

Role of Hepatic Stellate Cells on Morphometric Dynamics in a Three-dimensional Hepatocellular Carcinoma Coculture Model

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Jessica Obelar¹ | João G. Vasconcellos² | Natália B. do Nascimento¹ | Jade de Oliveira^{1,2} | Vera M. T. Trindade^{1,2} | Fátima T. C. R. Guma^{1,2}

¹ Programa de Pós-graduação em Ciências Biológicas: Bioquímica, Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil | ² Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

jessica.obelar@ufrgs.br

joaogarciavasc@gmail.com

shinataliabn@gmail.com

deoliveirajade10@gmail.com

vmtt@ufrgs.br

fatima.guma@ufrgs.br

<https://orcid.org/0000-0003-1213-4519>

<https://orcid.org/0009-0007-7009-606X>

<https://orcid.org/0000-0001-6820-7452>

<https://orcid.org/0000-0003-1454-2866>

<https://orcid.org/0000-0001-5602-6013>

<https://orcid.org/0000-0003-2369-7739>

Correspondence: Jessica Obelar (jessica.obelar@ufrgs.br)

Supplementary data

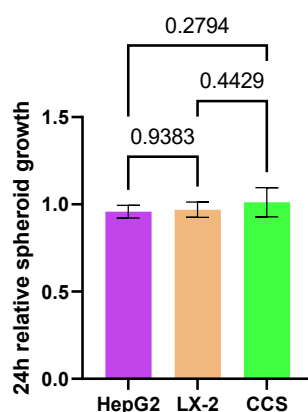


Figure S1. Relative spheroid growth after 24 hours in monoculture and co-culture systems. Spheroid growth was assessed after 24 hours in HepG2, LX-2, and co-culture spheroids (CCS), and expressed as relative growth normalized to HepG2. No statistically significant differences were observed between the groups (HepG2 vs. LX-2, $p = 0.9383$; HepG2 vs. CCS, $p = 0.2794$; LX-2 vs. CCS, $p = 0.4429$). Data are presented as mean $\pm$ SD.

To calculate the diameter of the spheroids we assumed their shapes as circles. The equation for the area of a circle is given by $A = \pi r^2$, where r is the radius. Since the diameter of a circle is given by $D = 2r$, we can write $D = 2\sqrt{\frac{A}{\pi}}$, which gives us the diameter of the spheroids in terms of the measured area (Fig. S2)

Figure S2. Temporal analysis of spheroid diameter in monoculture and co-culture systems. Spheroids derived from HepG2 (blue dashed line), LX-2 (gray dashed line), and co-culture (CCS; black solid line) were measured at 2, 24, 48, 72, and 96 hours. HepG2 spheroids showed a rapid increase in diameter after 2 h, maintaining a larger size throughout the experiment, while CCS spheroids exhibited a progressive reduction in diameter over time. Data are expressed as mean $\pm$ SD. Blue asterisks indicate statistical differences between CCS and HepG2 (* $p < 0.05$; **** $p < 0.0001$). Blue hashtags indicate statistical differences between CCS and LX-2 (# $p < 0.05$; ##### $p < 0.0001$).

Table S1. Comparison of the spheroid area over time for HepG2, LX-2, and coculture system (CCS) models. Mean area values (mm²) ± SEM are shown for each spheroid type at 2 h and at subsequent time points (24 h, 48 h, 72 h, and 96 h). The percentage variation in area (Δd%) between 2 h and each time point was calculated to assess spheroid growth or compaction. Statistical analysis was performed using two-way ANOVA, followed by Tuckey's post-hoc test. *p < 0.05 indicates statistical significance.

Area	Mean 1 (mm ²)	SEM1	Mean 2 (mm ²)	SEM2	Δd %	P-value
HepG2						
2h vs. 24h	1,718	0,27	1,354	0,11	-21,19	0,1165
2h vs. 48h	1,718	0,27	1,523	0,10	-11,35	0,5530
2h vs. 72h	1,718	0,27	1,335	0,03	-22,29	0,1084
2h vs. 96h	1,718	0,27	1,341	0,06	-21,94	0,1002
LX-2						
2h vs. 24h	1,419	0,26	0,3973	0,02	-72,00	<0,0001*
2h vs. 48h	1,419	0,26	0,314	0,03	-77,87	<0,0001*
2h vs. 72h	1,419	0,26	0,2878	0,03	-79,72	<0,0001*
2h vs. 96h	1,419	0,26	0,2778	0,02	-80,42	<0,0001*
CCS						
2h vs. 24h	1,303	0,12	0,3693	0,03	-71,66	<0,0001*
2h vs. 48h	1,303	0,12	0,33	0,03	-74,67	<0,0001*
2h vs. 72h	1,303	0,12	0,2863	0,03	-78,03	<0,0001*
2h vs. 96h	1,303	0,12	0,2882	0,03	-77,88	<0,0001*

Table S2. Comparative analysis of spheroid area between different culture models at 2 h and 24 h. Mean area values (mm²) ± SEM are shown for each pairwise comparison between HepG2, LX-2, and coculture system (CCS) spheroids at 2 h and 24 h. Percentage differences (Δd%) were calculated to assess relative area variations between models. Statistical analysis was performed using two-way ANOVA, followed by Tuckey's post-hoc test. *p < 0.05 indicates statistical significance.

Area	Mean 1 (mm ²)	SEM1	Mean 2 (mm ²)	SEM2	Δd %	P-value
2h						
CCS vs. HepG2	1,303	0,1224	1,718	0,27	31,8495779	0,0374
CCS vs. LX-2	1,303	0,1224	1,419	0,2607	8,902532617	0,6692
HepG2 vs. LX-2	1,718	0,27	1,419	0,2607	-17,40395809	0,1724
24h						

CCS vs. HepG2	0,3693	0,03048	1,354	0,1088	266,6395884	<0,0001*
CCS vs. LX-2	0,3693	0,03048	0,3973	0,02061	7,581911725	0,9765
HepG2 vs. LX-2	1,354	0,1088	0,3973	0,02061	-70,65731167	<0,0001*
Area 48h						
CCS vs. HepG2	0,33	0,0291	1,523	0,0965	361,5151515	<0,0001*
CCS vs. LX-2	0,33	0,0291	0,314	0,02833	-4,848484848	0,9923
HepG2 vs. LX-2	1,523	0,0965	0,314	0,02833	-79,38279711	<0,0001*
72h						
CCS vs. HepG2	0,2863	0,02963	1,335	0,03239	366,2940971	<0,0001*
CCS vs. LX-2	0,2863	0,02963	0,2878	0,02656	0,5239259518	>0,9999
HepG2 vs. LX-2	1,335	0,03239	0,2878	0,02656	-78,44194757	<0,0001*
96h						
CCS vs. HepG2	0,2882	0,02873	1,341	0,06086	365,3018737	<0,0001*
CCS vs. LX-2	0,2882	0,02873	0,2778	0,02299	-3,608605135	0,9968
HepG2 vs. LX-2	1,341	0,06086	0,2778	0,02299	-79,28411633	<0,0001*

Perimeter	Mean 1 (mm ²)	SEM1	Mean 2 (mm ²)	SEM2	Δd %	P-value
HepG2						
2h vs. 24h	17,230	4,690	12,600	1,230	-26,872	0,398
2h vs. 48h	17,230	4,690	9,710	0,650	-43,645	0,905
2h vs. 72h	17,230	4,690	12,900	2,270	-25,131	0,450
2h vs. 96h	17,230	4,690	13,130	2,140	-23,796	0,494
LX-2						
2h vs. 24h	11,900	1,861	4,335	0,553	-63,571	<0.0001*
2h vs. 48h	11,900	1,861	3,187	0,418	-73,218	<0.0001*
2h vs. 72h	11,900	1,861	3,530	0,681	-70,336	<0.0001*
2h vs. 96h	11,900	1,861	2,732	0,170	-77,042	<0.0001*
CCS						
2h vs. 24h	11,950	1,460	3,961	0,402	-66,854	<0.0001*
2h vs. 48h	11,950	1,460	3,922	0,511	-67,180	<0.0001*
2h vs. 72h	11,950	1,460	3,619	0,804	-69,715	<0.0001*
2h vs. 96h	11,950	1,460	3,487	0,609	-70,820	<0.0001*

Perimeter	Mean 1 (mm²)	SEM1	Mean 2 (mm²)	SEM2	Δd %	P-value
2h						
CCS vs. HepG2	11,950	1,460	17,230	4,690	44,184	0,0567
CCS vs. LX-2	11,950	1,460	11,900	1,861	-0,418	0,9995
HepG2 vs. LX-2	17,230	4,690	11,900	1,861	-30,934	0,0537
24h						

CCS vs. HepG2	3,961	0,402	12,600	1,230	218,101	<0.0001*
CCS vs. LX-2	3,961	0,402	4,335	0,553	9,442	0,9775
HepG2 vs. LX-2	12,600	1,230	4,335	0,553	-65,595	<0.0001*
48h						
CCS vs. HepG2	3,922	0,511	9,710	0,650	147,578	0,0068*
CCS vs. LX-2	3,922	0,511	3,187	0,418	-18,740	0,9162
HepG2 vs. LX-2	9,710	0,650	3,187	0,418	-67,178	0,0020*
72h						
CCS vs. HepG2	3,619	0,804	12,900	2,270	256,452	<0.0001*
CCS vs. LX-2	3,619	0,804	3,530	0,681	-2,459	0,9987
HepG2 vs. LX-2	12,900	2,270	3,530	0,681	-72,636	<0.0001*
96h						
CCS vs. HepG2	3,487	0,609	13,130	2,140	276,541	<0.0001*
CCS vs. LX-2	3,487	0,609	2,732	0,170	-21,652	0,9194
HepG2 vs. LX-2	13,130	2,140	2,732	0,170	-79,193	<0.0001*

Circularity	Mean 1	SEM1	Mean 2	SEM2	Δd %	P-value
HepG2						
2h vs. 24h	0,115	0,067	0,122	0,024	5,811	0,99980
2h vs. 48h	0,115	0,067	0,217	0,030	87,771	0,31450
2h vs. 72h	0,115	0,067	0,160	0,050	38,595	0,85970
2h vs. 96h	0,115	0,067	0,137	0,036	19,081	0,98580
LX-2						
2h vs. 24h	0,148	0,038	0,346	0,095	134,056	0,24850
2h vs. 48h	0,148	0,038	0,447	0,077	202,640	0,0414*
2h vs. 72h	0,148	0,038	0,398	0,992	169,668	0,10360
2h vs. 96h	0,148	0,038	0,405	0,067	173,866	0,09270
CCS						
2h vs. 24h	0,105	0,021	0,323	0,048	206,167	0,26320
2h vs. 48h	0,105	0,021	0,337	0,082	219,924	0,21660
2h vs. 72h	0,105	0,021	0,435	0,112	312,429	0,0477*
2h vs. 96h	0,105	0,021	0,411	0,113	290,133	0,07060

Circularity	Mean 1	SEM1	Mean 2	SEM2	Δd %	P-value
2h						
CCS vs. HepG2	0,105	0,021	0,115	0,067	9,393	0,9966
CCS vs. LX-2	0,105	0,021	0,148	0,038	40,133	0,9150
HepG2 vs. LX-2	0,115	0,067	0,148	0,038	28,101	0,9626
24h						

CCS vs. HepG2	1,206	0,044	1,181	0,027	-2,073	0,9722
CCS vs. LX-2	1,206	0,044	1,495	0,160	23,964	0,0286*
HepG2 vs. LX-2	1,181	0,027	1,495	0,160	26,588	0,0107*
48h						
CCS vs. HepG2	1,186	0,066	1,159	0,016	-2,277	0,9668
CCS vs. LX-2	1,186	0,066	1,239	0,066	4,469	0,8830
HepG2 vs. LX-2	1,159	0,016	1,239	0,066	6,903	0,7296
72h						
CCS vs. HepG2	1,224	0,094	1,191	0,016	-2,696	0,9469
CCS vs. LX-2	1,224	0,094	1,139	0,021	-6,944	0,6956
HepG2 vs. LX-2	1,191	0,016	1,139	0,021	-4,366	0,8719
96h						
CCS vs. HepG2	1,204	0,080	1,188	0,017	-1,329	0,9872
CCS vs. LX-2	1,204	0,080	1,120	0,016	-6,977	0,6996
HepG2 vs. LX-2	1,188	0,017	1,120	0,016	-5,724	0,7901

Table S11. Comparative analysis of spheroid geometric solidity evolution across different cell types during 96h. The comparison of mean spheroid areas with SEM, the size of the percentage difference, p-values, and significance of the differences between mean areas across different cultivation times is represented with “*”

Geometric solidity	Mean 1	SEM1	Mean 2	SEM2	Δd %	P-value
HepG2						
2h vs. 24h	0,561	0,196	0,636	0,134	13,273	0,94190
2h vs. 48h	0,561	0,196	0,824	0,024	46,766	0,19090
2h vs. 72h	0,561	0,196	0,800	0,026	42,473	0,25650
2h vs. 96h	0,561	0,196	0,807	0,026	43,684	0,23640
LX-2						
2h vs. 24h	0,684	0,063	0,786	0,068	14,862	0,36330
2h vs. 48h	0,684	0,063	0,882	0,032	28,935	0,02050
2h vs. 72h	0,684	0,063	0,931	0,013	36,110	0,00530
2h vs. 96h	0,684	0,063	0,936	0,012	36,782	0,00450
CCS						
2h vs. 24h	0,737	0,068	0,869	0,050	17,963	0,20010
2h vs. 48h	0,737	0,068	0,871	0,041	18,303	0,18760
2h vs. 72h	0,737	0,068	0,885	0,042	20,095	0,13180
2h vs. 96h	0,737	0,068	0,898	0,038	21,955	0,08940

Table 12. Comparative analysis of geometric solidity evolution across different cell types during 96h. The comparison of mean spheroid areas with SEM, the size of the percentage difference, p-values, and significance of the differences between mean areas across different cultivation times is represented with “*”

Geometric solidity	Mean 1	SEM1	Mean 2	SEM2	Δd %	P-value
2h						
CCS vs. HepG2	0,737	0,068	0,561	0,196	-23,788	0,1950
CCS vs. LX-2	0,737	0,068	0,684	0,063	-7,088	0,8304
HepG2 vs. LX-2	0,561	0,196	0,684	0,063	21,913	0,4412

24h

CCS vs. HepG2	0,869	0,050	0,636	0,134	-26,819	0,0303
CCS vs. LX-2	0,869	0,050	0,786	0,068	-9,530	0,6274
HepG2 vs. LX-2	0,636	0,134	0,786	0,068	23,624	0,2222

48h

CCS vs. HepG2	0,871	0,041	0,824	0,024	-5,452	0,8571
CCS vs. LX-2	0,871	0,041	0,882	0,032	1,262	0,9917
HepG2 vs. LX-2	0,824	0,024	0,882	0,032	7,101	0,7918

72h

CCS vs. HepG2	0,885	0,042	0,800	0,026	-9,587	0,6134
CCS vs. LX-2	0,885	0,042	0,931	0,013	5,302	0,8723
HepG2 vs. LX-2	0,800	0,026	0,931	0,013	16,469	0,3463

96h

CCS vs. HepG2	0,898	0,038	0,807	0,026	-10,209	0,5656
CCS vs. LX-2	0,898	0,038	0,936	0,012	4,208	0,9148
HepG2 vs. LX-2	0,807	0,026	0,936	0,012	16,057	0,3586

Table S13. Comparative analysis of spheroid normalized solidity evolution across different cell types during 96h. The comparison of mean spheroid areas with SEM, the size of the percentage difference, p-values, and significance of the differences between mean areas across different cultivation times is represented with “*”

Normalized solidity	Mean 1	SEM1	Mean 2	SEM2	Δd %	P-value
HepG2						
2h vs. 24h	0,356	0,091	0,514	0,034	44,669	0,17720
2h vs. 48h	0,356	0,091	0,551	0,029	54,880	0,07880
2h vs. 72h	0,356	0,091	0,510	0,065	43,404	0,19450
2h vs. 96h	0,356	0,091	0,510	0,047	43,404	0,19480
LX-2						
2h vs. 24h	0,326	0,067	0,660	0,022	102,640	<0,0001*
2h vs. 48h	0,326	0,067	0,823	0,036	152,809	<0,0001*
2h vs. 72h	0,326	0,067	0,909	0,047	178,938	<0,0001*
2h vs. 96h	0,326	0,067	0,809	0,023	148,419	<0,0001*
CCS						
2h vs. 24h	0,406	0,044	0,772	0,029	90,081	<0,0001*
2h vs. 48h	0,406	0,044	0,840	0,029	106,768	<0,0001*
2h vs. 72h	0,406	0,044	0,820	0,032	101,772	<0,0001*
2h vs. 96h	0,406	0,044	0,811	0,037	99,582	<0,0001*

Table S14. Comparative analysis of spheroid normalized solidity evolution across different cell types during 96h. The comparison of mean spheroid areas with SEM, the size of the percentage difference, p-values, and significance of the differences between mean areas across different cultivation times is represented with “*”

Normalized solidity	Mean 1	SEM1	Mean 2	SEM2	Δd %	P-value
2h						
CCS vs. HepG2	0,406	0,044	0,356	0,091	-12,503	0,7634
CCS vs. LX-2	0,406	0,044	0,326	0,067	-19,838	0,3655

HepG2 vs. LX-2	0,356	0,091	0,326	0,067	-8,383	0,9109
24h						
CCS vs. HepG2	0,772	0,029	0,514	0,034	-33,407	0,0001*
CCS vs. LX-2	0,772	0,029	0,660	0,022	-14,541	0,1460
HepG2 vs. LX-2	0,514	0,034	0,660	0,022	28,330	0,0421*
48h						
CCS vs. HepG2	0,840	0,029	0,551	0,029	-34,460	<0,0001*
CCS vs. LX-2	0,840	0,029	0,823	0,036	-1,988	0,9569
HepG2 vs. LX-2	0,551	0,029	0,823	0,036	49,546	<0,0001*
72h						
CCS vs. HepG2	0,820	0,032	0,510	0,065	-37,814	<0,0001*
CCS vs. LX-2	0,820	0,032	0,909	0,047	10,820	0,2966
HepG2 vs. LX-2	0,510	0,065	0,909	0,047	78,207	<0,0001*
96h						
CCS vs. HepG2	0,811	0,037	0,510	0,047	-37,132	<0,0001*
CCS vs. LX-2	0,811	0,037	0,809	0,023	-0,222	0,9995
HepG2 vs. LX-2	0,510	0,047	0,809	0,023	58,709	<0,0001*

Table S15. Comparative analysis of spheroid density evolution across different cell types during 96h.

The comparison of mean spheroid areas with SEM, the size of the percentage difference, p-values, and significance of the differences between mean areas across different cultivation times is represented with “**”

Density	Mean 1	SEM1	Mean 2	SEM2	Δd %	P-value
HepG2						
2h vs. 24h	1841,000	175,900	1536,000	66,250	-16,567	0,17720
2h vs. 48h	1841,000	175,900	1466,000	56,590	-20,369	0,07880
2h vs. 72h	1841,000	175,900	1544,000	125,600	-16,133	0,19450
2h vs. 96h	1841,000	175,900	1545,000	90,270	-16,078	0,19480
LX-2						
2h vs. 24h	1773,000	40,910	1255,000	43,010	-29,216	<0,0001*
2h vs. 48h	1773,000	40,910	941,000	68,380	-46,926	<0,0001*
2h vs. 72h	1773,000	40,910	690,100	27,530	-61,077	<0,0001*
2h vs. 96h	1773,000	40,910	968,500	43,840	-45,375	<0,0001*
CCS						
2h vs. 24h	1744,000	84,590	1039,000	56,480	-40,424	<0,0001*
2h vs. 48h	1744,000	84,590	908,900	54,880	-47,884	<0,0001*
2h vs. 72h	1744,000	84,590	947,900	60,700	-45,648	<0,0001*
2h vs. 96h	1744,000	84,590	965,000	70,960	-44,667	<0,0001*

Table S16. Comparative analysis of spheroid density evolution across different cell types during 96h.

The comparison of mean spheroid areas with SEM, the size of the percentage difference, p-values, and significance of the differences between mean areas across different cultivation times is represented with “**”

Density	Mean 1	SEM1	Mean 2	SEM2	Δd %	P-value
2h						
CCS vs. HepG2	1744,000	84,590	1841,000	175,900	5,562	0,7051
CCS vs. LX-2	1744,000	84,590	1773,000	40,910	1,663	0,9567

HepG2 vs. LX-2	1841,000	175,900	1773,000	40,910	-3,694	0,8528
24h						
CCS vs. HepG2	1039,000	56,480	1536,000	66,250	47,834	<0,0001*
CCS vs. LX-2	1039,000	56,480	1255,000	43,010	20,789	0,0847
HepG2 vs. LX-2	1536,000	66,250	1255,000	43,010	-18,294	0,0176*
48h						
CCS vs. HepG2	908,900	54,880	1466,000	56,590	61,294	<0,0001*
CCS vs. LX-2	908,900	54,880	941,000	68,380	3,532	0,9445
HepG2 vs. LX-2	1466,000	56,590	941,000	68,380	-35,812	<0,0001*
72h						
CCS vs. HepG2	947,900	60,700	1544,000	125,600	62,886	<0,0001*
CCS vs. LX-2	947,900	60,700	690,100	27,530	-27,197	0,0427
HepG2 vs. LX-2	1544,000	125,600	690,100	27,530	-55,304	<0,0001*
96h						
CCS vs. HepG2	965,000	70,960	1545,000	90,270	60,104	<0,0001*
CCS vs. LX-2	965,000	70,960	968,500	43,840	0,363	0,9993
HepG2 vs. LX-2	1545,000	90,270	968,500	43,840	-37,314	<0,0001*