Therapeutic enhancement of a cytotoxic agent using Photochemical internalisation in 3D compressed collagen constructs of ovarian cancer

Layla Mohammad Hadi*, Elnaz Yaghini, Katerina Stamati, Marilena Loizidou*, Alexander J. MacRobert

Division of Surgery & Interventional Science, University College London, London, UK

* Corresponding authors: layla.hadi.13@ucl.ac.uk; m.loizidou@ucl.ac.uk

Supplementary Material and Methods:

Cellular uptake and localisation of $TPPS_{2a}$

To investigate the uptake of $TPPS_{2a}$ within the SKOV3 and HEY cells in non-spheroid constructs (75,000 cells/construct), the constructs were incubated with $TPPS_{2a}$ (1μg/mL), for 20 hours. Afterwards, the constructs were washed with PBS and incubated with fresh cell culture medium without photosensitiser for a further 4 hours before imaging. The constructs were imaged with a fluorescence microscope with quasi-confocal structured illumination capability (20x objective, Apotome.2, Carl Zeiss). The optical sectioning capability of this microscope enabled imaging within the 3D gels with retention of imaging resolution. Fluorescence from the photosensitiser was recorded within the range of 600-700 nm using Alexa Fluor 647 channel (Figure S2).

To determine the intracellular localisation of $TPPS_{2a}$ within non-spheroid constructs, separate constructs of both cell lines were prepared (10,000 cells/model). The constructs were incubated with $TPPS_{2a}$ (3 μg/mL) for 20 hours before washing with PBS and incubating with fresh medium for 3.5 hours. The medium was then replaced with fresh medium containing LysoTracker Green (Molecular Probes, Thermo Fisher Scientific) at 100 nM concentration for 30 minutes before microscope imaging. The constructs were washed 2 times with PBS and incubated with drug-free medium for imaging with Olympus fluorescence microscope (20x objective, Olympus BX63). The fluorescence from LysoTracker green and $TPPS_{2a}$ were recorded at 520 nm and 650nm respectively. A lower number of cells were used in these constructs as it allowed the colocalisation of Lysotracker Green and $TPPS_{2a}$ to be observed more clearly (Figure S3).

Initial screening studies to determine the dosage of $TPPS_{2a}$ and cytotoxic agent saporin

The initial concentrations of $TPPS_{2a}$ used in PDT studies to determine the appropriate dose of the photosensitiser were 0.4- 0.6 μg/mL in HEY and 0.2- 0.4 μg/mL in SKOV3 monolayer cultures. For the PDT study the viabilities were measured using the MTT assay, with the aim of identifying sub-lethal photosensitiser and light doses. Based on the MTT results, the $TPPS_{2a}$ concentration ranges were identified. In the 3D non-spheroid cultures, the same $TPPS_{2a}$ concentration ranges as those for the monolayer cultures were used. In spheroid cultures the $TPPS_{2a}$ concentration range used was 0.4-0.6 μg/mL in HEY cell cultures and 0.3-0.6 μg/mL and 0.5-0.7 μg/mL (conducted in two separate PDT studies) in SKOV3 cells cultures. The saporin concentrations used in initial toxicity studies were 10-20nM.

Figures and Tables:

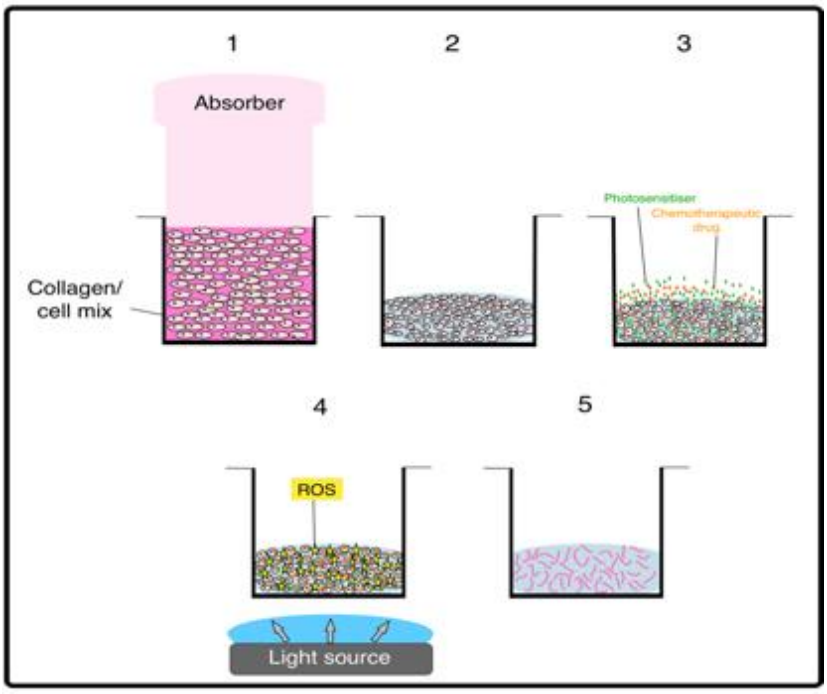


Figure S1. The application of PCI in a 3D non-spheroid compressed collagen cancer constructs after plastic compression of the hydrogels. The compression ratio is sufficient (and higher than depicted) to remove > 90 % of the excess interstitial fluid. Step 1: the compression of the Type 1 collagen hydrogel containing cancer cells via plastic compression. Step 2: the developed compressed 3D collagen cancer construct with cancer cells embedded in the extracellular matrix. Step 3: the photosensitiser and chemotherapeutic drugs are added to the 3D construct and undergo incubation. Step 4: the cells absorb the drugs and the 3D construct undergoes illumination from the underside by light of a suitable wavelength after removal of excess drug and washing of the constructs. Step 5: cancer cells have been destroyed by the effect of treatment.

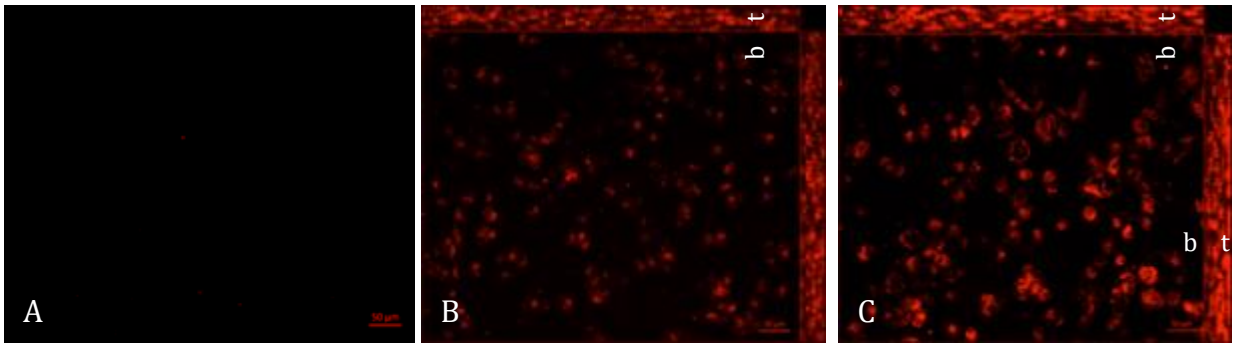


Figure S2. Intracellular uptake of $TPPS_{2a}$ in HEY and SKOV3 non-spheroid 3D compressed collagen constructs. (A): control constructs for SKOV3 cells, (B): intracellular uptake of $TPPS_{2a}$ in HEY non-spheroid 3D compressed collagen construct; (C): intracellular uptake of $TPPS_{2a}$ in SKOV3 non-spheroid 3D compressed collagen construct. Images (B and C) were obtained through stacking with fluorescence microscope (20x objective, Apotome.2, Carl Zeiss). The fluorescence of the photosensitiser was measured between 600-700 nm using Alexa Fluor 647 channel. The scale bar presented in each image is 50 μm. t: top; b: bottom. As shown in Figure S2, in comparison to the control 3D construct (A), bright fluorescence signals of the $TPPS_{2a}$ were detected in both HEY and SKOV3 constructs incubated with $TPPS_{2a}$, indicating the intracellular uptake of the photosensitiser in the 3D constructs (Figure S2 B and C). These results show that in the treated 3D constructs, the $TPPS_{2a}$ was taken up by the cells rather than becoming bound in the collagen. The control collagen construct for SKOV3 cells without photosensitiser showed negligible autofluorescence levels (Figure S2 A). Negligible cell autofluorescence was also observed in control constructs with the HEY cells (data not shown).

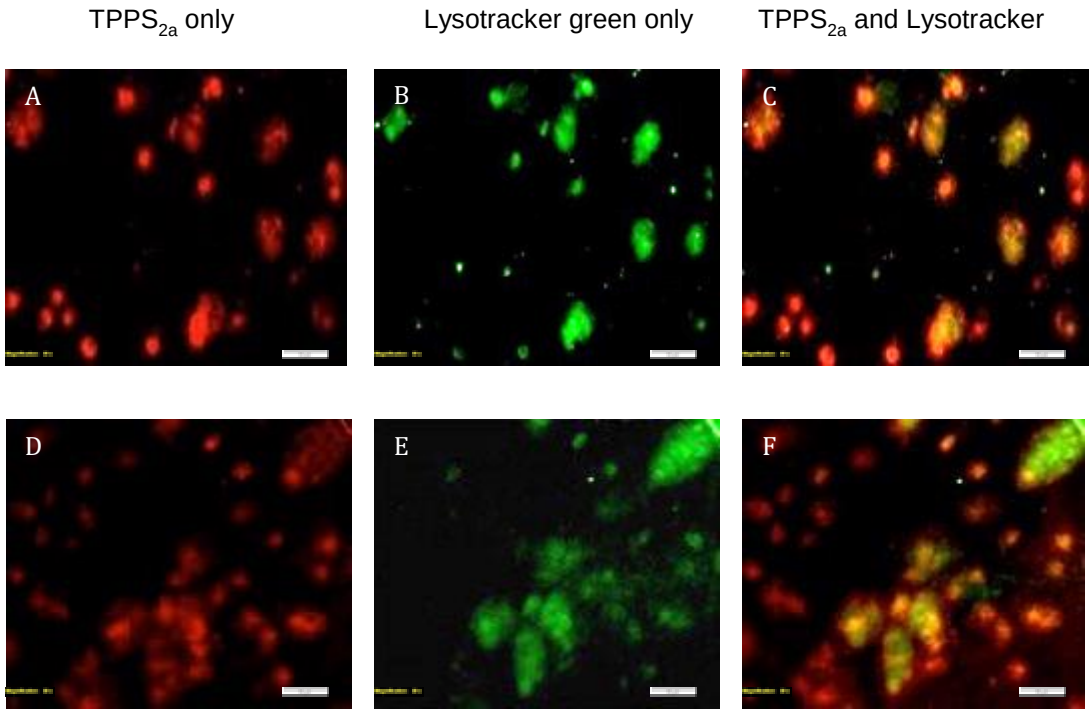


Figure S3. Intracellular localisation of $TPPS_{2a}$ in 3D compressed collagen non-spheroid constructs of SKOV3 (A-C) and HEY (D-F) cells. The cells were seeded at density of 10,000 cells/construct. The constructs were treated with $TPPS_{2a}$ (3μg/ml) and Lysotracker green (100nM) and imaged using a fluorescence microscope (20x objective, Olympus BX63). A and D show the localisation of $TPPS_{2a}$ and B and E show the localisation of Lysotracker green stain within the small cellular aggregates. The co-localisation of both $TPPS_{2a}$ and Lysotracker green is shown in C and F. The scale bar presented in each image is 50μm.

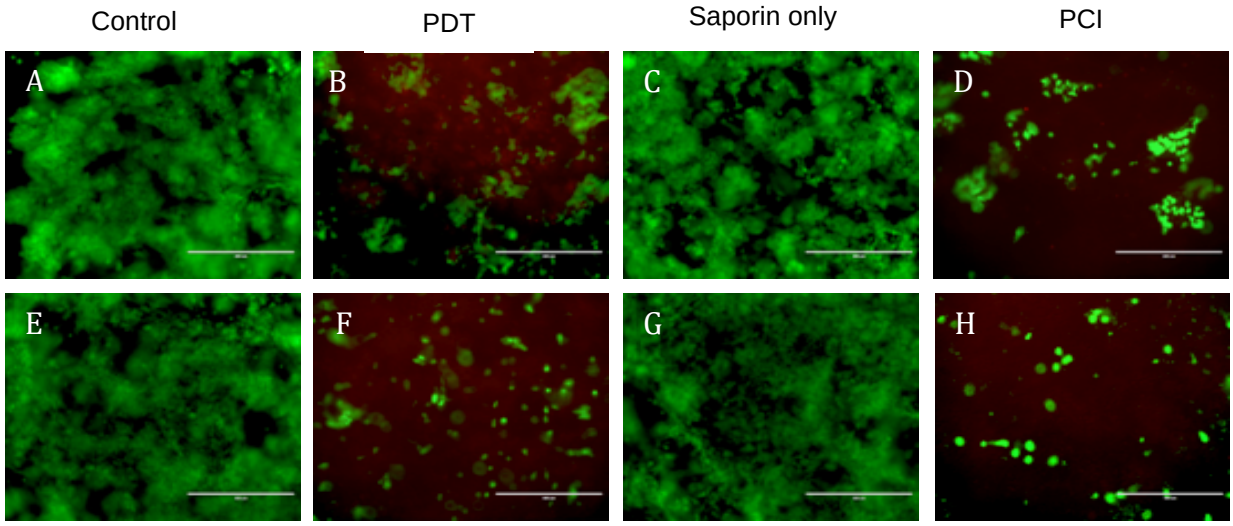
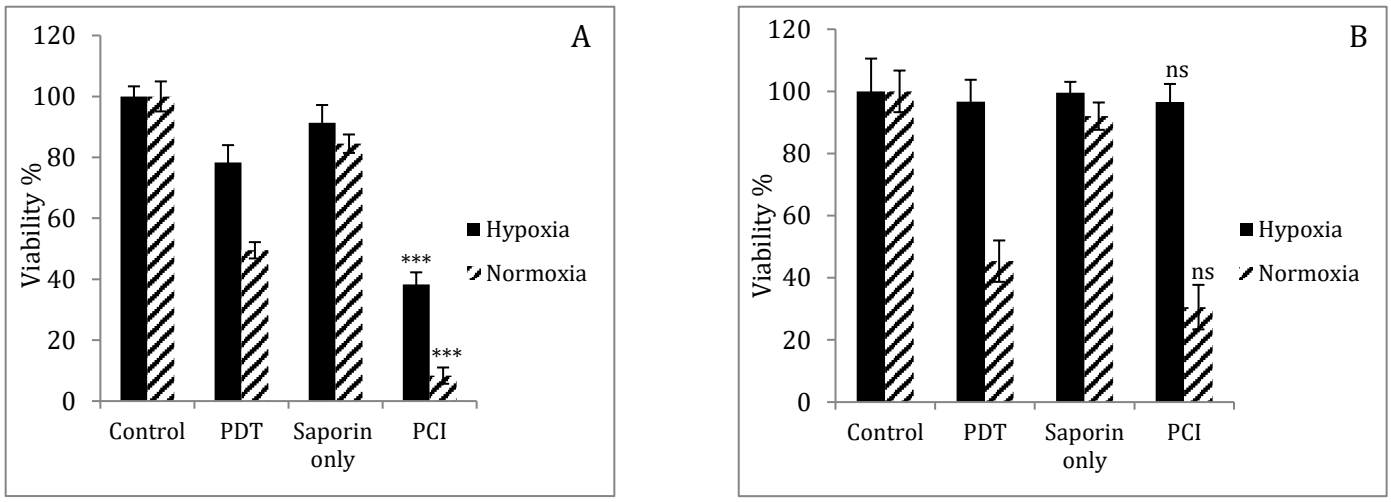


Figure S4. Live-dead images of spheroid 3D compressed collagen constructs of HEY (A-D) and SKOV3 (E-H) cells after undergoing treatment with $TPPS_{2a}$ only (0.5μg/ml for HEY cells) (B) and (0.7μg/ml for SKOV3 cells) (F), saporin only (20nM for HEY cells) (C) and (40nM for SKOV3 cells) (G) and a combination of both drugs (D and H) following 3 minutes of exposure to light and a 48 hour post-illumination assay time point. 3D constructs were incubated with the Live/dead solution containing Calcein-AM to stain live cells (green) and Ethidium homodimer-1 to stain dead cells (red). The scale bar presented in each image is 400μm.



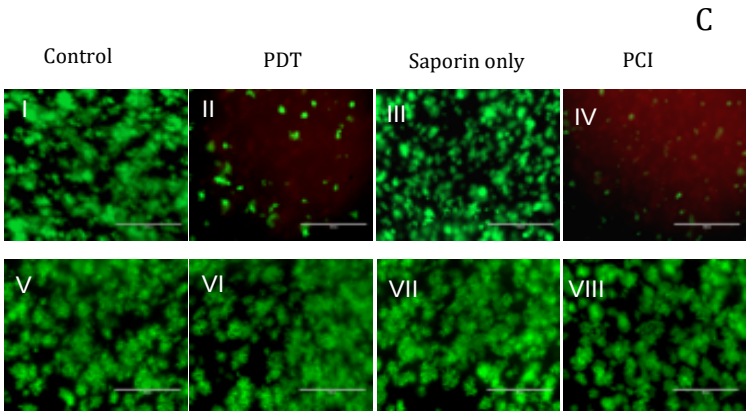


Figure S5. A. Percentage viability of SKOV3 cell in 2D monolayer experiments after PCI treatment in normoxic condition compared to hypoxic condition using $TPPS_{2a}$ only (0.3 µg/ml) only for PDT, saporin 40 nM only and a combination of both drugs for PCI as well as a light irradiation period of 7 minutes. ***p<0.001, the p values show the significance difference between PDT and PCI. B. Percentage viability of SKOV3 cell 3D spheroid constructs after PCI treatment in normoxic condition compared to hypoxic condition using $TPPS_{2a}$ only (0.7 µg/ml) only for PDT, saporin 40 nM only and a combination of both drugs for PCI as well as a light irradiation period of 7 minutes. Alamar Blue assay was carried out 48 hours after exposure to light. No significance difference (ns) between PDT and PCI can be observed; C. Live-dead images of SKOV3 cell spheroid constructs after PCI treatment in normoxic condition (I-IV) compared to hypoxic condition (V-VIII). The scale bar presented in each image is 400µm.

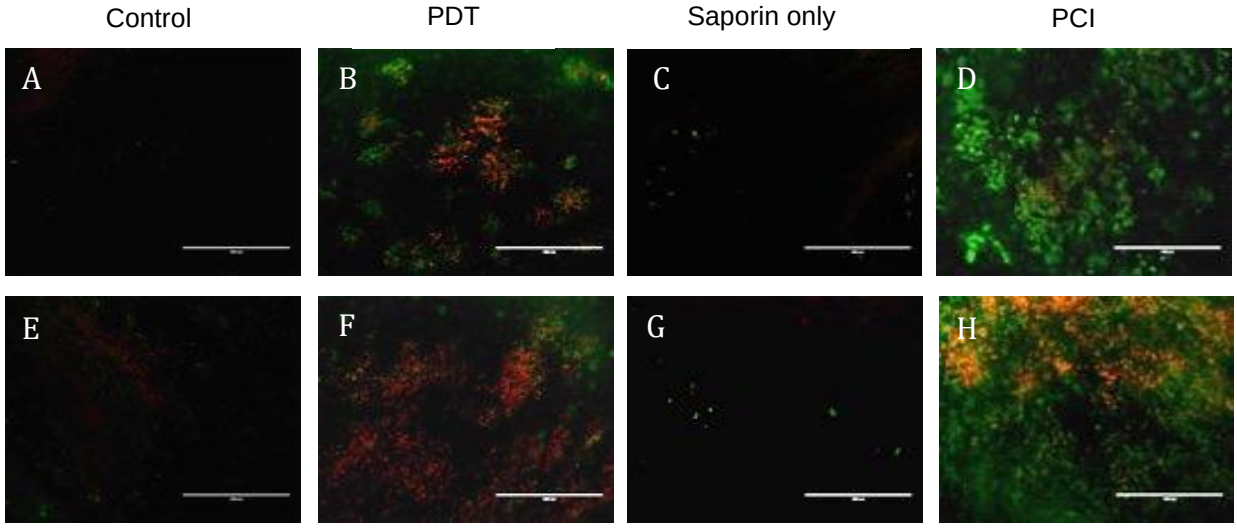


Figure S6: Necrosis and apoptosis in PDT (B and F) and PCI (D and H) treated 3D compressed spheroid constructs of HEY cells 24 hours (A- D) and 48 hours (E- H) post illumination. In PDT only treated constructs $TPPS_{2a}$ was used at concentrations of 0.5 µg/ml (B and F). The saporin concentration used in this experiment was 20nM. Annexin V- was used to indicate apoptosis (green) and propidium iodide was used to indicate necrosis (red). The scale bar presented in each image is 400µm.

Cell line	Small	Medium	Large
	Area: < 5000	Area: 5000 -12000	Area: >12000
SKOV3	1870- 4930 (3610)	5000 - 11720 (7520)	12170- 13710 (12780)
HEY	3460- 4740 (4100)	5310-11990 (790)	12670-82930 (18570)

Table S1. The size classification of SKOV3 and HEY cell spheroids based on the cross-sectional area (μm^2) measured at day 7. The ranges of measured values obtained by image analysis are listed for each size range with median values shown in parentheses.

	2D culture							
Cell line	Incubation period post light exposure (hour)	Light Exposure period (minute)	Saporin concentration (nM)	PDT only (% mean viability ± % SD)	Saporin only (% mean viability ± % SD)	PCI (% mean viability ± %SD)	PCI efficacy ratio vs. PDT	PCI efficacy ratio vs. saporin only
SKOV3	48	3	10	74±4	95±4	44±5	2	2
HEY	48	3	10	69±7	92±8	38±6	2	2
SKOV3	48	5	10	36±6	95±7	24±5	2	4
HEY	48	5	10	40±7	98±1	19±5	2	5
SKOV3	48	7	10	24±5	93±3	16±3	2	6
HEY	48	7	10	26±5	92±4	16±2	2	6
SKOV3	48	3	20	76±8	91±7	27±2	3	3
HEY	48	3	20	75±6	91±5	24±2	3	4
SKOV3	48	5	20	52±8	92±8	18±8	3	5
HEY	48	5	20	40±4	88±5	11±3	4	8
SKOV3	48	7	20	34±9	92±5	8±3	5	12
HEY	48	7	20	30±2	92±5	6±2	6	17
SKOV3	96	3	20	66±5	94±9	21±3	3	5
HEY	96	3	20	64±5	90±5	14±6	5	7
SKOV3	96	5	20	40±6	95±7	13±3	3	7
HEY	96	5	20	32±6	93±6	8±4	4	12
SKOV3	96	7	20	36±7	94±7	4±1	9	23
HEY	96	7	20	20±4	87±10	3±1	7	32

Table S2. Summary of percentage viabilities ± %SD and PCI efficacies in 2D monolayer cultures of SKOV3 and HEY cells.

	3D culture							
Cell line	Incubation period post light exposure (hour)	Light exposure period (minute)	Saporin concentration (nM)	PDT only (% mean viability ± %SD)	Saporin only (% mean viability ± %SD)	PCI (% mean viability ± %SD)	PCI efficacy vs. PDT	PCI efficacy vs. saporin only
SKOV3	48	3	10	77±8	93±6	58±8	1	2
HEY	48	3	10	74±7	96±5	48±2	2	2
SKOV3	48	5	10	58±7	91±6	41±7	1	2
HEY	48	5	10	46±5	94±5	29±5	2	3
SKOV3	48	7	10	49±4	92±8	29±6	2	3
HEY	48	7	10	37±6	97±5	22±4	2	5
SKOV3	48	3	20	81±5	96±3	40±6	2	2
HEY	48	3	20	81±6	98±7	29±4	3	3
SKOV3	48	5	20	58±5	92±4	21±7	3	5
HEY	48	5	20	44±6	96±5	23±3	2	4
SKOV3	48	7	20	48±6	93±5	11±2	4	8
HEY	48	7	20	37±3	97±5	12±1	3	8
SKOV3	96	3	20	69±3	98±2	28±1	3	4
HEY	96	3	20	67±2	99±4	18±2	4	5
SKOV3	96	5	20	52±3	99±2	17±2	3	6
HEY	96	5	20	37±2	98±7	12±3	3	8
SKOV3	96	7	20	45±1	99±3	6±2	7	16
HEY	96	7	20	25±4	90±8	7±1	4	13

Table S3. Summary of percentage viabilities ± %SD and PCI efficacies in 3D non-spheroid cultures of SKOV3 and HEY cells.

	3D culture			
Cell line	Incubation period post light exposure (hour)	Light exposure period (minute)	Saporin concentration (nM)	Alpha values
SKOV3	48	3	10	1.2
HEY	48	3	10	1.5
SKOV3	48	5	10	1.3
HEY	48	5	10	1.5
SKOV3	48	7	10	1.6
HEY	48	7	10	1.6
SKOV3	48	3	20	1.9
HEY	48	3	20	2.7
SKOV3	48	5	20	2.5
HEY	48	5	20	1.8
SKOV3	48	7	20	4.1
HEY	48	7	20	3.0
SKOV3	96	3	20	2.4
HEY	96	3	20	3.7
SKOV3	96	5	20	3.0
HEY	96	5	20	3.0
SKOV3	96	7	20	7.4
HEY	96	7	20	3.2

Table S4. Summary of alpha values for non-spheroid cultures of SKOV3 and HEY cells.