Supplementary Information (SI) for RSC Pharmaceutics.
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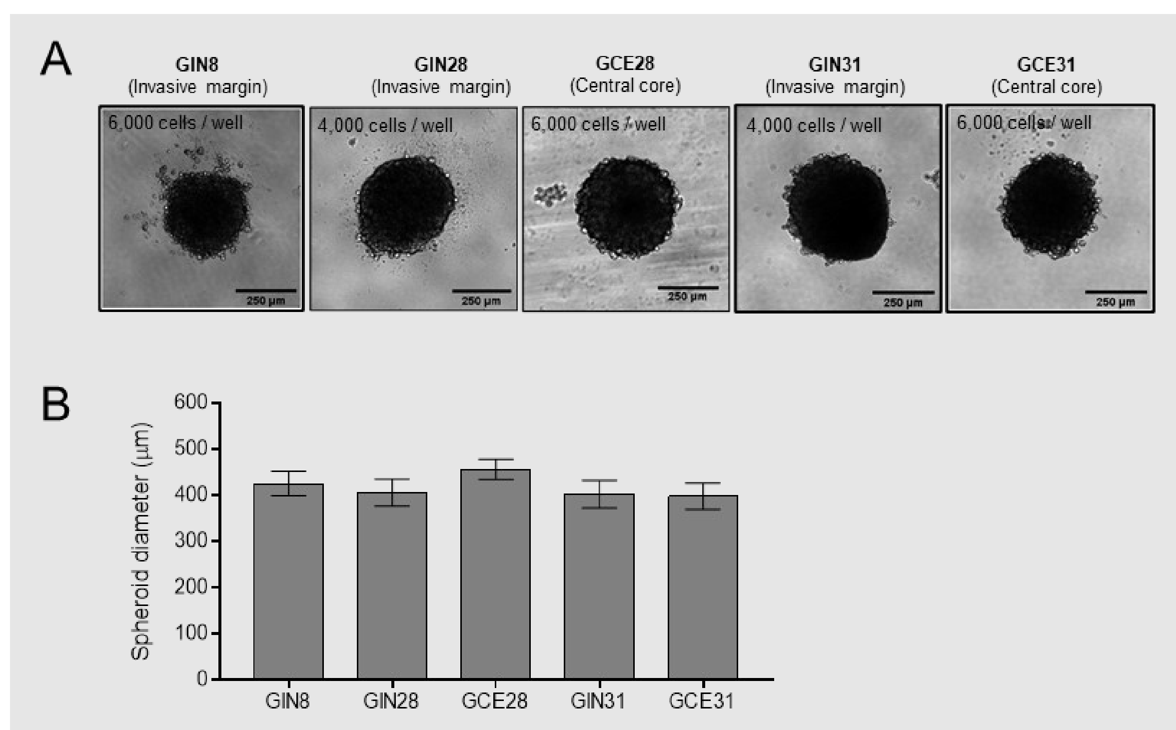
Supramolecular hydrogels enable co-delivery of chemotherapeutics with synergistic efficacy against patient-derived glioblastoma cells and spheroids

Robert J. Cavanagh,[a,b]* Saif Baquain,[c] Cameron Alexander,[a] Oren A. Scherman[c] and Ruman Rahman [b]*

[a] School of Pharmacy, University of Nottingham, NG7 2RD, UK, [b] School of Medicine, Biodiscovery Institute, University of Nottingham, NG7 2RD, UK, [c] Melville Laboratory for Polymer Synthesis, Yusuf Hamied Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW.

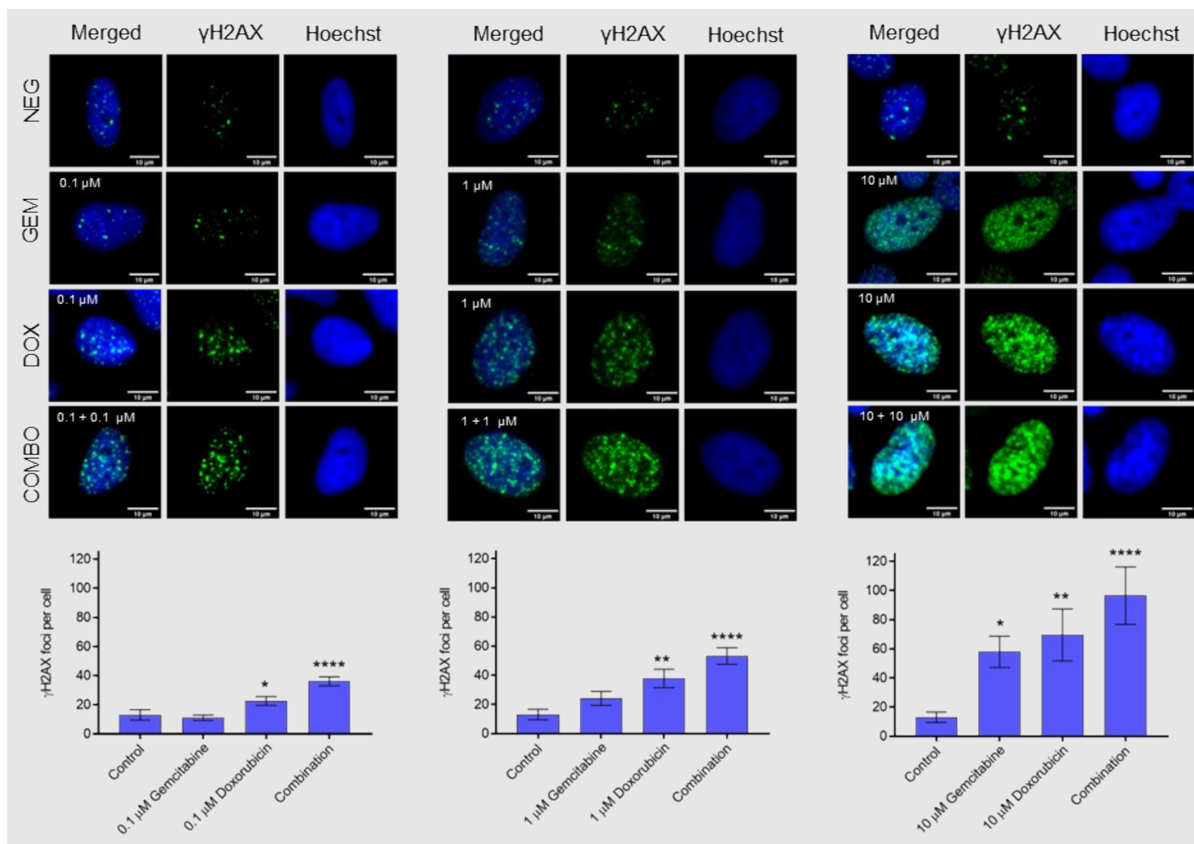
Supporting information



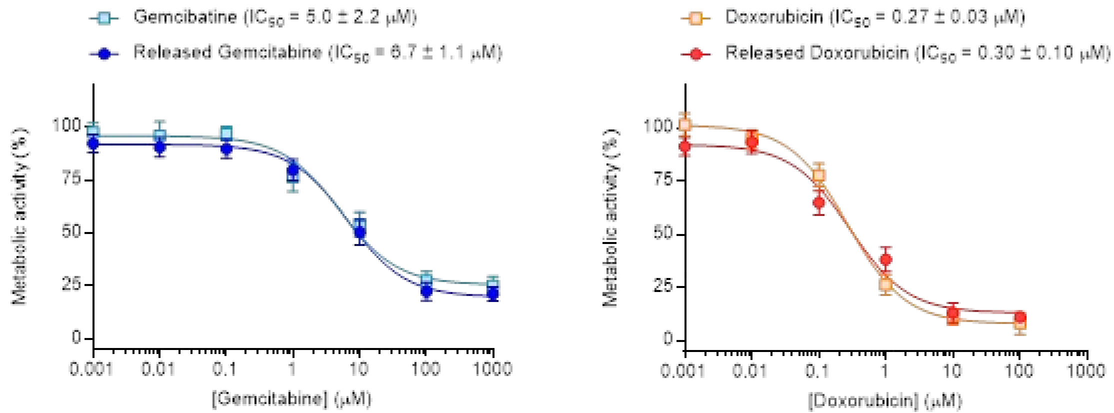
Supplemental Figure 1. Spheroids sizes produced from patient derived GBM cells. Cell spheroids produced using ULA 96 well plates. Images are of Day 3 spheroids. GIN8, GCE28 and GCE31 seeded at 6,000 cells per well (spheroid), and GIN28 and GIN31 seeded at 4,000 cells per spheroid. Spheroid diameter calculated using ImageJ analysis and plotted as mean ± S.D. Images shown are representative of 3 independent repeats with six technical repeats per seeding density. Scale bar, 250 µm.

Drug	GIN8	GIN28	GCE28	GIN31	GCE31
Temozolomide	522 ± 47 μM	436 ± 36 μM	84.3 ± 12 μM	741 ± 51 μM	791 ± 45 μM
Olaparib	77.2 ± 11.1 μM	31.6 ± 10 μM	25.6 ± 4.2 μM	221 ± 19 μM	75.9 ± 12 μM
Etoposide	90.9 ± 13.5 μM	77.8 ± 17 μM	43.3 ± 5.2 μM	53.6 ± 5.12 μM	38.5 ± 7.57 μM
Irinotecan	83.5 ± 17.1 μM	65.8 ± 7.8 μM	48.6 ± 5.6 μM	38.5 ± 8.69 μM	33.2 ± 8.01 μM
Gemcitabine	6.13 ± 3.44 μM	2.08 ± 0.34 μM	3.94 ± 0.25 μM	9.35 ± 0.51 μM	23.2 ± 1.98 μM
Doxorubicin	0.52 ± 0.05 μM	0.30 ± 0.02 μM	0.15 ± 0.06 μM	0.43 ± 0.09 μM	0.58 ± 0.26 μM

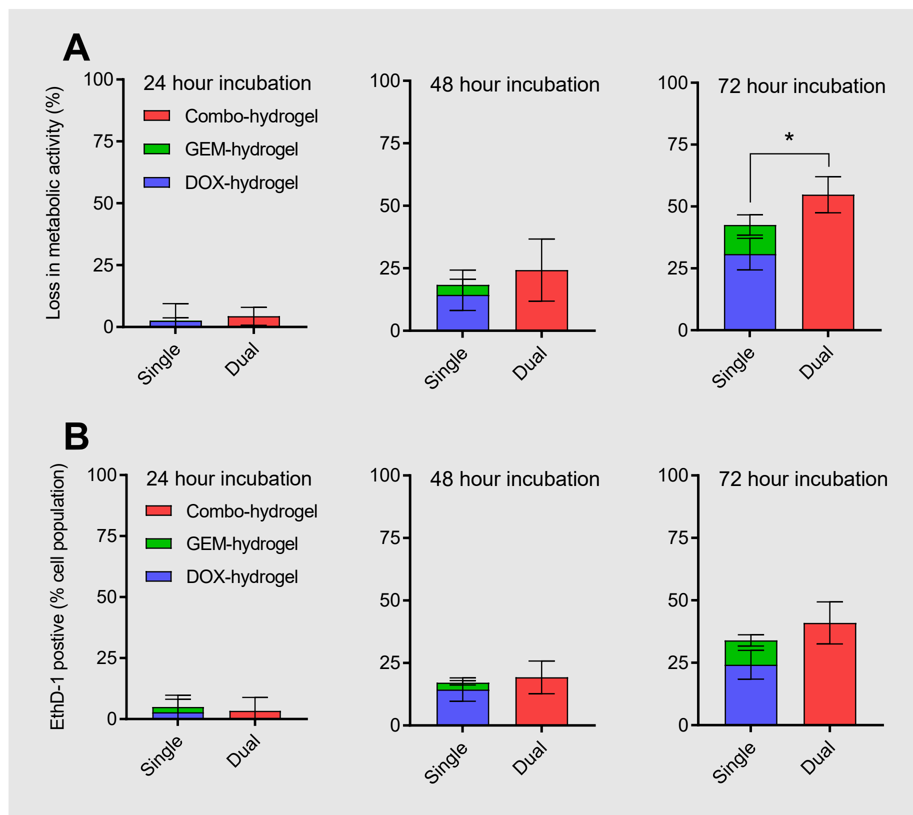
Supplemental Figure 2. Drug potency in 2D cultured GIN8, GIN28 and GCE28. Cells grown for 24 hours prior to assaying in 96 well plates and exposed to drug applied in 10% FBS:DMEM. Metabolic activity measured using PrestoBlue™ assay. IC_{50} values calculated via GraphPad prism. Data is presented as mean ± S.D.



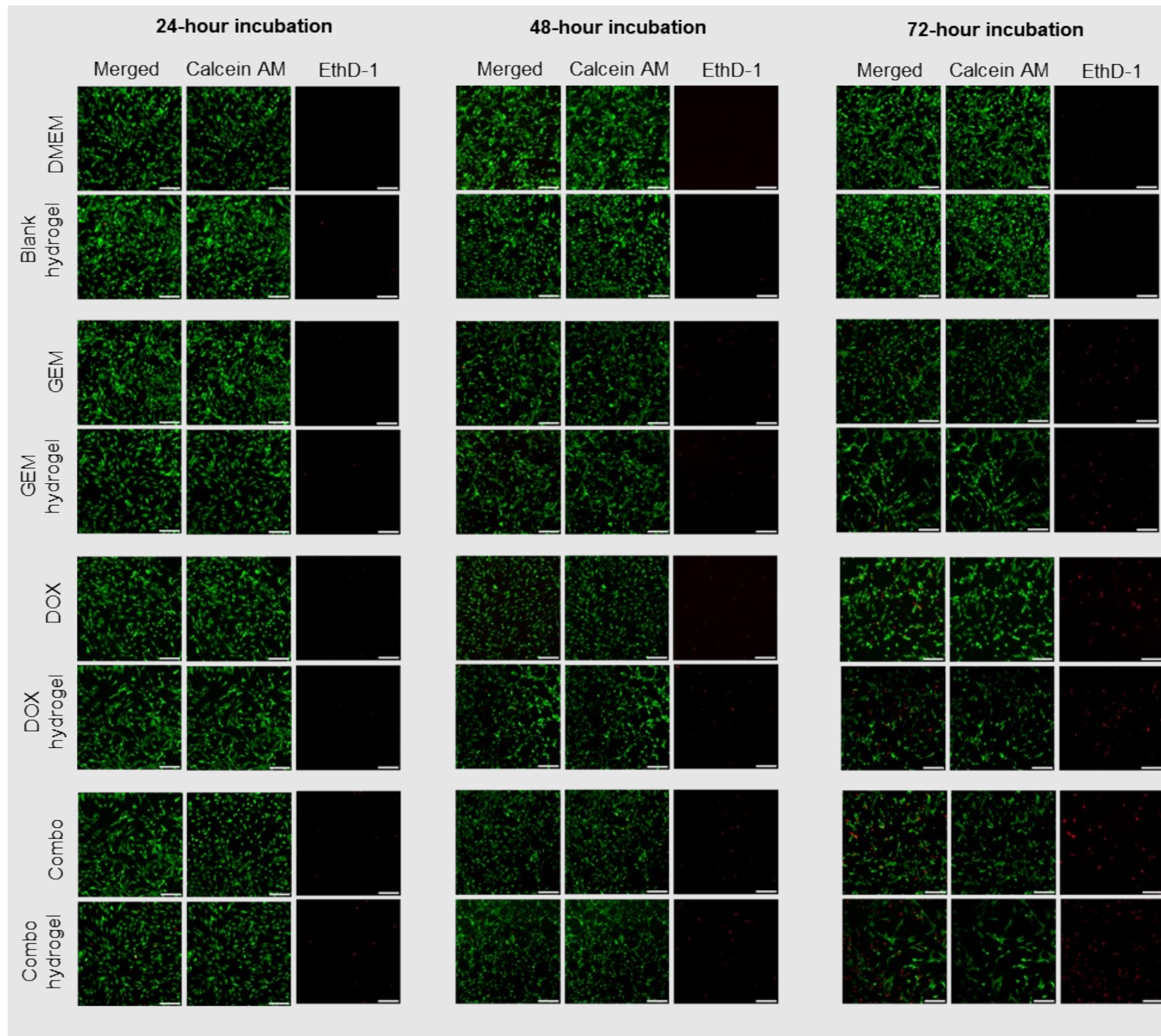
Supplemental Figure 3. Assessment of dsDNA breaks using γH2AX immunostaining. GIN28 cells exposed to single dosed DOX and GEM drugs and 1:1 DOX:GEM combinations for 24 hours. Cells were dosed with drugs applied at 0.1, 1 or 10 μM. Cells were fixed and stained for γH2AX (green) and nuclei (DAPI, blue) and imaged on an EVOS M5000 fluorescent microscope. Scale bar is 10 μm. Foci quantification was performed using ImageJ and data presented in bar charts (mean ± S.D). Statistical significance tested using one-way ANOVA and Dunnett's multiple comparisons tests. (*, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0005$; ****, $p < 0.0001$).



Supplemental Figure 4. Potency of released drugs in GIN28 cells. Drug loaded hydrogels incubated for 72h in PBS on semipermeable membrane. Released drug was collected and drug concentration measured. Solutions were diluted to appropriate drug concentrations and applied to GIN28 cells for 72h followed by viability assessment with PrestoBlue™ assay. Data presented as mean ± S.D. IC_{50} values (mean ± S.E.M) shown in graph keys and calculated using GraphPad Prism.



Supplemental Figure 5. Response summation graphs for drug-hydrogel *in vitro* testing using (A) metabolic assessment and (B) EthD-1 staining as indicators of cellular toxicity. Treatments tested for 24, 48 and 72 hours. Data presented as mean ± S.D. Statistical significance tested using one-way ANOVA and Dunnett's multiple comparisons tests. (*, $p < 0.05$).



Supplemental Figure 6. Images of LIVE/DEAD staining of blank and drug loaded hydrogels applied of 24, 48 and 72 hours. Quantification of live dead images is presented in main text Figure 5.