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Supplementary Materials for

Peptide-protein coassembling matrices as a biomimetic 3D model of ovarian cancer

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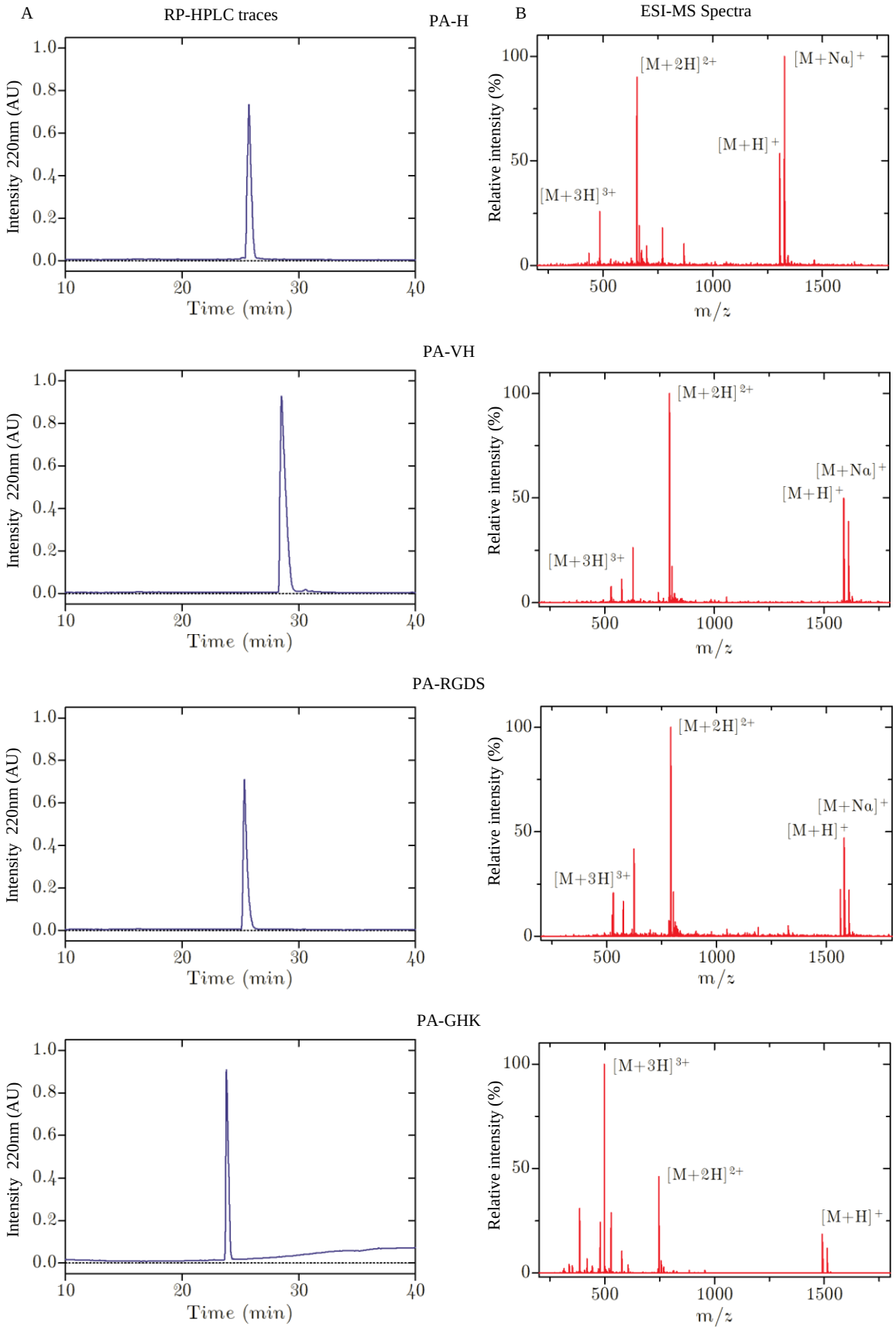


Fig. S1. Peptide purity of in-house synthesized sequences PA-H, PA-VH, PA-RGDS, and PA-GHK. (**A**) Reverse-phase high-performance liquid chromatography (RP-HPLC) traces. (**B**) Electrospray ionization mass spectra (ESI-MS).

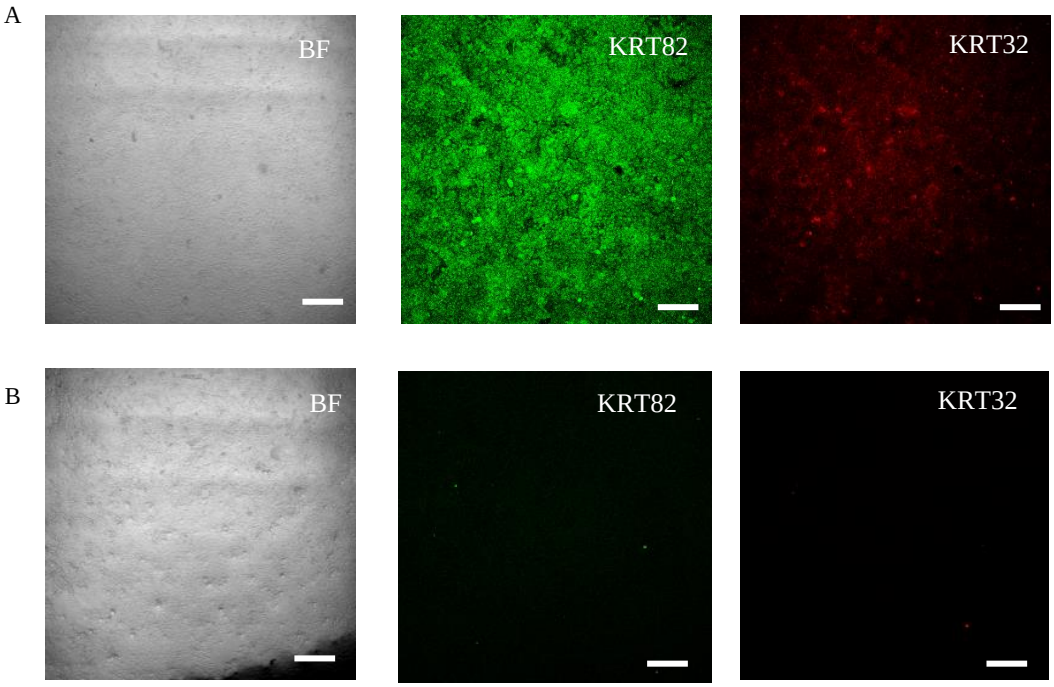


Fig. S2. Immunohistochemical (IHC) staining for Keratin type I (KRT32) and Keratin type II (KRT82). (**A**) IHC staining for KRT82 and KRT32 in a confluent monolayer of epithelial ovarian cancer cells. (**B**) IHC controls of the monolayers stained with only secondary antibody. Scale bar = 100 µm.

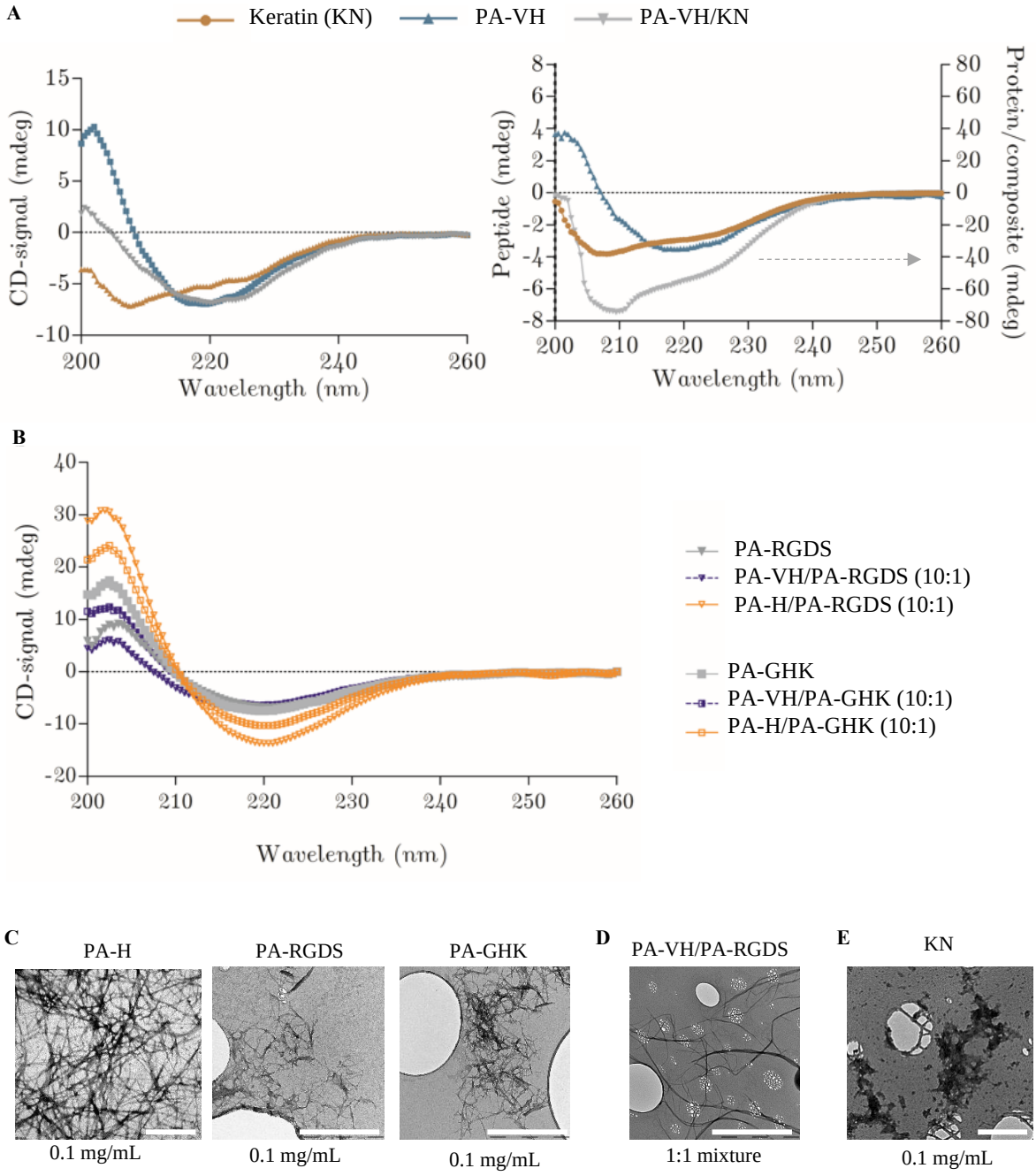


Fig. S3. Hydrogel (PA/KN) characterization at the nanoscale. (**A**) Circular dichroism (CD) Spectra of pure **KN** and **PA-VH**, as well as, **PA-VH/KN** (1:1) mixed in equal concentration (0.1 mg/mL in HEPES; right) and equimolar (0.02 mmol/dm^3 in HEPES; left). (**B**) CD-Spectra of pure **PA-RGDS** and **PA-GHK**, and in mixtures with **PA-H** and with **PA-VH** (All PA solutions were pre-prepared at 0.1 mg/mL in HEPES). (**C**) Transmission electron microscopy (TEM) micrographs of pure peptide **PA-H**, **PA-RGDS**, and **PA-GHK**. (**D**) Representative TEM micrograph of a mixture of two peptides, in this case **PA-VH/PA-RGDS**, mixed 1:1 at equal concentration (0.1 mg/mL in HEPES). (**E**) TEM micrograph of pure keratin (**KN**) prepared in HEPES (0.1 mg/mL). Scale bars = 500 nm.

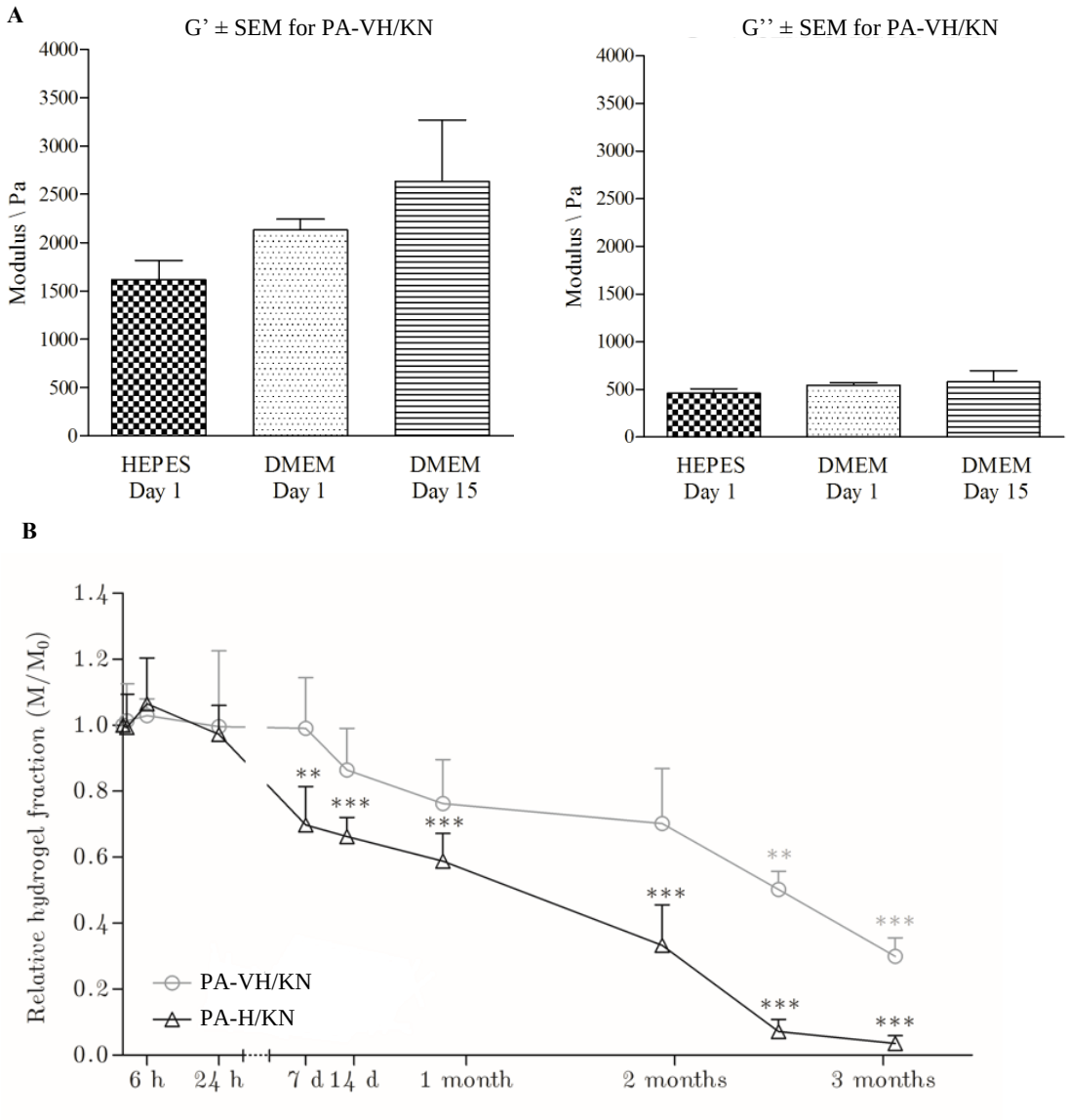
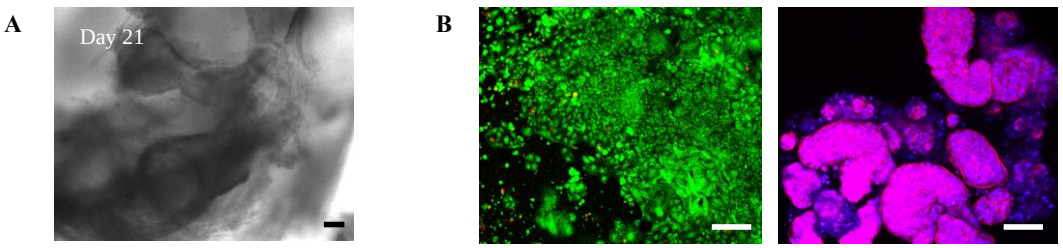
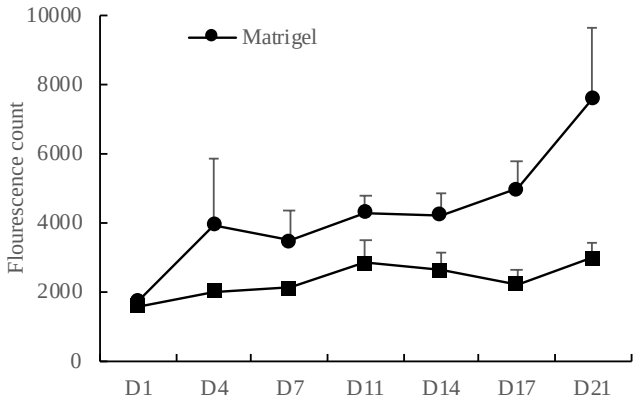


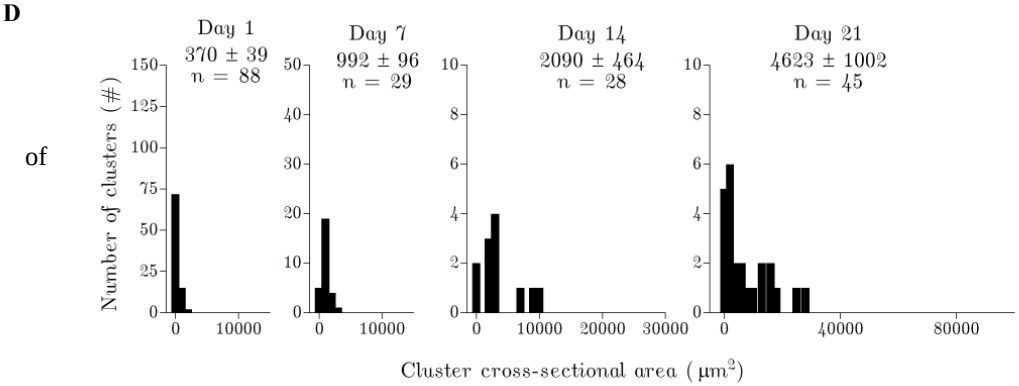
Fig. S4. Hydrogel (PA/KN) stiffness and characterization at the macroscale. (**A**) G' (storage modulus) and G'' (loss modulus) were measured at 25°C at a constant frequency of 1 Hz in the 0.01-10% strain during the amplitude sweep, while the oscillation frequency experiments were carried out at a 0.1% fixed strain along 0.1-100 Hz, using a dynamic rheology (DHR-3, TA Instruments, USA). Graphical plot shows data for **PA-VH/KN** hydrogels after 1 day in HEPES buffer and after 1 and 15 days in cell medium at 37°C. (**B**) Graphical plot showing **PA/KN** hydrogel stability in HEPES 10 mM over a period of 3 months. The hydrogel mass was measured after a 4-hour hydrogel formation period, and recorded as 'M_0', with all subsequent timepoints (t) reported as 'M_0-M_t/M_0'. Data shown for **PA-VH/KN** (n=4) and **PA-H/KN** (n=4).





	Matrigel		
	Mean	SD	n
D1	1708	169	5
D4	3954	1929	3
D7	3492	895	3
D10	4289	522	3
D14	4236	601	3
D17	4988	801	3
D21	7600	2037	3

	PA-VH/KN		
	Mean	SD	n
D1	1568	138	4
D4	2015	207	5
D7	2119	163	5
D10	2833	699	5
D14	2615	531	5
D17	2219	404	5
D21	2961	491	5



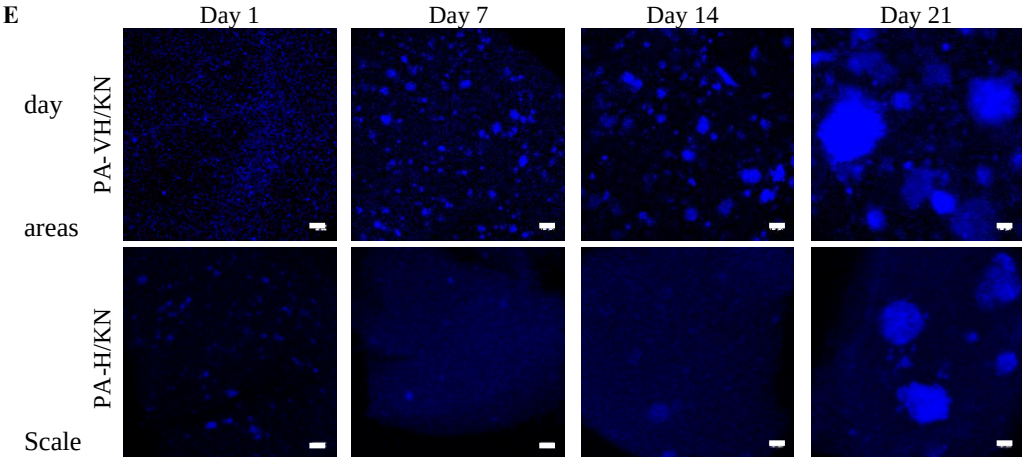


Fig. S5. Monocultures of ovarian cancer cells in PA-VH/ KN hydrogels and Matrigel. (**A**) Brightfield image of a **PA-VH/KN** hydrogel after 21 days of incubation in cell medium, but without cells. Image used as reference to identify cells in **PA/KN** hydrogel matrix. (**B**) Live (green) / Dead (red) staining of ovarian cancer cells detached from Matrigel and now adhering to the well bottom 21 days after encapsulation. IF image staining for F-actin (Phalloidin, red) and nuclei (DAPI, blue) of ovarian cancer mono-culture spheroids in remaining Matrigel fragmentations at day 21. Scale bars = 100 μm. (**C**) Readout of fluorescence values from the AlamarBlue assay of monocultures ovarian cancer cells in **PA-VH/KN** hydrogels. Data represented graphically and in a table with Mean, SD, and n (number of samples). (**D**) Spheroid size distribution plot, shown as the cross-sectional area against number of tumours, in **PA-H/KN** hydrogels at day 1, 7, 14 and 21. IF images from *(D)* were used to determine the cross-sectional tumour areas each time point. (**E**) IF images stained for nuclei (DAPI, blue), of ovarian cancer spheroids growing in **PA-H/KN** and **PA-VH/KN** hydrogels. Scale bars = 100 μm.

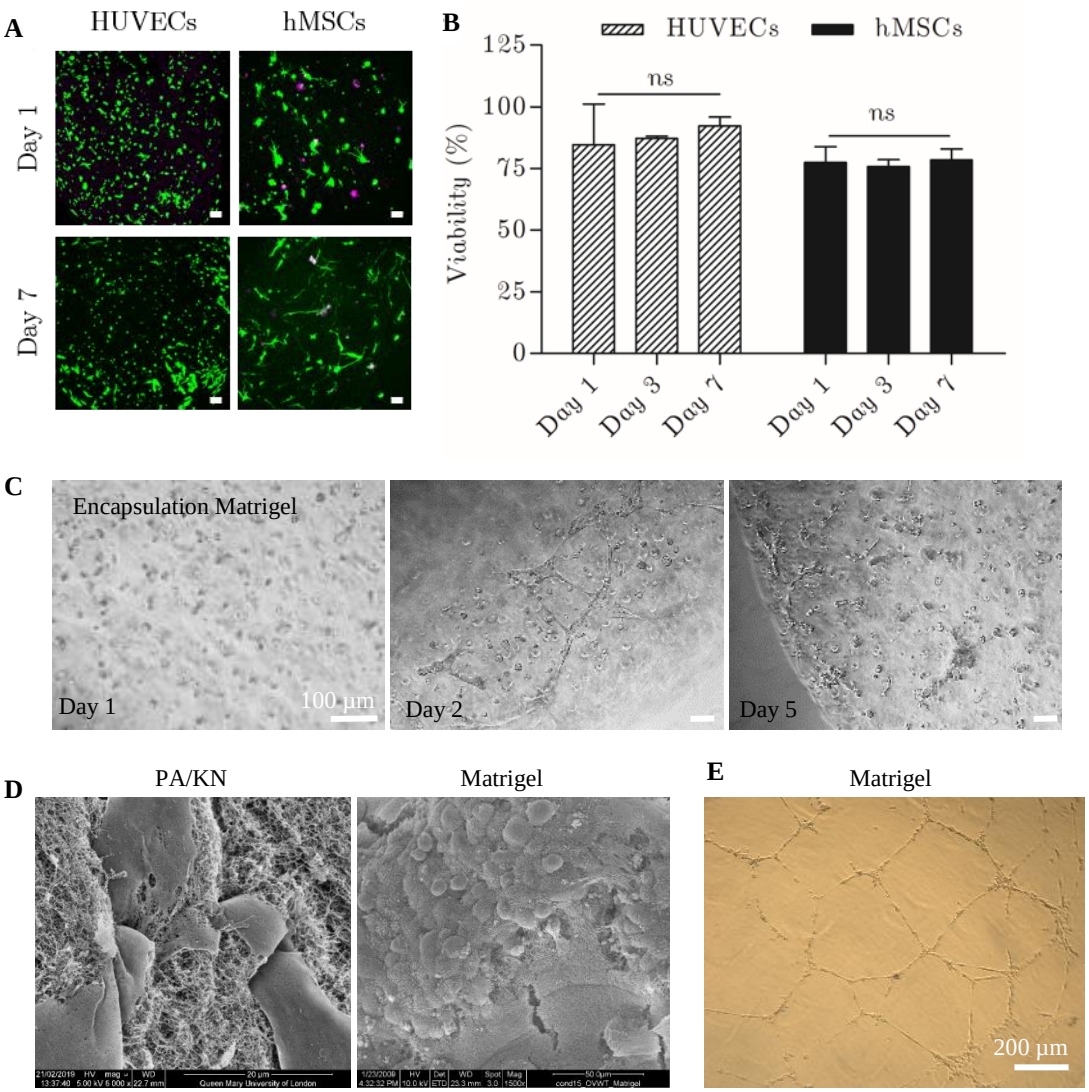
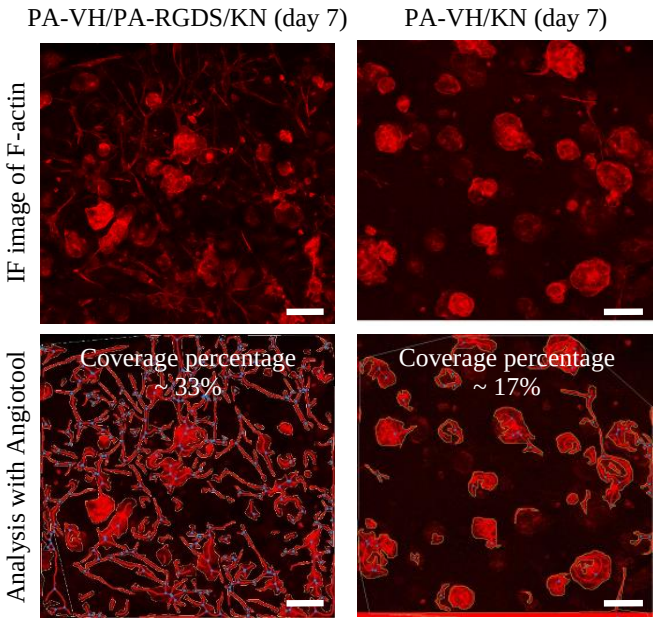


Fig. S6. Hydrogel (PA/KN) compatibility with stromal cells. (**A**) Live (green) / Dead (red, pseudo colored magenta) fluorescent assay images of HUVECs or MSCs encapsulated in **PA-VH/KN** hydrogels. Live/Dead assay was carried out after 1 and 7 days of incubation. Scale bars = 100 µm. (**B**) Graphical plot of the cell viability in percentage, as determined by analysis of the Live/Dead assay images shown in (A). (**C**) Brightfield images of HUVECs encapsulated in Matrigel at three time-pointes post-encapsulation; day 1, 2, and 4. (**D**) Scanning electron microscopy (SEM) images of encapsulated HUVECs interacting with the nanofibrous hydrogel network in **PA-VH/KN** hydrogels (left) and with Matrigel (right). (**E**) Brightfield image of a HUVEC network formed on top of a Matrigel at day 8 post cell seeding.

A



B

	Matrigel			PA-VH/PA-RGDS/KN/FN		
	Mean	SD	n	Mean	SD	n
D1	27694	612	3	19135	1759	3
D4	20610	2140	3	14159	1070	3
D7	29422	6035	3	26070	8246	3
D10	23986	3349	3	27281	4890	3
D14	18536	3127	3	24424	2151	3

C

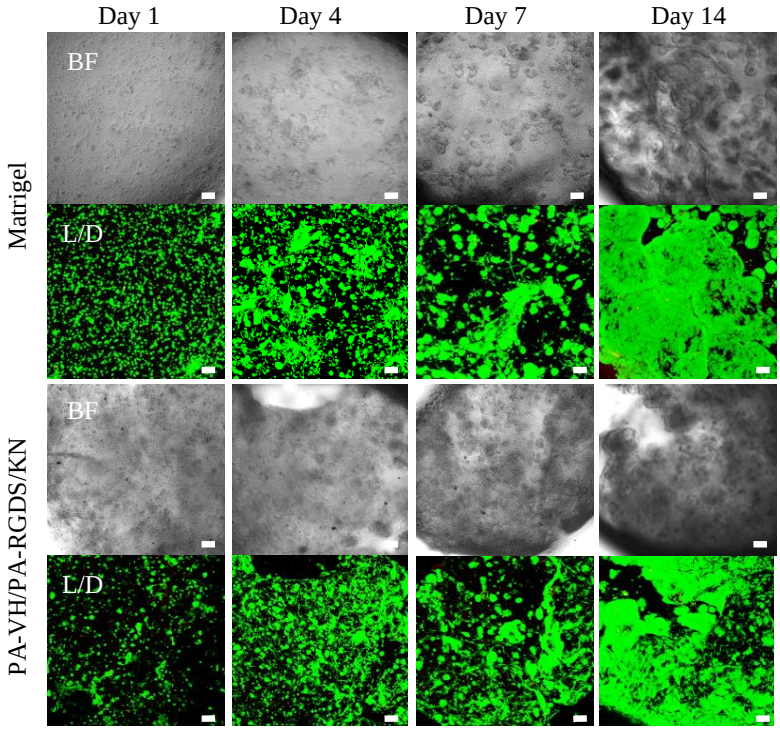


Fig. S7. Tricultures of ovarian cancer cells, HUVECs, and MSCs in PA-VH/PA-RGDS/KN/FN hydrogels and Matrigel. (**A**) Analysis of F-actin network coverage in tri-cultures within **PA/KN** hydrogels using Angiotool and IF stained images. Red stain is F-actin. Scale bars = 100 μm. (**B**) Readout fluorescence values from the AlamarBlue assay for treatment study of tricultures in **PA-VH/PA-RGDS/KN/FN** hydrogels and Matrigel. Data shown as Mean, SD, and n (number of samples) before correcting for background (AB+Media+hydrogel, no cells). Graphical plot in Manuscript Fig. 5B shows values after correcting for the background. (**C**) Brightfield and Live (green) / Dead (red) assay images of tri-cultures in Matrigel and **PA-VH/PA-RGDS/KN** and Matrigel. Scale bars = 100 μm.

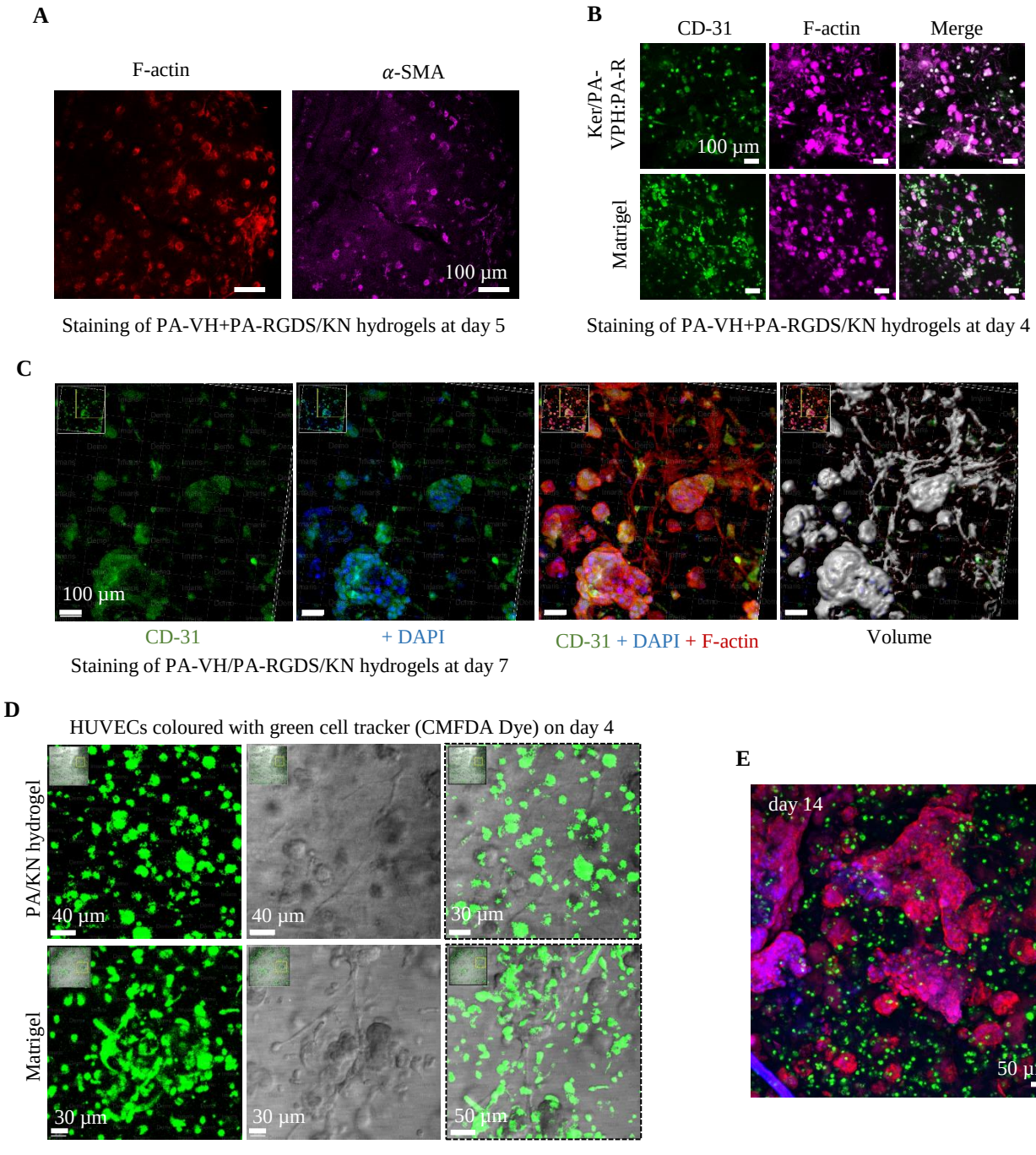


Fig. S8. Immunofluorescent (IF) staining of tricultures (560 Ovarian cancer cells/µL, 6000 HUVECs/µL, and 600 MSCs/µL) in PA/KN hydrogels and Matrigel. (**A**) IF staining for F-actin (Phalloidin, red, left) and α-SMA (magenta, right) in a **PA-VH/PA-RGDS/KN** hydrogel at day 5 post encapsulation. (**B**) IF staining for CD-31 (green) and F-actin (Phalloidin, red pseudo coloured magenta) in a **PA-VH/PA-RGDS/KN** hydrogel and **Matrigel**, at day 4 post encapsulation. (**C**) Still image sequence showing IF staining for CD-31 (green), nuclei (DAPI, blue), and F-actin (red) in a **PA-VH/PA-RGDS/KN** hydrogel at day 7 post encapsulation. Still images taken from 3D-reconstructions of stacked IF imaging. Far right, a volume reconstruction based on the IF images showing the spherical tumour formations with cell-cell networks between adjacent tumours. (**D**) Fluorescent and brightfield image of HUVECs tagged with green cell-tracker encapsulated in a **PA/KN** hydrogel and Matrigel at day 4 post encapsulation. Merged brightfield and fluorescent image show HUVECs spreading. (**E**) IF staining for nuclei (DAPI, blue), F-actin (Phalloidin, red), and HUVECs (green, cell-tracker dye) at day 14 post-encapsulation in a tri-culture **PA-VH/PA-RGDS/KN** hydrogel.

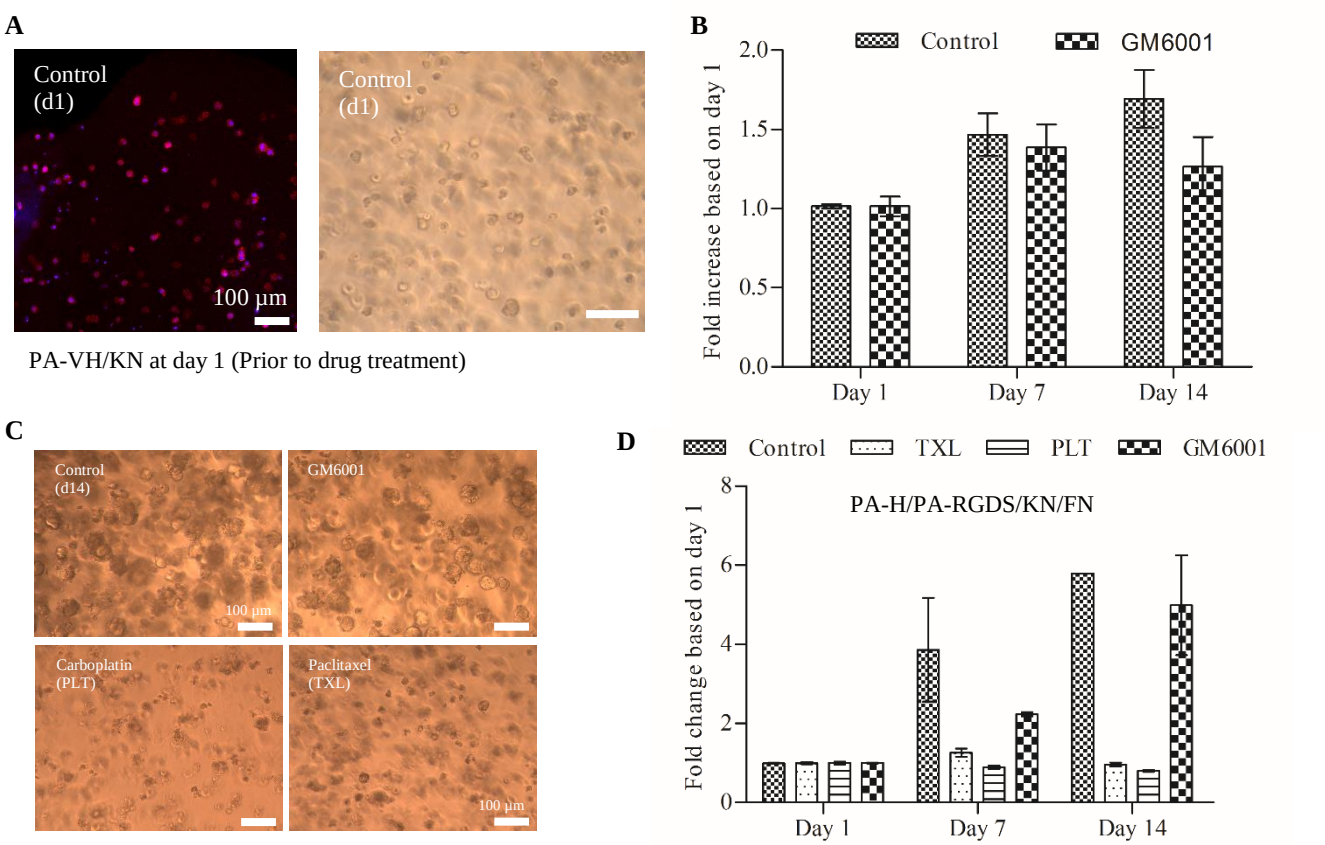
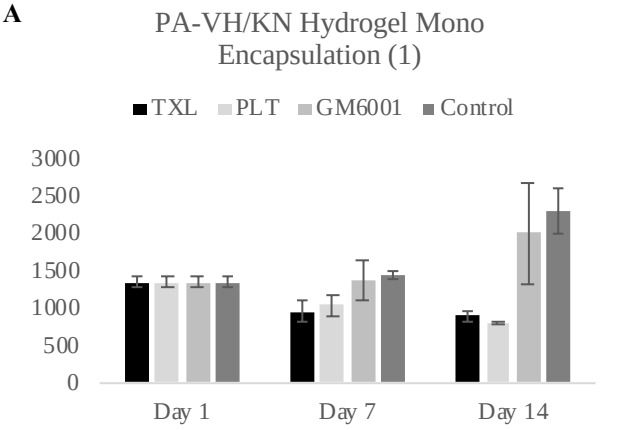
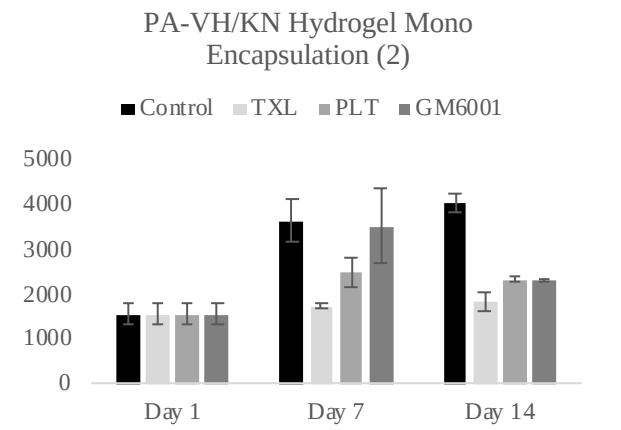


Fig. S9. **Treatment study with cytotoxic drugs and inhibitor (I).** (**A**) IF and brightfield image from day 1 post encapsulation, and prior to drug treatment of ovarian cancer cells in **PA-VH/KN** hydrogel. (**B**) Metabolic activity of ovarian cancer cells in **PA-VH/KN** hydrogels (values normalised to day 1), measured using an AlamarBlue assay. Values shown for the control (no treatment) and GM6001 (treatment with inhibitor) at three timepoints, day 1, 7, and 14 post cell-encapsulation. (**C**) Brightfield images of ovarian cancer cells in Matrigel at day 14 for four treatment conditions; control, GM6001, Carboplatin, and Paclitaxel. (**D**) Metabolic activity of a triculture (Ovarian cancer cells, HUVECs, and MSCs) in a **PA-H/PA-RGDS/KN/FN** hydrogel (values normalised to day 1), measured using an AlamarBlue assay. Values shown for four conditions; control (no treatment), GM6001 (treatment with inhibitor), TXL (treatment with Paclitaxel) and PLT (treatment with Carboplatin), at three timepoints, day 1, 7 and 14 post cell-encapsulation.

A

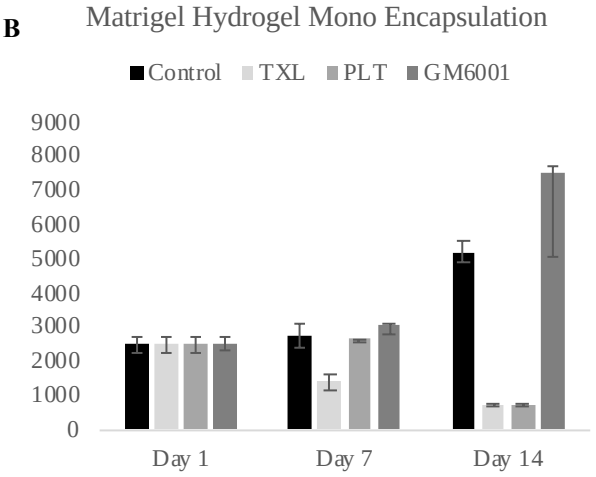


	Control			TXL			PLT			GM6001		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
D1	1353	81	7	1353	81	7	1353	81	7	1353	81	7
D7	1450	57	5	956	142	6	1046	142	6	1375	268	7
D14	2308	299	5	908	70	6	812	19	6	2010	695	7



	Control			TXL			PLT			GM6001		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
D1	1548	251	10	1548	251	10	1548	251	10	1548	251	10
D7	3641	468	6	1730	68	3	2498	322	3	3519	827	3
D14	4032	185	2	1815	205	2	2325	42	1	2321	30	1

B



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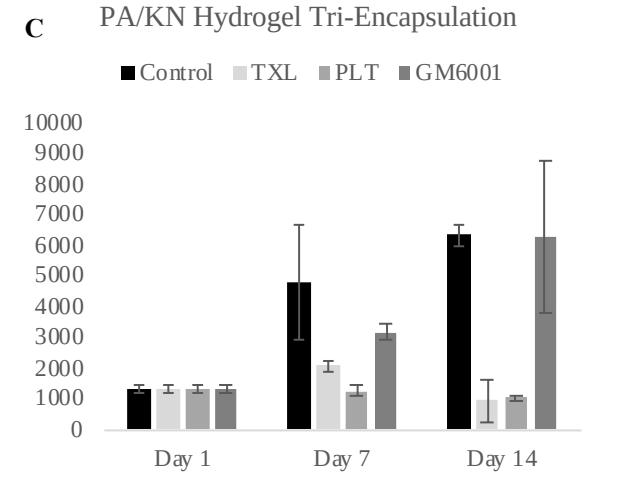

Fig. S10. Treatment study with cytotoxic drugs and inhibitor (II). (**A**) Readout fluorescence values from the AlamarBlue assay for treatment study of monocultures in **PA-VH/KN** hydrogels. Data represented graphically and in a table with Mean, SD, and n (number of samples). (**B**) Readout fluorescence values from the AlamarBlue assay for treatment study of monocultures in Matrigel. (**C**) Readout fluorescence values from the AlamarBlue assay for treatment study of tricultures in **PA-H/KN** hydrogels.