

Synthesis, characterisation and evaluation of hyperbranched N-(2-hydroxypropyl) methacrylamides for transport and delivery in pancreatic cell lines *in vitro* and *in vivo*

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Table S1 Reaction conditions used for the synthesis of the hyperbranched HPMA polymers

Polymer	HPMA (mg)	EDMA (mg)	RAFT agent (mg)	Initiator (mg)	Solvent (mL)	Time (hr)	% conversion	Yield (%)
HPMA-HB-7	200	13.71	21	2.3	1.6	24	90	76
HPMA-HB-20	200	13.71	21	2.3	1.2	18	93	74
HPMA-HB-40	200	13.71	21	2.3	0.8	14	97	82

Table S2 Details of physical properties of different batches of the hyperbranched polymers.

	HB-HPMA-7			HB-HPMA-20			HB-HPMA-40	
	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2
M_n GPC (g/mol)	1.8×10^4	2.4×10^4	1.5×10^4	1.1×10^5	9.4×10^4	1.6×10^5	1.8×10^6	1.1×10^6
\bar{D}	2.1	2.2	2.1	2.5	1.3	2.0	3.1	3.9
Size DLS (nm)	7	7	7	20	20	19	40	39

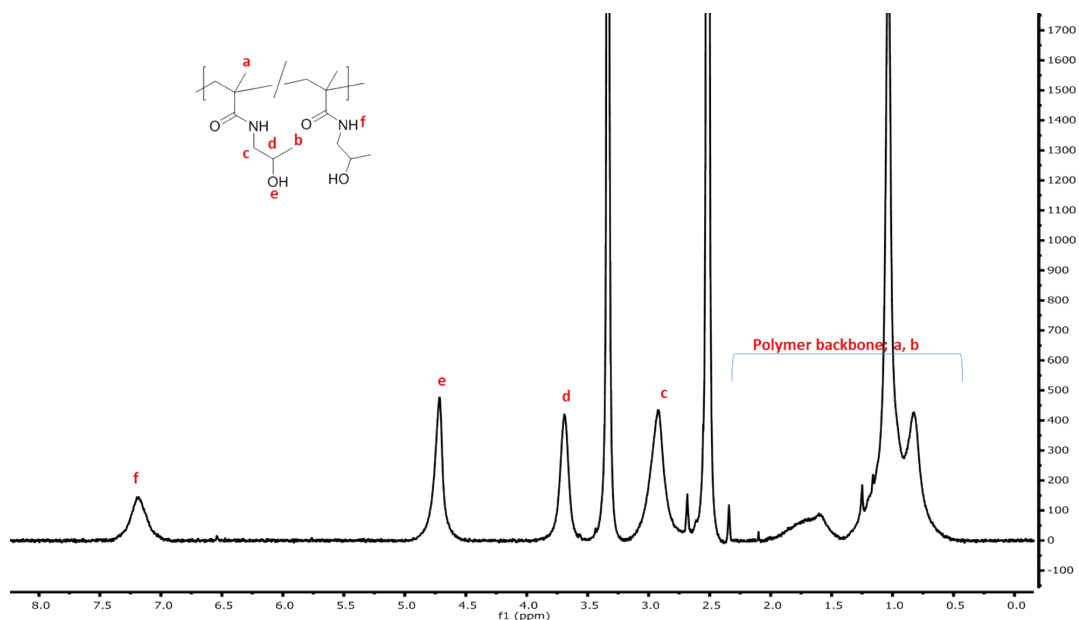


Figure S1 Representative ¹H NMR spectrum of hyperbranched HPMA polymer (HB-HPMA) in DMSO-d₆.

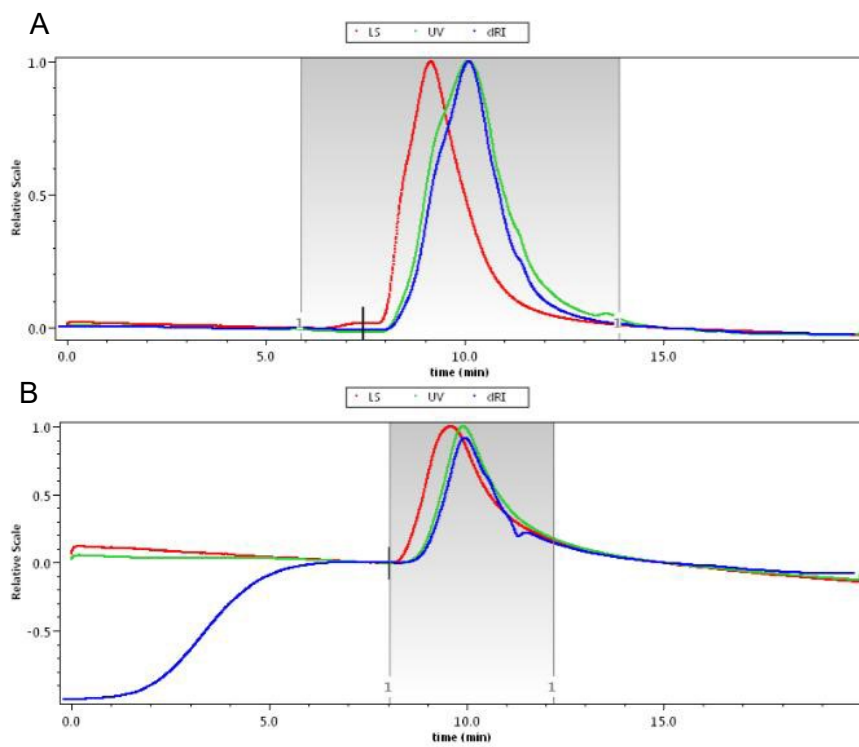


Figure S2 Representative SEC-MALLS traces of (A) HB-HPMA polymers (HB-HPMA-20) and (B) HB-HPMA-GEM polymers.

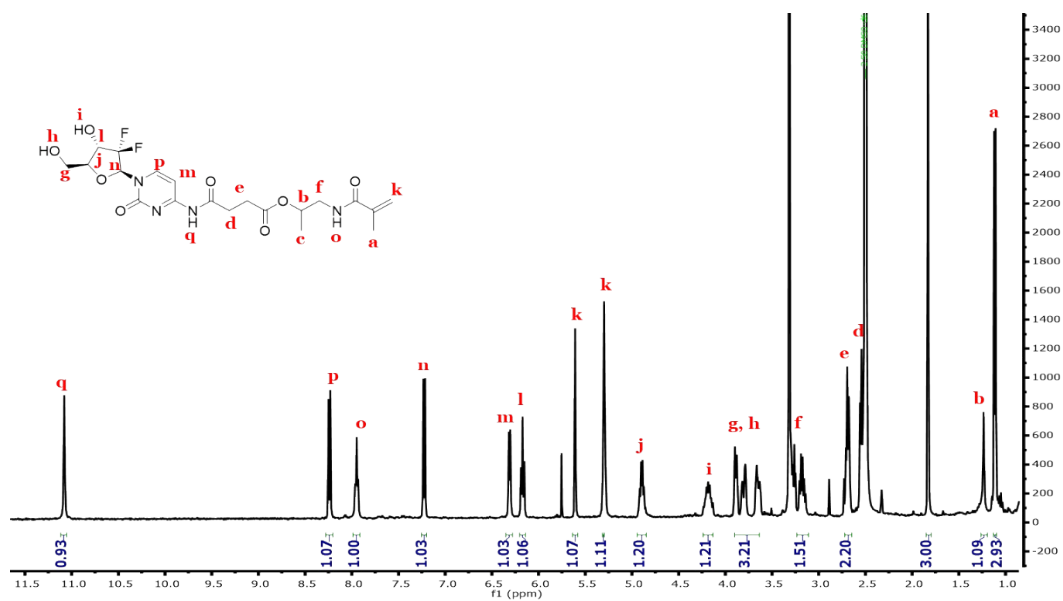


Figure S3 ^1H NMR spectrum of HPMA-GEM monomer in DMSO-d_6

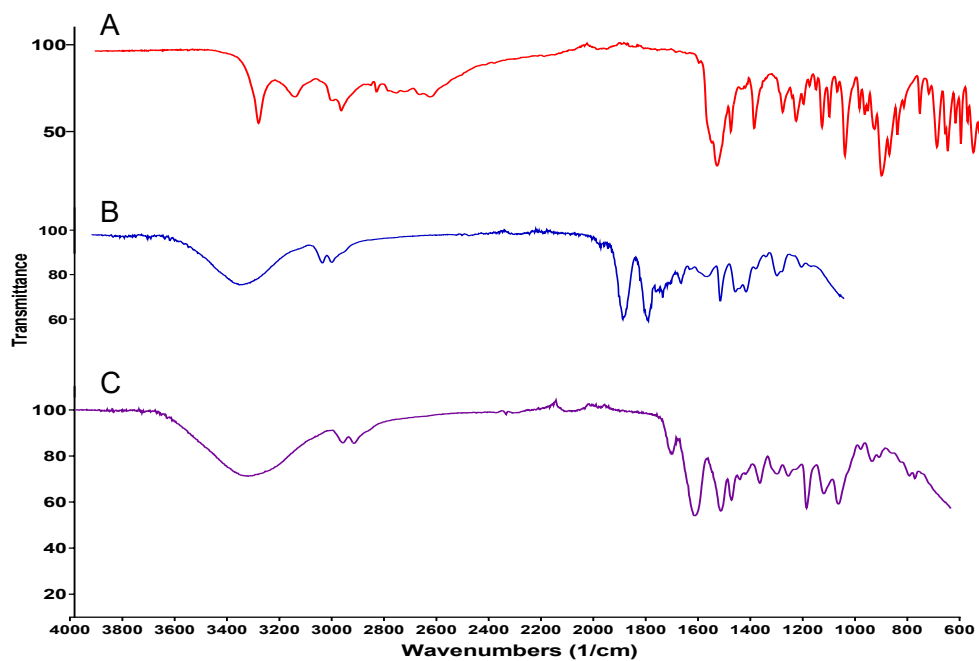


Figure S4 FT-IR spectra of (A) GEM, (B) control HPMA polymer and (C) HPMA-GEM prodrug polymer.

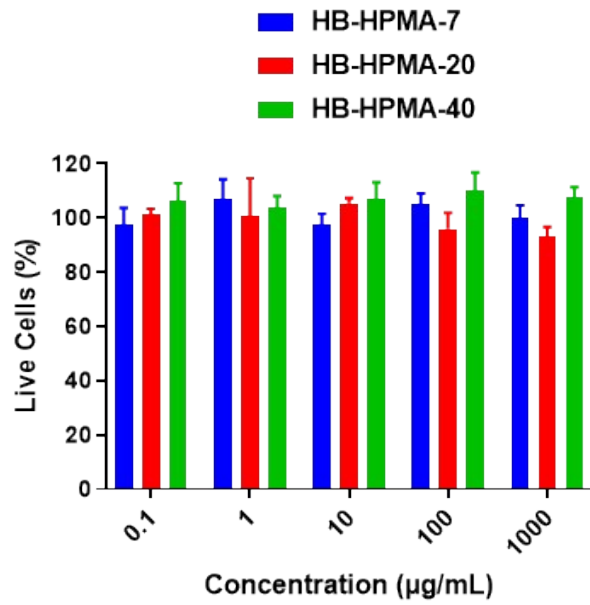


Figure S5. In vitro 2D cell viability assay at 72h post-treatment with different concentrations of the three HB-HPMA polymers on MIA PaCa-2 pancreatic cancer cell line using trypan blue dye exclusion test of cell viability.

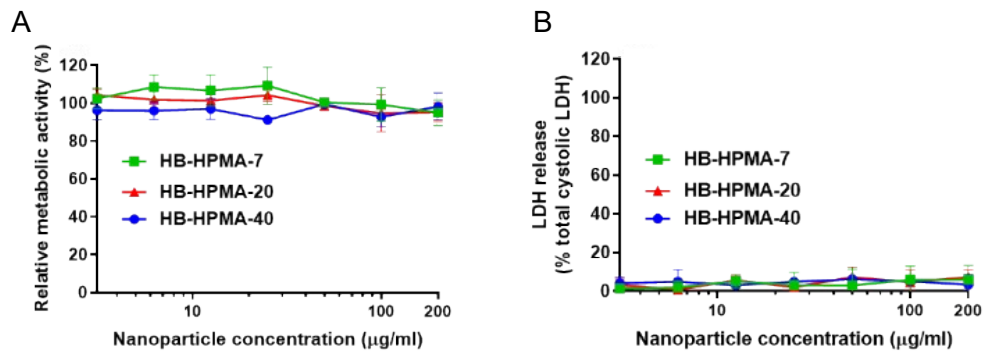


Figure S6 Evaluation of HB-HPMA polymers biocompatibility in RAW 264.7 macrophages polymers applied in 10 % FBS/DMEM for 24 hours, (1) PrestoBlue™ cell metabolic assay (2) LDH release assay. Data are presented as mean ± S.D (N = 3, n = 3).

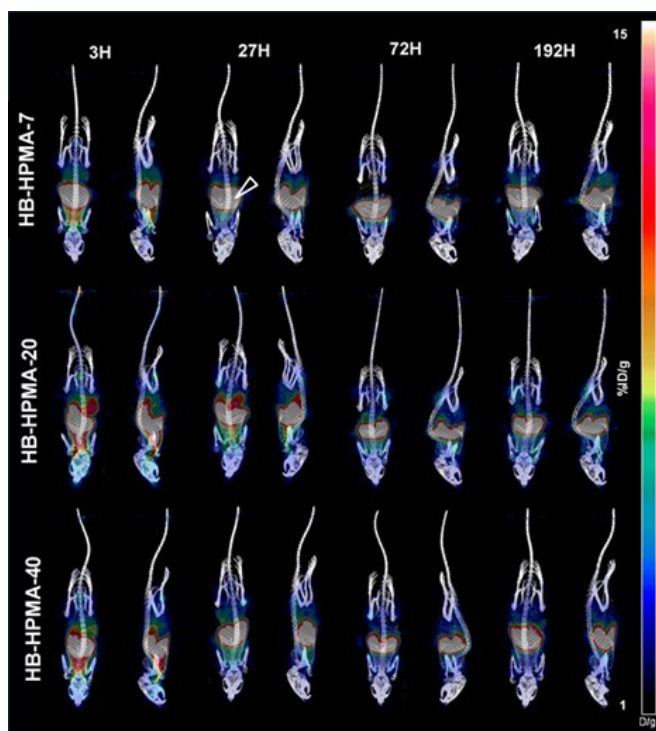


Figure S7 PET-CT imaging of ^{89}Zr -labelled HB-HPMA polymers in MIA PaCa-2 xenograft models. Representative two orientation maximum intensity projection (MIP) images of all three polymers showing the majority of the particles in circulation at 3 H post-injection. This is followed by the retention of the particles mostly in the liver in the later time points. The intensity bar represents the %ID/g whereby black = 0 %, white = 15 %ID/g, dark blue = 1 %ID/g and red = approx. 10 %ID/g. Hollow arrowhead highlights the accumulation of particles in the liver.

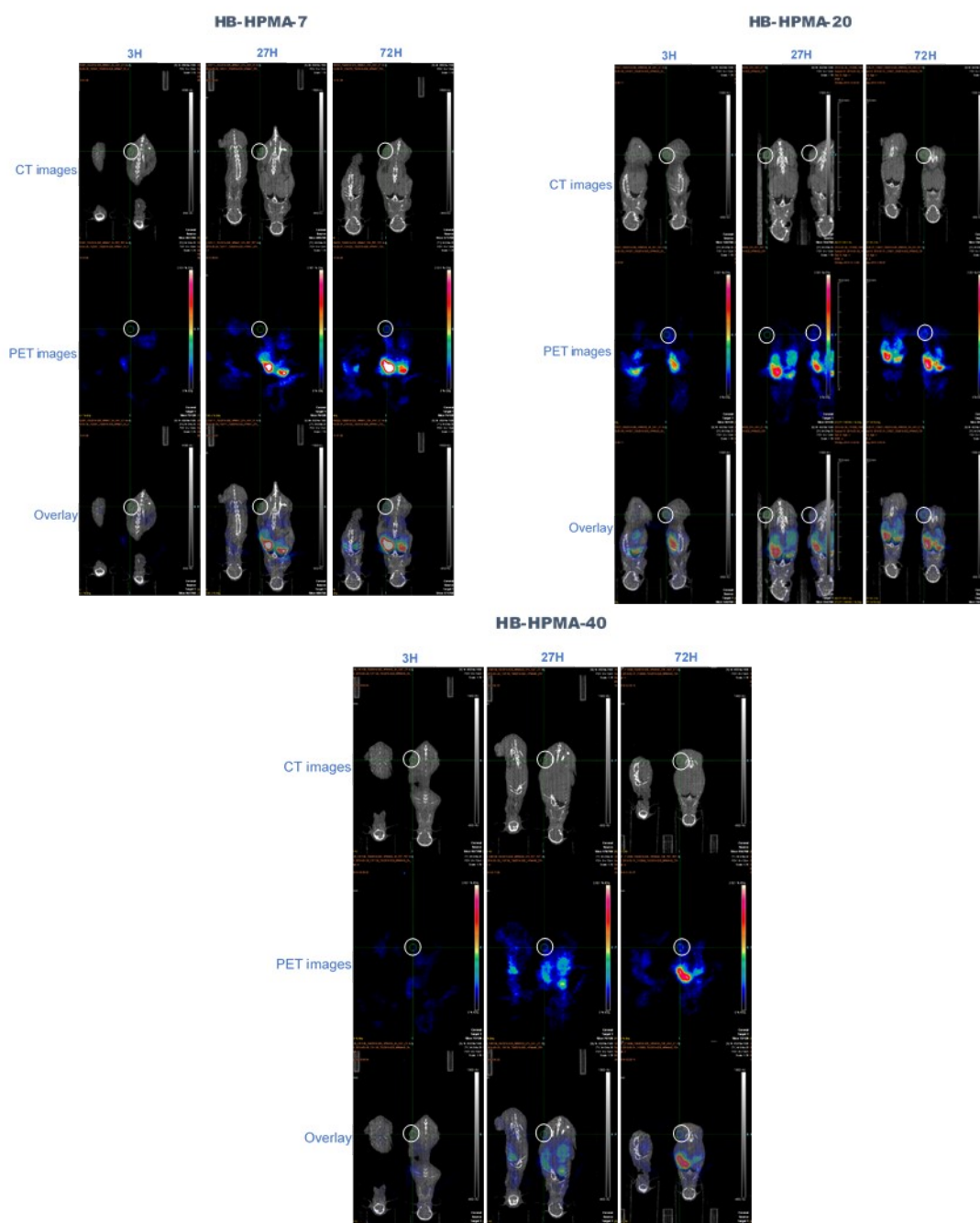


Figure S8 Representative in vivo PET-CT images of the ^{89}Zr -labelled HB-HPMA polymers at 3H, 27H and 72H post-injection in MIA PaCa-2 xenograft mice models. (A-C) Distribution behaviour of HB-HPMA-7, HB-HPMA-20 and HB-HPMA-40 at the indicated time points post-injection. White circles highlight the tumour location in each image. The intensity bar of the PET images represents the %ID/g whereby black = 0 %, blue = approx. 4 % and red = approx. 16 %.

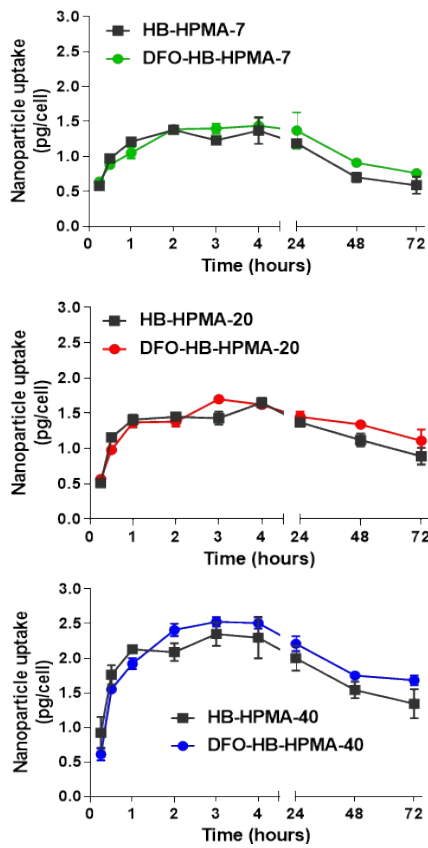


Figure S9 Comparison between the in vitro time-dependent uptake of HB-HPMA and HB-HPMA-DFO polymers in RAW264.7 macrophage cells at a concentration of 50 $\mu\text{g}/\text{m}$. Cells were cultured for 24h before assay. Hyperbranched polymers were applied in DMEM containing 10 % (v/v) FBS. Data are presented as mean \pm S.D (N = 3, n=3).

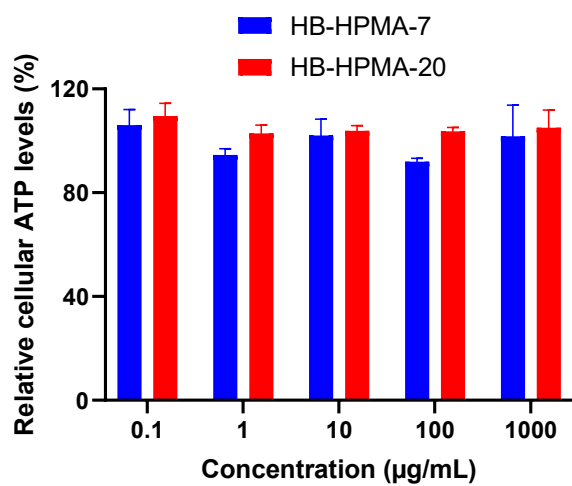


Figure S10 In vitro CellTiter-Glo[®] 3D cell viability assay for HB-HPMA-7 and HB-HPMA-20 polymers in MIA PaCa-2 pancreatic cancer cell line, (N = 4).

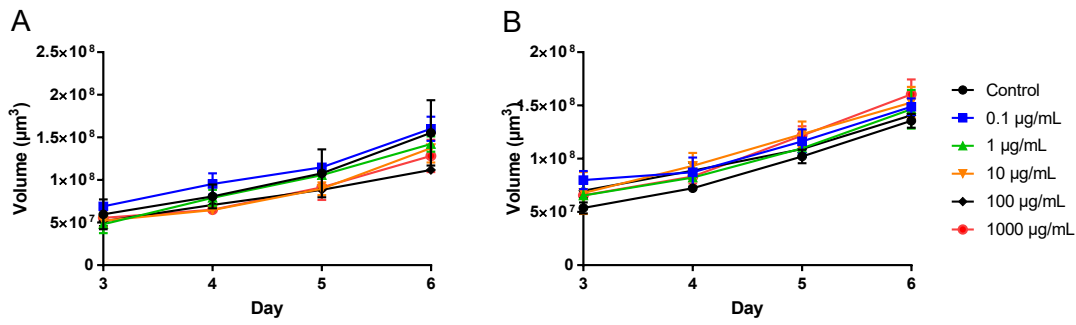


Figure S11 Graphs showing changes in spheroid volume over the 72 hours of incubation with: (A) HB-HPMA-7 and (B) HB-HPMA-20 polymers, (N = 4).

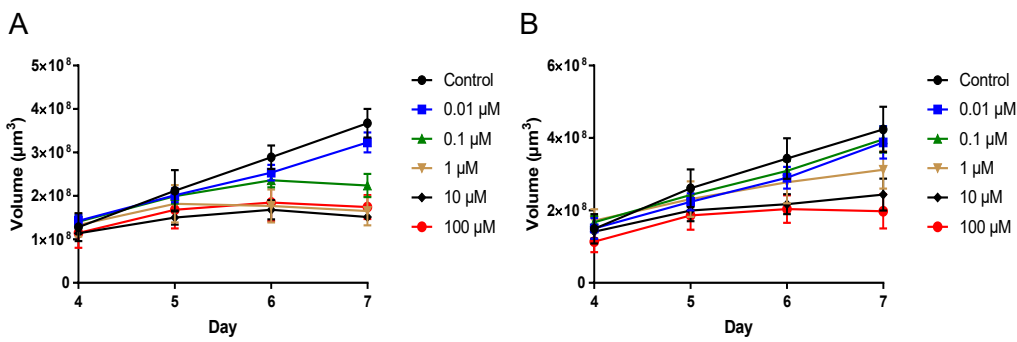


Figure S12 Graphs showing changes in spheroid volume over the 72 hours of incubation with: (A) Free GEM and (B) HB-HPMA GEM with monoculture MIA PaCa-2 pancreatic cancer cell line, (N = 4).

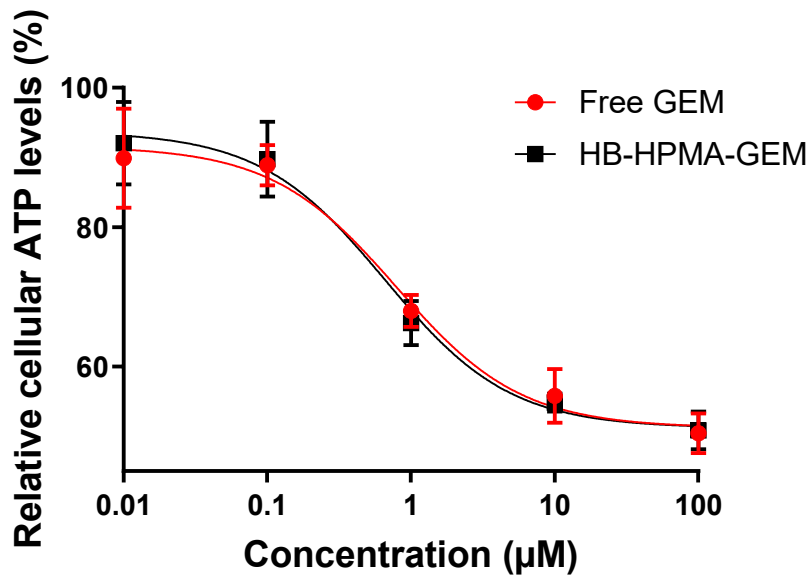


Figure S13 *In vitro* CellTiter-Glo® 3D cell viability assay for free GEM and HB-HPMA-GEM in monoculture MIA PaCa-2 pancreatic cancer cell line, (N = 4)

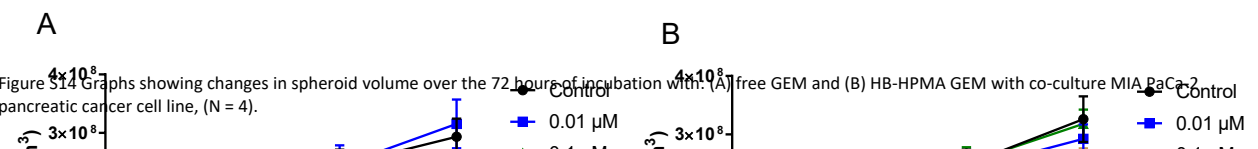


Figure S14 Graphs showing changes in spheroid volume over the 72 hours of incubation with: (A) free GEM and (B) HB-HPMA GEM with co-culture MIA PaCa-2 pancreatic cancer cell line, (N = 4).

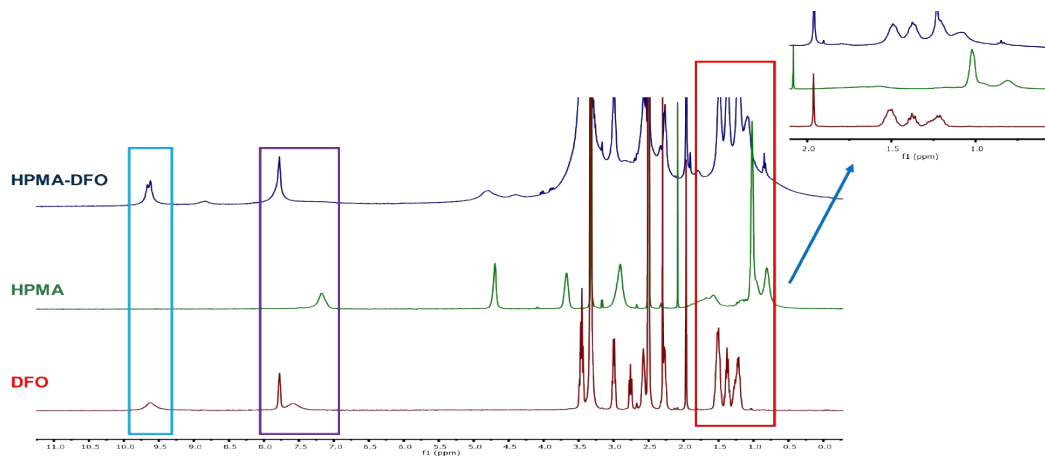


Figure S15 Representative ^1H NMR spectra for HPMA-DFO conjugation. Top) HPMA-DFO polymer. Middle) HPMA polymer and Bottom) DFO.

Figure S16 Radiographic TLC of all three polymers after ^{89}Zr labelling +/-DTPA. The signals at the bottom of the TLC represent polymer-bound radioisotope and any movement up the plate represents unbound ^{89}Zr (+DTPA plate) or free DFO in the sample bound to ^{89}Zr (-DTPA plate).

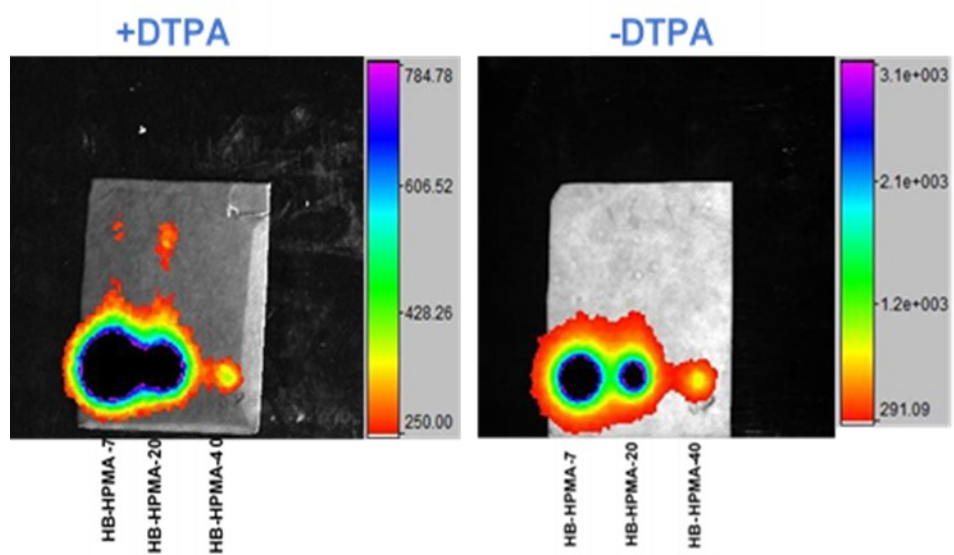


Figure S17 ¹H NMR spectrum of HPMA monomer in CDCl₃.

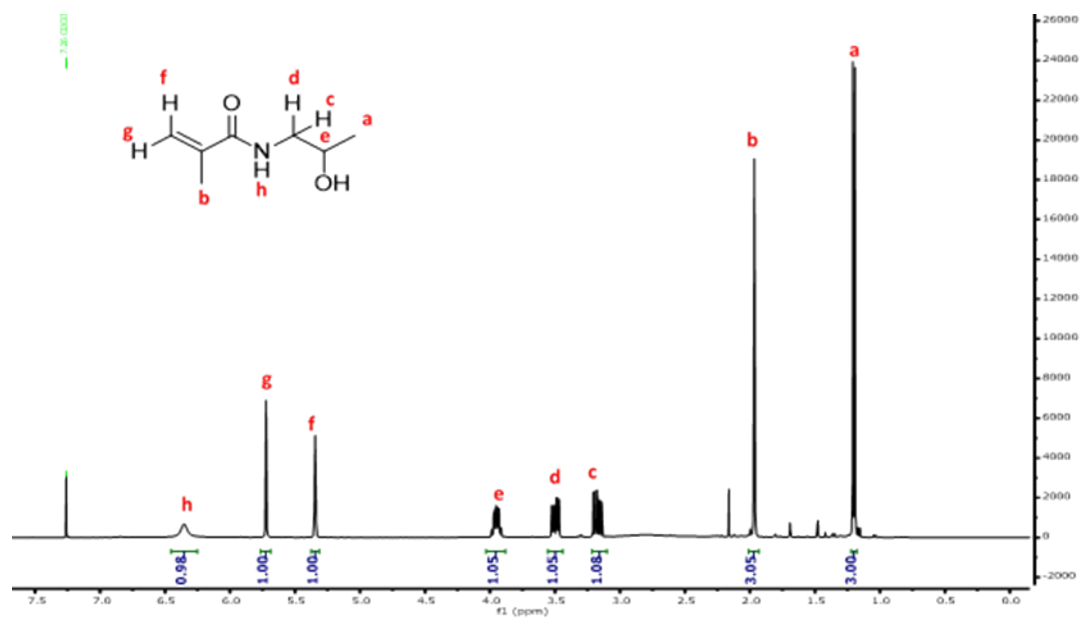


Figure S18 ¹³C NMR spectrum of HPMA monomer in CDCl₃.

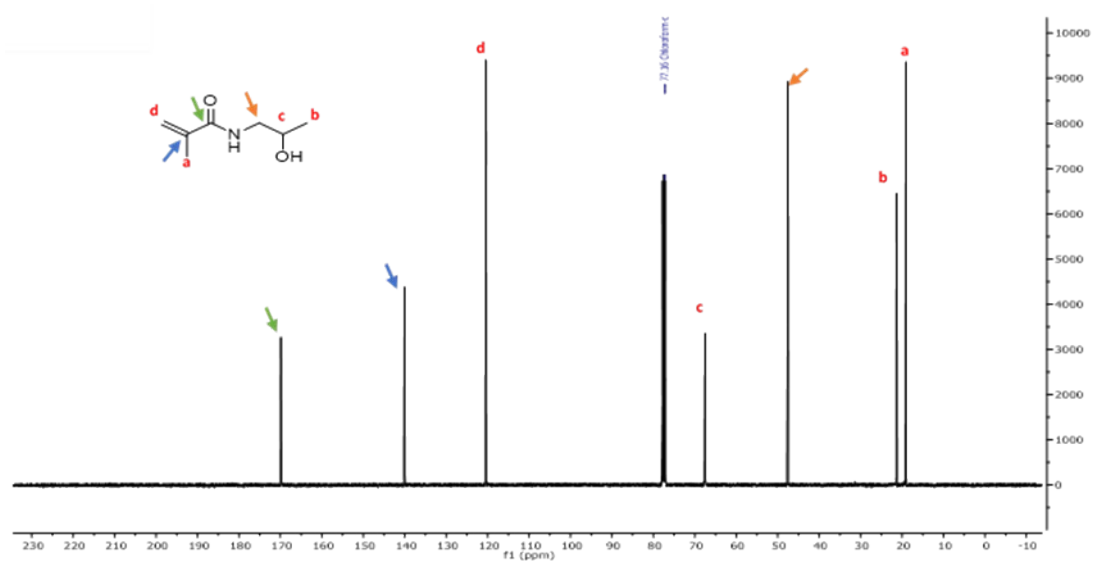


Figure S19 ^1H NMR spectrum of EDMA in CDCl_3 .

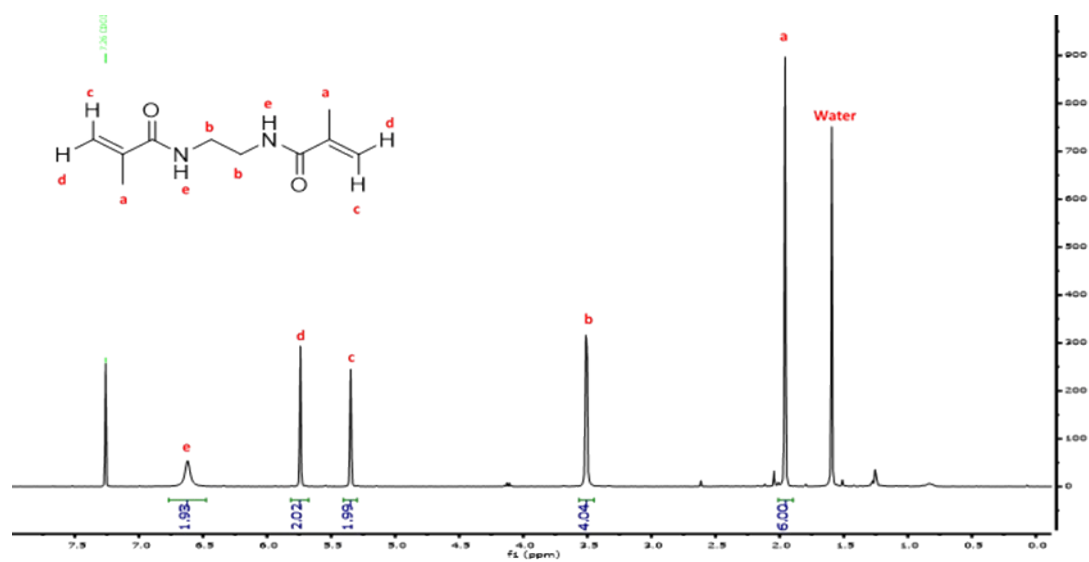


Figure S20 ^{13}C NMR spectrum of EDMA in CDCl_3 .

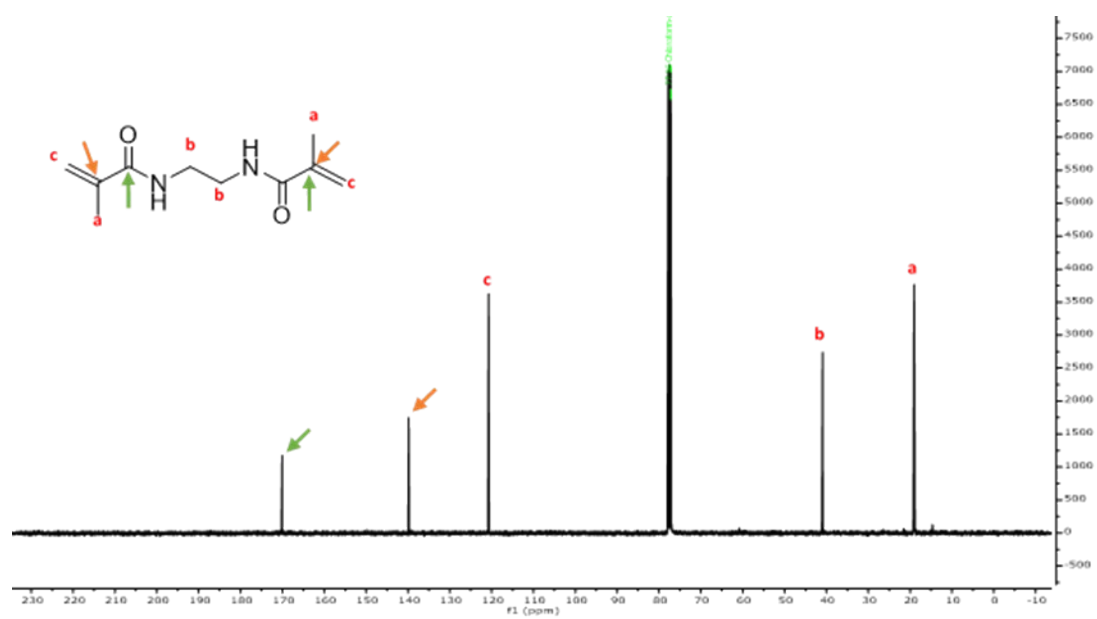
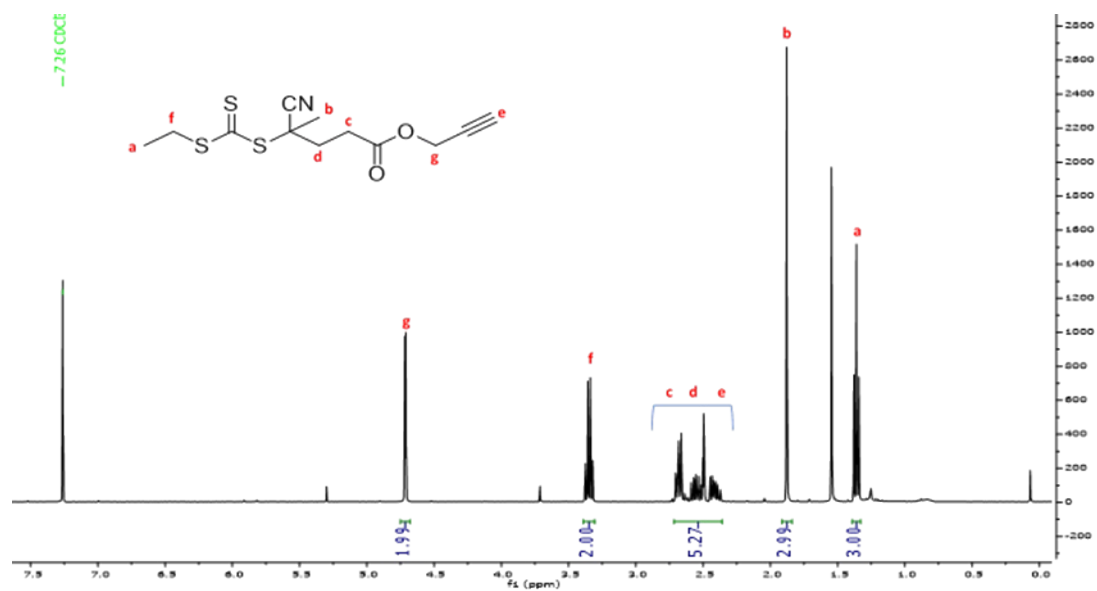


Figure S21 ^1H NMR spectrum of Alkyne-CTA in CDCl_3 .



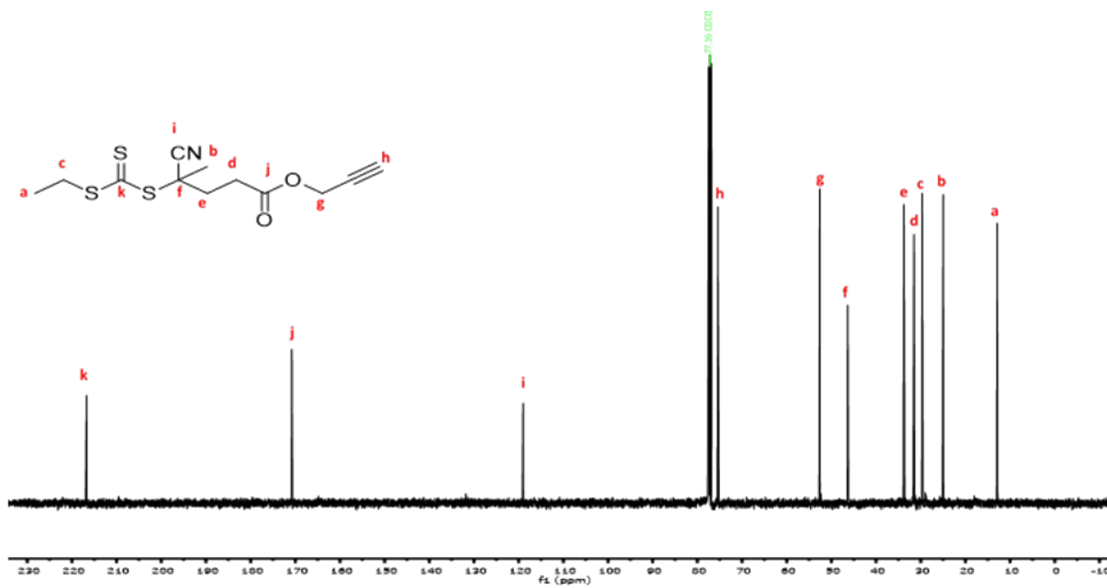


Figure S22 ^{13}C NMR spectrum of Alkyne-CTA in CDCl_3 .