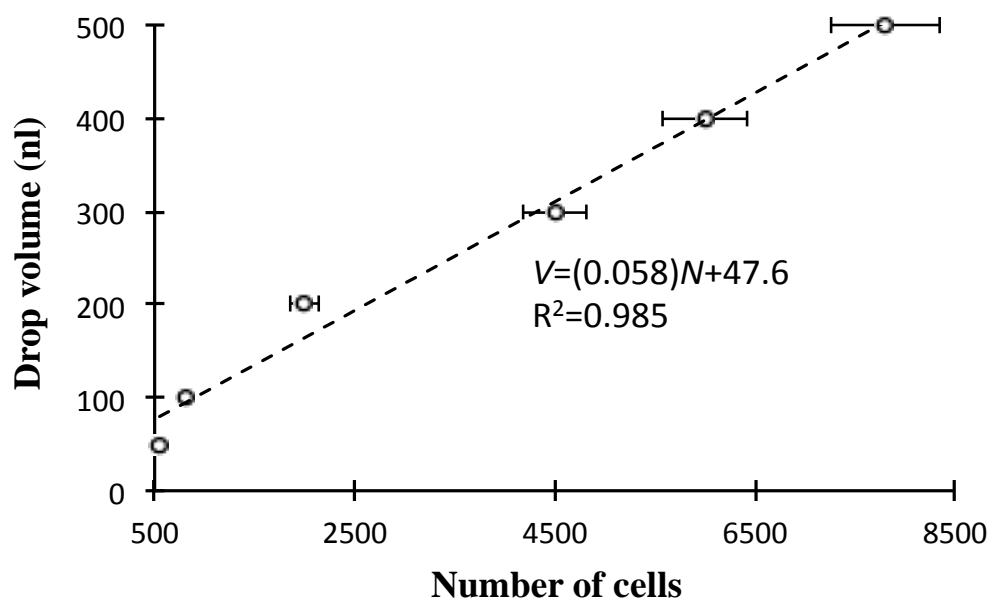
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Spheroid\$Forma9on\$

**Figure SI□1.** Six polymers were selected to first study formation of aqueous two-phase system (ATPS). For each polymer, aqueous solutions were prepared at phase concentrations of 5%, 10%, 15%, and 20% (w/w). Then equal volumes from solutions of each two polymers were mixed in 1.5 ml conical tubes to evaluate ATPS formation. For each pair of polymers, this resulted in 16 combinations. Systems resulting in at least one ATPS are shown by green colored boxes in the upper right diagonal of the table. Within each green box, the combinations that gave an ATPS are noted with “x” symbol. At the bottom of each green box, the bottom phase solution is noted in blue. Based on this screening, we narrowed down the combinations of polymers to those whose aqueous solutions form an ATPS. Next we evaluated the feasibility of spheroid formation using aqueous solutions of pairs of polymers that resulted in ATPS. From each polymer pairs, we selected only one system whose aqueous solutions have the lowest polymer concentrations (e.g., 10% Peg 8k-5% DEX 500k from the Peg 8k-Dex 500k combinations). Systems that resulted in successful spheroid formation are shown with green colored boxes in the lower left diagonal of the table.



**Figure SI-2.** Distribution of diameter of spheroids generated from a density of  $1.5 \times 10^4$  cells per DEX drop in three separate 96-well plates. Data show an average of  $392.2 \pm 32.5$  µm, i.e., a standard deviation of 8.2%.



**Figure SI-3.** The volume of DEX drops ( $V$ ) to facilitate formation of a single spheroid from a desired number of cells ( $N$ ) follows a linear relationship within the range of cell density studied.