Electronic Supplementary Material (ESI) for Integrative Biology

Growth of confined cancer spheroids: a combined experimental and mathematical modelling approach

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Figure S1. Proliferative and apoptotic protein expression of cancer cell spheroids grown within medium stiff (G' = 637 ± 93 Pa) hydrogels. Expression of proliferation (Ki67, integrin α6) and apoptosis (caspase–8) markers of multicellular spheroids were analysed performing maximal projections of CLSM images. A distinct Ki67 staining of a cell undergoing division and transmembrane integrin α6 staining were detected, while cytoplasmic caspase–8 staining was more pronounced at the outer spheroid area. Scale bars, 5 µm.

Figure S2. Cancer cell spheroid formation within hydrogels as a function of biomaterial stiffness. A. Maximal projections of CLSM images depicted that protein expression in less stiff (G' = 241 ± 19 Pa) microenvironments was more pronounced regarding survival stimuli (Ki67) in the centre and apoptotic (annexin V, caspase–8) events in outer areas of large spheroids. Scale bars, 50 µm **B.** Protein expression in stiff (G' = 1201 ± 121 Pa) microenvironments was reflected by weak staining of proliferative (Ki67) and apoptotic (annexin V, caspase–8) markers using maximal projections of CLSM images. Scale bars, 50 µm.

Figure S3. Time–lapse microscopy of spheroid formation dependent on biomaterial stiffness. Time–lapse microscopy of live cell spheroid survival and formation in soft (G' = 241 ± 19 Pa), medium stiff (G' = 637 ± 93 Pa) and stiff (G' = 1201 ± 121 Pa)

microenvironments over 4.5 days showed that multicellular spheroids were formed from single cells (supplementary movies). Scale bars, overview – 100 µm, zoom – 50 µm.

Figure S4. Time–lapse microscopy of spheroid formation dependent on biomaterial stiffness. Representative time–lapse experiments (avi–files) of spheroids grown within different stiff (elastic shear modulus G' = 241 ± 19, 637 ± 93, 1201 ± 121 Pa) hydrogels are shown using a widefield microscope over a time frame of 4.5 days with images taken every 20 min with a 10x air objective.

Figure S5. Spheroid–based ovarian cancer mouse model using medium stiff (G' = 637 ± 93 Pa) hydrogels. A. All intraperitoneal organs, including tumours, were weighted after 4 and 8 weeks respectively (green blots) and paclitaxel treatment in week 4 for another 4 weeks (red blot). Then, the visible tumour mass was removed and weighed separately indicated as ratio between tumour weight and total weight. Spheroid–induced tumour growth was significantly enhanced after 8 weeks compared to 4 weeks (p=0.00015). Paclitaxel treatment significantly reduced tumour growth compared to non–treated controls (p=0.002). **B.** H/E immunohistochemistry confirmed tumour formation and presence of spheroids within hydrogels after *in vivo* implantation: a) tumour mass and b) spheroid after 4 weeks of *in vivo* growth; c) tumour mass and d) spheroid after 8 weeks of *in vivo* growth; e) tumour mass and f) spheroid after paclitaxel treatment. Scale bars, 25 µm.