Secretome-based prediction of 3D hepatic microtissue physiological relevance

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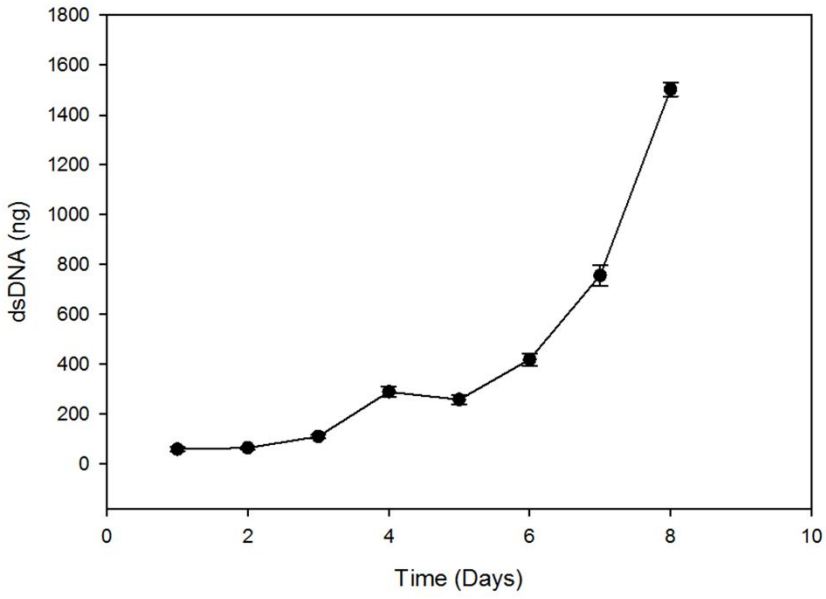

Figure S1-Sn: Growth of HepG2 cells over 8 days on 2D tissue culture plastic

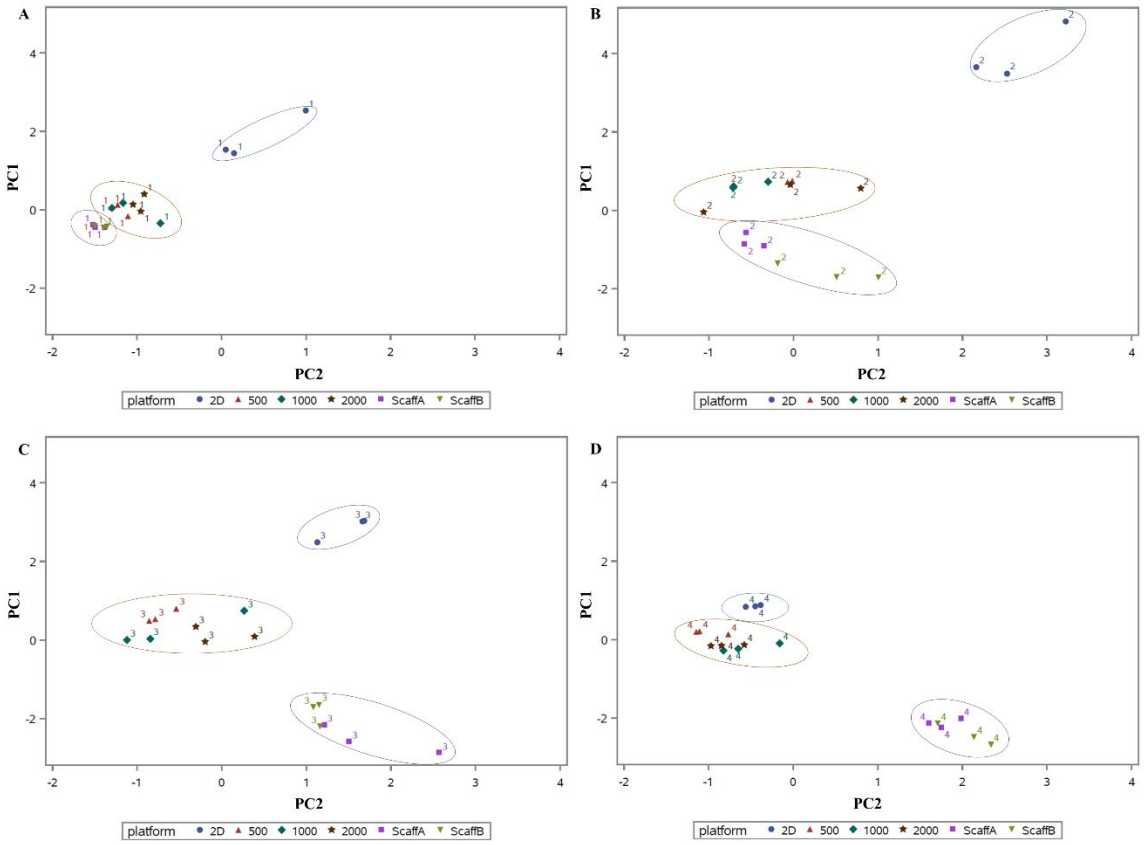

Figure S2-Sn: Principal component analysis (PCA) of cytokine measurements for the top two principal components (PCs), which explained >95% of the results, split into subplots by day (day 1 - 4, A - D) to make it easier to visualize the differences between culture platforms on each day. Observations were color-coded by culture platform and represents an independent biological replicate. Each data point has been labeled with the day the sample was collected. Ellipses have been added to emphasize co-clustering within the same culture platform (i.e., 2D, spheroid, scaffold) and relative to each other. A time-dependent difference in clustering of the culture platforms is apparent on day 3 (C).

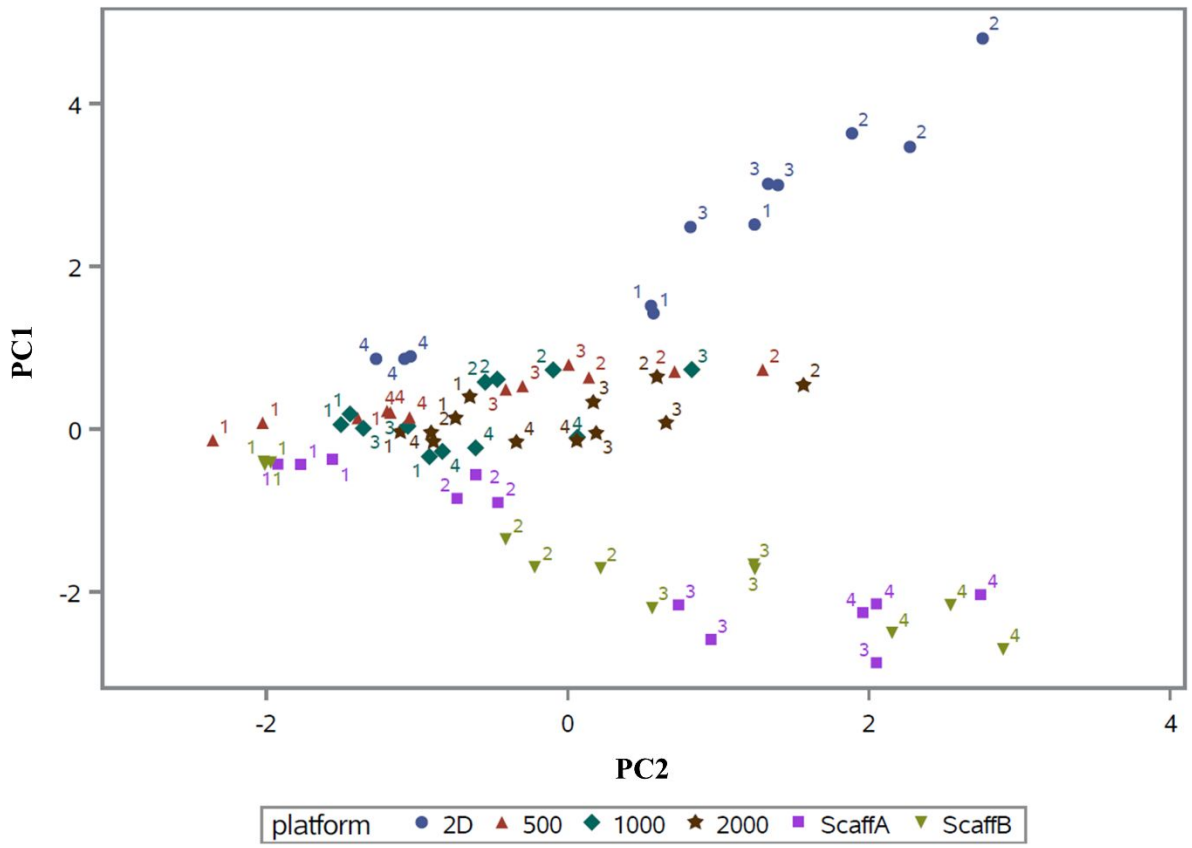

Figure S3-Sn: Principal component analysis (PCA) of a "signature" that includes the cytokines secretion profile and albumin production, a hepatocyte-specific function, for the first four days of culture. PC1 and PC2 explained >80% of the results. Observations were color-coded by culture platform and represents an independent biological replicate. Each data point has been labeled with the day the sample was collected. The addition of albumin production did not appear to change the clustering of the data (also see Fig. 5).

	Days in Culture							
	1	2	3	4	5	6	7	8
2D/3D [1]	- / 1.8	NS	NS	- / 1.6	- / 0.8	NS	NS	NS
2D/Sph	1.0 / -	NS	NS	- / 1.8	- / 0.8	NS	NS	NS
2D/Scaff	5.7 / -	2.6 / -	0.9 / -	- / 1.4	- / 0.8	- / 1.0	- / 1.9	- / 3.8
Sph/Scaff [2]	2.3 / -	3.2 / -	1.4 / -	NS	NS	- / 0.7	- / 2.3	- / 3.3
500/1000	- / 1.7	NS	NS	NS	NS	NS	NS	NS
1000/2000	NS	NS	NS	NS	NS	NS	- / 0.7	NS
500/2000	- / 1.8	NS	NS	NS	NS	NS	- / 0.7	NS
Scaff A/B	NS	NS	- / 0.7	- / 0.5	- / 0.6	- / 0.8	- / 0.7	- / 0.5

Table S1-Sn: Albumin secretion fold change and statistical significance.

[1] All spheroid and scaffold platforms are pooled together as "3D" and compared to 2D cultures to determine statistical significance using One Way ANOVA and Student-Newman-Keuls Methods. For example, on day 2 the difference between 2D cultures and 3D cultures with respect to albumin (ng) production per dsDNA (ng) was not significant (i.e., NS).

[2] Spheroid cultures produced significantly more albumin (ng) per dsDNA (ng) when compared to scaffold cultures for the first three days. There was no statistical significance between the albumin production on day 4 and 5. On day 6, scaffolds produced significantly more albumin, being 0.7-fold higher than spheroids.