Supplemental data

A: Effect of rinsing prior to bioluminescence assay

We conducted additional experiments to confirm that the observed difference in chemosensitivity between cells in 3-D microgel and 2-D monolayer is not due to the presence of “ghost”, which is defined as cells that are detachable during wash but carry residual luciferase activities. Since these ghost are more likely to be retained in 3-D microgel even after wash due to the structure provided by the matrix, we see to confirm if ghost cell is a problem in our system. We seeded 2 sets of plates with 231-LUC cells in 2-D monolayer then subjected to both to the usual chemotherapeutics treatment regime. Prior to adding luciferin into each plate, one plates was rinsed 3-times (50% volume exchange) while the control plate was not rinsed. Bioluminescence assay revealed that the additional rinsing step did not alter the observed chemosensitivity of 231-LUC cells, suggesting the observed differences between 3-D and 2-D culture platforms is not due to the presence of ghost cells.

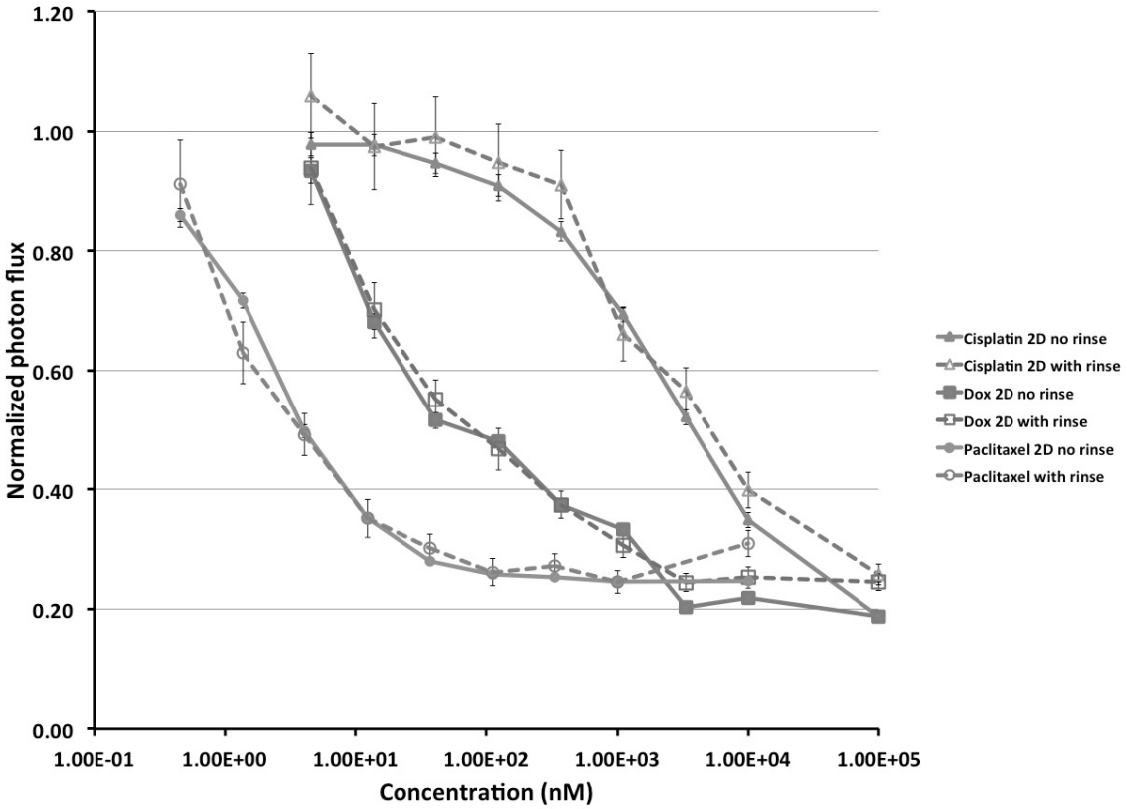

Figure S1: Bioluminescence based cytotoxicity assay against three common chemotherapeutics (cisplatin, doxorubicin and paclitaxel). Pre-luciferin rinse in 384-well plates seeded with 231-LUC 2-D monolayer did not lead to significant difference in observed chemosensitivities toward drug treatments.

B: Effects of dispensing height, speed and volume

Automated liquid handling routines were crated and executed as scripts in CyBio Composer software. The scripts used for spotting collagen-DEX in PEG solution and rinsing PEG out from 384-well plate are included as supplemental files:

ATPS collagen gel spotting.bms – This script load collagen ATPS from the reservoir and delivers it to a 384-well plate already filled with 50μL of PEG per well.

ATPS PEG rinse.bms – This script perform the half volume serial rinse of PEG after ATPS. Each run of the script perform 2x rinse so must run script 3.5 time to complete 7x rinse.

Variables in scripts	Parameter	Note	Optimization method
DispXY	Tip position within a well	Use to fine tune printing position of microgel within well	Empirical
DispSpeed	Dispense Speed	Adjust to ensure proper dispensing volume for viscous DEX solution	Empirical, but should be kept to minimum speed if possible
DispHeight	Dispense Height	Adjust to ensure symmetrical DEX microgel delivery	Empirical, but can be estimated based on well-plate schematics
DispVol	Dispense volume	Adjust to create different size microgel	Empirical
VDriveSpeed	Vertical drive speed	Adjust to minimize disturbance of microgel during medium change and rinses	Empirical, but should be kept to minimum speed if possible

Table S1: Parameter description for ATPS collagen microgel printing.

Most parameters are optimized empirically. In this study we tested the effects of dispensing height, dispensing volume and dispensing speed. As expected larger dispensing volume created larger microgel. Dispensing speed has minimal effects on microgel morphology, but not dispensing height. Dispensing too close to the bottom of the well lead to non-circular microgel, potentially caused by lateral jetting of collagen-DEX.

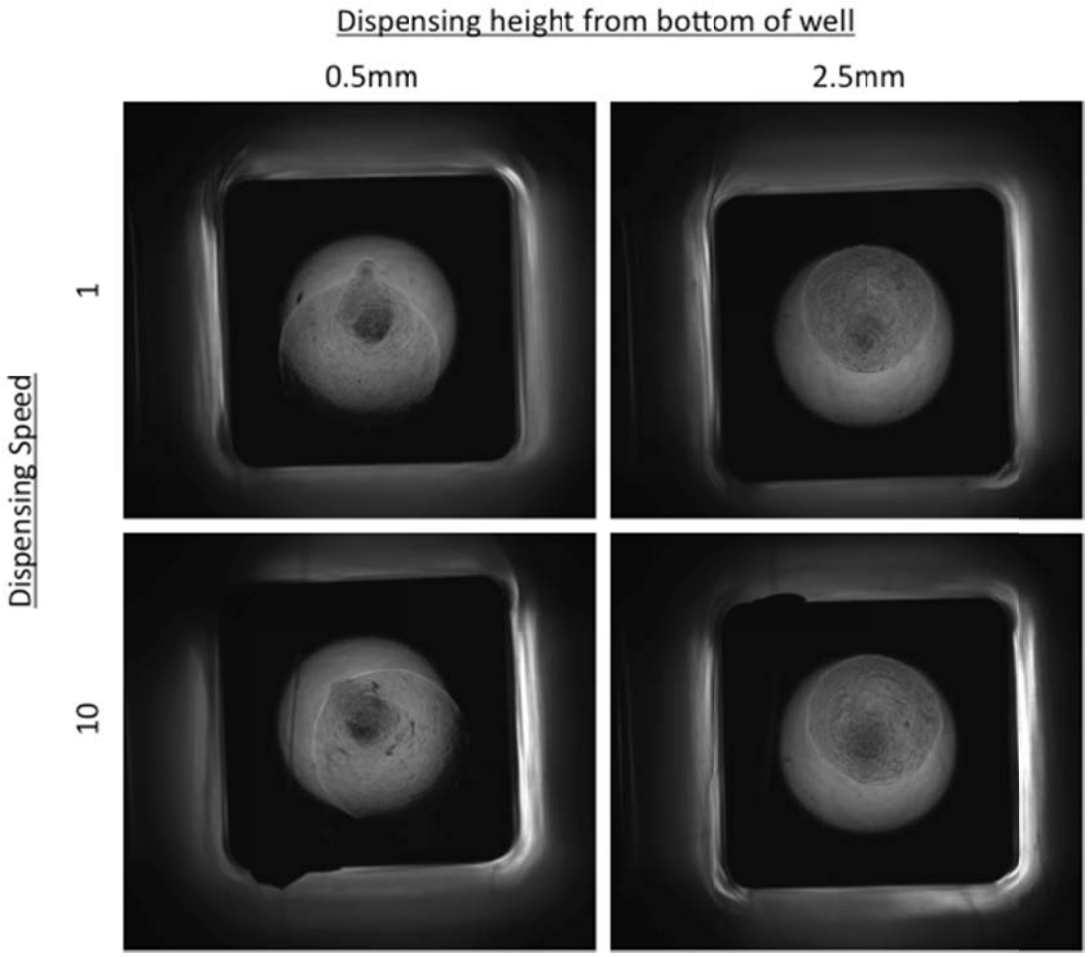

Figure S2: Morphologies of ATPS collagen microgel (1μL) dispensed at difference heights and speeds. Low dispensing height (0.5mm) lead to the formation of non-circular gel protrusions at the edge of the microgel, which are not seen in high dispensing height (2.5mm). Dispensing speed setting of high (10) and low (1) has no discernable effect on microgel morphology

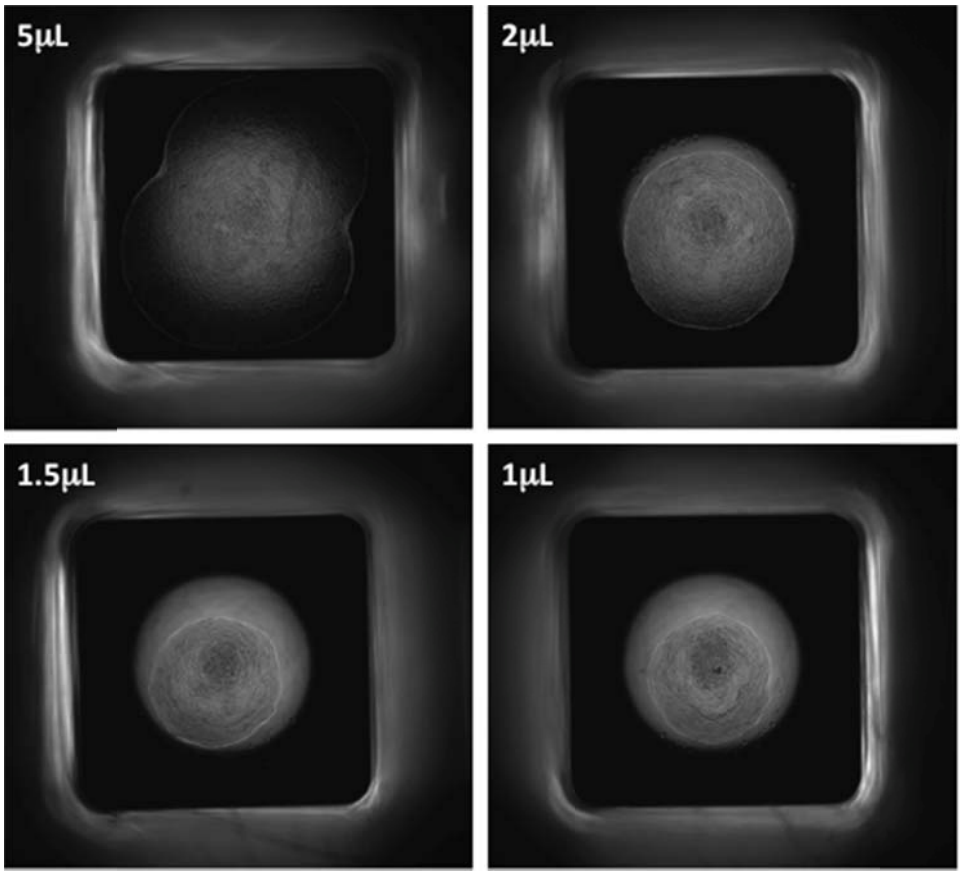

Figure S3: ATPS collagen microgels with different dispensing volume in 384-well plate. ATPS collagen microgel remained circular up to 2µL.

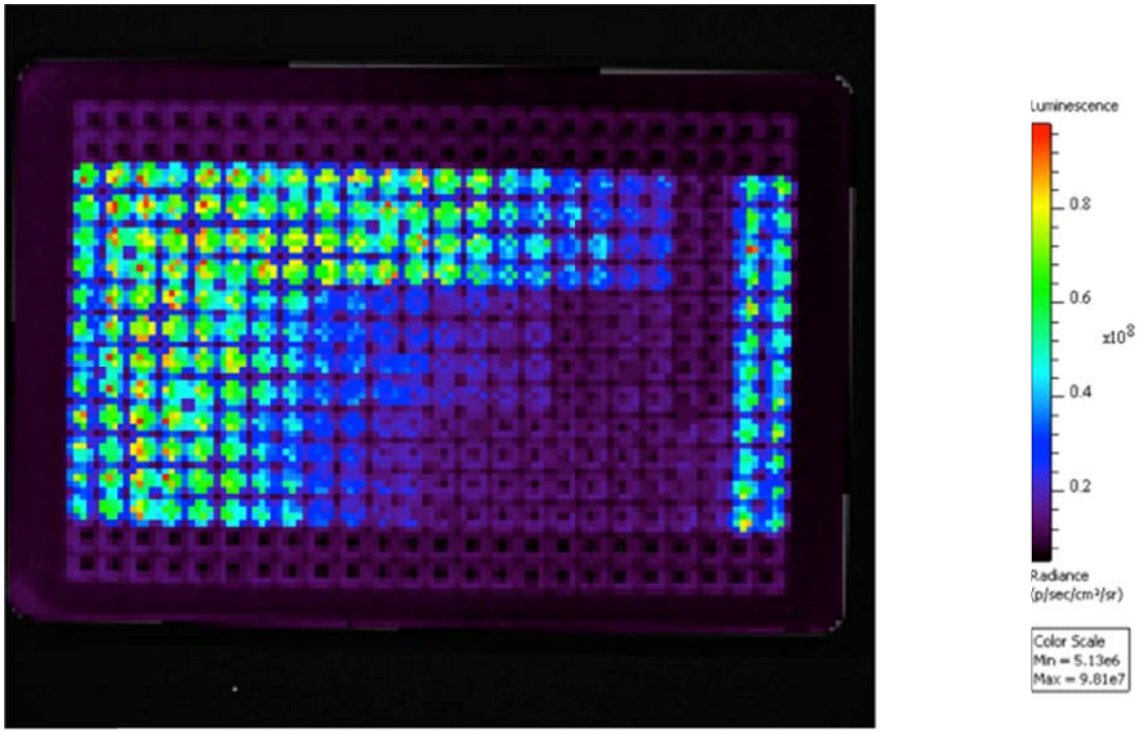

Figure S4: Sample bioluminescence image taken by the IVIS Spectrum imager