Supporting Information

Biomaterials-Based Approaches to Tumor Spheroid and Organoid Modeling

*Pradip Shahi Thakuri, Chun Liu, Gary D. Luker, Hossein Tavana**

Table S1. A summary of natural and synthetic materials used for 3D tumor modeling as spheroids and organoids, types of cancer cells used in each study, and major conclusion of the studies are shown.

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-1α and VEGF angiogenic factors	51
	Mono-culture of breast cancer cells MDA-MB-231	Upregulation of HIF-1α 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 (lrECM)	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 lrECM	Increased α_6- and/or β_1-integrin in the co-culture compared to the mono-culture of PC-3 cells; Blocking β1 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	71

		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	
	Mono-culture of oral cancer cells	High level of pro-angiongneic 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 VGF	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-angiongenic factors $VEGEF_{165}$ 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	Osteosacroma	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 functionalized PEG hydrogels	Mono-cultures of ovarian cancer cells OV-MZ-6 and SKOV-3	Proliferation dependent on integrin binding capacity; Significantly upregulated α_3, α_5, β_1 integrins and MMP-9 levels; resistance to paclitaxel	109

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 hydrogels	Mono-culture of liver cancer cells HepG2	Enhanced albumin secretion and urea synthesis over a three-week culture period	145

Biomaterials for Tumor organoid cultures			
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