



Supporting Information

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Biomaterials-Based Approaches to Tumor Spheroid and Organoid Modeling

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BIOMATERIAL	3D TUMOR MODEL	OUTCOMES	REFERENCE
Natural materials for tumor spheroid cultures			
Collagen	Mono-culture of breast cancer cells MDA-MB-231	Reduced spheroid invasion in stiffer matrices; paclitaxel resistance	47
	Co-culture of breast cancer cells MCF7 and mammary fibroblasts	Formation of tight clusters with distinct border, apical-basal polarity, and lumen	49
	Co-culture of colon cancer cells LS174T and cancer associated fibroblasts	Co-culture promoted LS174T spheroid invasion in collagen matrix	50
	Co-culture of liver cancer cells HEPG2 and fibroblasts NIH 3T3	Co-culture spheroids were highly resistant to doxorubicin compared to the mono-culture cancer cells spheroids	53
	Mono-culture of colon cancer cells HT-29	Upregulation of HIF-1 α and VEGF angiogenic factors	51
	Mono-culture of breast cancer cells MDA-MB-231	Upregulation of HIF-1 α and VEGF-A; delayed upregulation of both markers at lower cell density	40
	Mono-culture of ovarian cancer cells SK-OV-3	Mimicked oxygen gradients by 3D spatial localization of cells in a six-layered scaffold	54
Laminin rich ECM (IrECM)	Mono-culture of various prostate and breast cancer cells	Malignant sub-types displayed disorganized, proliferative and non-polar colonies and were distinguishable from non-malignant cells based on their morphology; Gene expression of malignant cancer with distinct morphology frequently clustered together	56,57,58
	Mono-culture of Lewis lung carcinoma LLC1	Cytoskeleton arrangement without formation of stress fibers; LLC1 clusters had marked differences in metabolic, MAPK, cell adhesion, and immune response genes compared to the 2D culture of the cells	60
	Co-culture of prostate cancer cells PC-3 and bone stromal cells; Pre-formed breast cancer spheroids in IrECM	Increased α_6 - and/or β_1 -integrin in the co-culture compared to the mono-culture of PC-3 cells; Blocking β_1 integrin inhibited the growth of the spheroids	70, 66

Alginate	Mono-culture spheroids of hepatocarcinoma cells	Preserved acini, apical morphogenesis, stem cell markers and β -catenin signaling; Wnt/ β -catenin signaling pathway activity promoted spheroid formation and maintaining cancer cells stemness; Cells in spheroid culture were highly tumorigenic in mouse compared to cells cultured in 2D	71
	Mono-culture of oral cancer cells	High level of pro-angiogenic maker IL-8 but moderate alteration of VEGF expression	72
	Mono-culture of hepatocarcinoma cells	Actin reorganization to facilitate spheroid formation; Cell in spheroids expressed tight junctions, canaliculi like structures, showed microvilli on their surfaces and were arranged in trabecular form	73
	Mono-culture of prostate cancer cells PC-3	Enriched expression of stem cell maker genes NANOG, OCT4, CD44, and CD133	74
Chitosan	Mono-culture of colon cancer and hepatocarcinoma cells	Upregulated cancer stem cell genes (OCT4, NANOG, CD133, CD44), epithelial maker EpCAM, and non-canonical Wnt-STAT3 signaling in CD44 ⁺ hepatocellular carcinoma cells	79
Chitosan-Alginate (CA)	Mono-culture of Hepatocarcinoma cells	High expression of Glypican-3 in spheroids compared to 2D cultures; cells were more tumorigenic, formed large tumors, and expressed pro-angiogenic growth factor such as IL-8, bFGF and VEGF	83
	Mono-culture of glioblastoma cells U-87 MG	Increased in vivo angiogenic capability of CA pre-cultured cells	85
Hyaluronic acid (HA)	Mono-culture of prostate patient derived cells	Continued expression of androgen receptor in long term culture; resistance to docetaxel treatment	94
	Mono-culture of prostate cancer cells LNCaP	Higher mRNA level of E-cadherin, and integrins α_5 and β_1	95
	Mono-culture of prostate cancer cells LNCaP	Spheroids showed cortically organized F-actin, and increased protein and mRNA expression of pro-angiogenic factors VEGF ₁₆₅ and IL-8	98
Chitosan-hyaluronan (CH)	Mono-cultures of A549 and H1299 small cell lung cancers	Strong upregulation of N-cadherin, vimentin, fibronectin, anti-apoptotic genes BCRC5 and BCL2, EMT-related transcription factor TWIST1, and cancer stem cell genes CD44, CD133, SOX2, NANOG, POU5F1	99
Silk	Osteosarcoma	Level of Cyclin B, E2F1, Ki67, and PcNA were similar to those in a SCID mouse model	104
	Breast cancer MDA-MB-231 cells	Spheroids displayed proliferation gradients of cells and growth that followed Gompertz law; Upregulated IL-8 and VEGF markers	105
Synthetic materials for tumor spheroid cultures			
RGD	Mono-cultures of ovarian	Proliferation dependent on integrin	109

functionalized PEG hydrogels	cancer cells OV-MZ-6 and SKOV-3	binding capacity; Significantly upregulated α_3 , α_5 , β_1 integrins and MMP-9 levels; resistance to paclitaxel	
PEG-fibrinogen	Mono-cultures of breast cancer cells MCF-7, SK-BR-3, and MDA-MB-231; Mono-cultures of breast and prostate cancers	MCF-7, SK-BR-3 cells formed compact spheroids in large range of hydrogel stiffness; MDA-MB-231 cells showed elongated morphology in softer matrices but round spheroids at higher stiffness; Cells in PEG-fibrinogen microspheres showed loss of apico-basal polarity, cellular and nuclear atypia, increased disorganization, elevated nuclear cytoplasmic ratio and nuclear volume density, and reduced length of cell-cell junctions	110, 111
Cysteine responsive PEG hydrogel	Mono-culture of liver cancer cells HEPG2	Recovered HEPG2 spheroid secreted higher level of urea and albumin compared to the 2D culture of cells; Level of secreted albumin was similar to the physiologic level in the body	113
HA-PEGDA, HA-SH/PEGDA	Layered co-culture of uterine with ESS1 endometrial stromal sarcoma cells or prostate cancer cell with HS27A bone marrow stroma cells	Prostate cancer spheroid preserved PSA and EGFR in the co-culture; Cells in uterine cancer spheroid expressed mucin1 and estrogen-induced gene 121 protein in the co-culture	114
	PDX cells (MDA PCa 183 and MDA PCa118b)	Preserved epithelial phenotype of the native tumors; Resistance to docetaxel compared to the spheroid that were generated from bone metastatic prostate cancer cell line (C42B)	94
PEG-DEX ATPS	Mono-culture of breast cancer cells MDA-MB-157	Spheroids showed normal growth over time, secreted and deposited major ECM proteins such as collagen I, fibronectin, and laminin; showed proliferation gradients, size and density dependent hypoxia, expressed stem cell markers (CD24, CD133, NANOG) and displayed hypoxia mediated docorubicin resistance	124
Polycaprolactone (PCL)	Mono-culture of TC-71 Ewing sarcoma cells	Spheroids preserved major marker such as CD99 ⁺ , keratin ⁻ and smooth muscle actin; Significantly upregulated phospho-IGF-1R	127
PLGA	Mono-culture of ovarian cancer cells HO1980	Expressed E-cadherin and proliferated in the microsphere	137
	Mono-culture of glioblastoma cells U-251	High expression of angiogenic factors, and resistance to doxorubicin; resistance to apoptosis (low caspase activity) by upregulating apoptosis-resistance proteins such as survivin and BCL-2	138
PLG	Mono-culture of oral squamous cell carcinoma OSCC-3	3D PLG pre-cultured OSCC-3 cells contained more blood vessels relative to the density of blood vessels in tumors formed by implanting 2D pre-cultured cells; tumors formed from 3D PLG pre-cultured spheroids expressed higher α_5 -integrin receptors	141
Thermoresponsive	Mono-culture of liver cancer	Enhanced albumin secretion and urea	145

hydrogels	cells HepG2	synthesis over a three-week culture period	
<i>Biomaterials for Tumor organoid cultures</i>			
PEG-Matrigel hydrogel	Mammary carcinoma	Enhanced the stiffness of the Matrigel for 50 50 to 4000 Pa; Functionalizing PEG with adhesive peptides promoted migratory capacity of mammary carcinoma	184
Recombinant matrix	Intestine organoids	Precisely controlled biochemical and biomechanical cues for intestinal organoids	185