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Hepatocyte Spheroid Models

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DVDMDG Vendor Show

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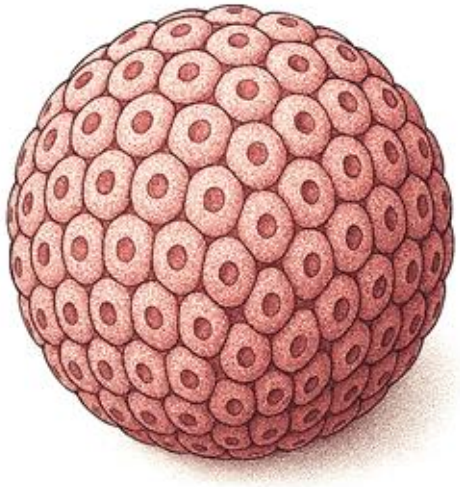
New Approach Methodologies

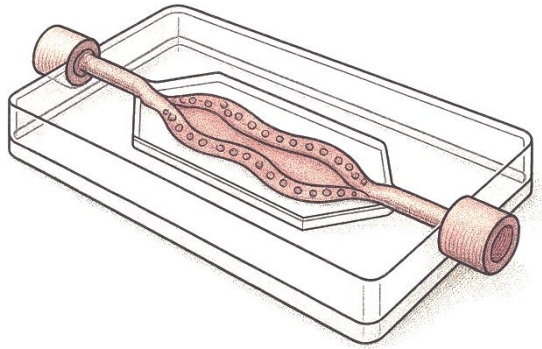
NAMs supported by NIH

Need more predictive *in vitro* models for drug efficacy, toxicity and metabolism

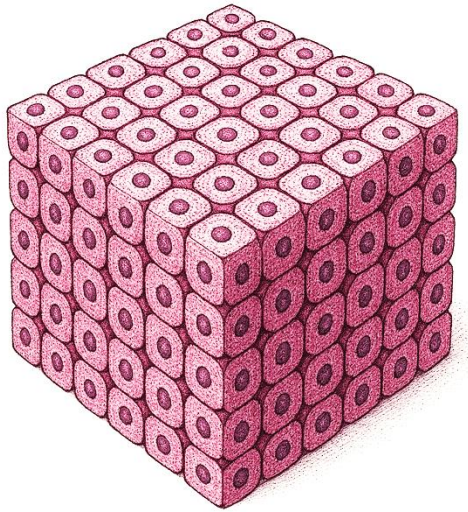
Scalability → Complexity



Spheroid



Microphysiological System



Engineered Tissue

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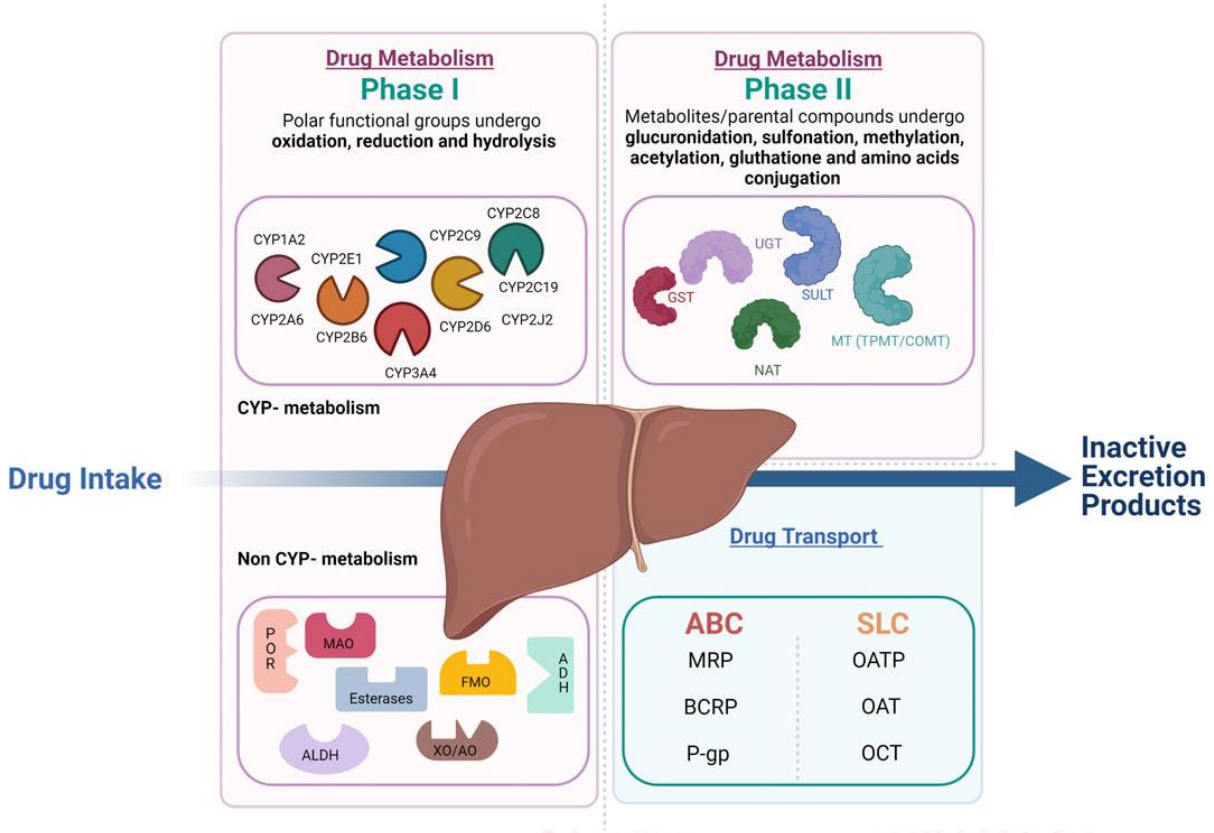
Drug Metabolism Models

Clearance evaluation in the liver

- Liver Microsomes, up to 1 hour
- Hepatocytes in suspension, up to 4 hours
- Hepatocyte in suspension relay method, 1-2 days
- Plated hepatocytes, 2-3 days
- Complex models (co-cultures) such as Hepatopac, TruVivo, Hurel, 2-4 weeks
- Hepatic spheroid cultures, 2-4 weeks



Abouir et al. DOI:10.3389/fphar.2021.708299

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Hepatocyte Spheroid Model

Scaling and throughput are challenging for more complex models

Needs to make an assay efficient:

- Minimize reagents and manipulations
- Long viability in culture with minimal media changes
- Maintained metabolic activity
- Measurable activity
- 96-well or higher format for high throughput applications

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Donor Screening is Key

Not all hepatocyte lots form spheroids despite plating well in 2D

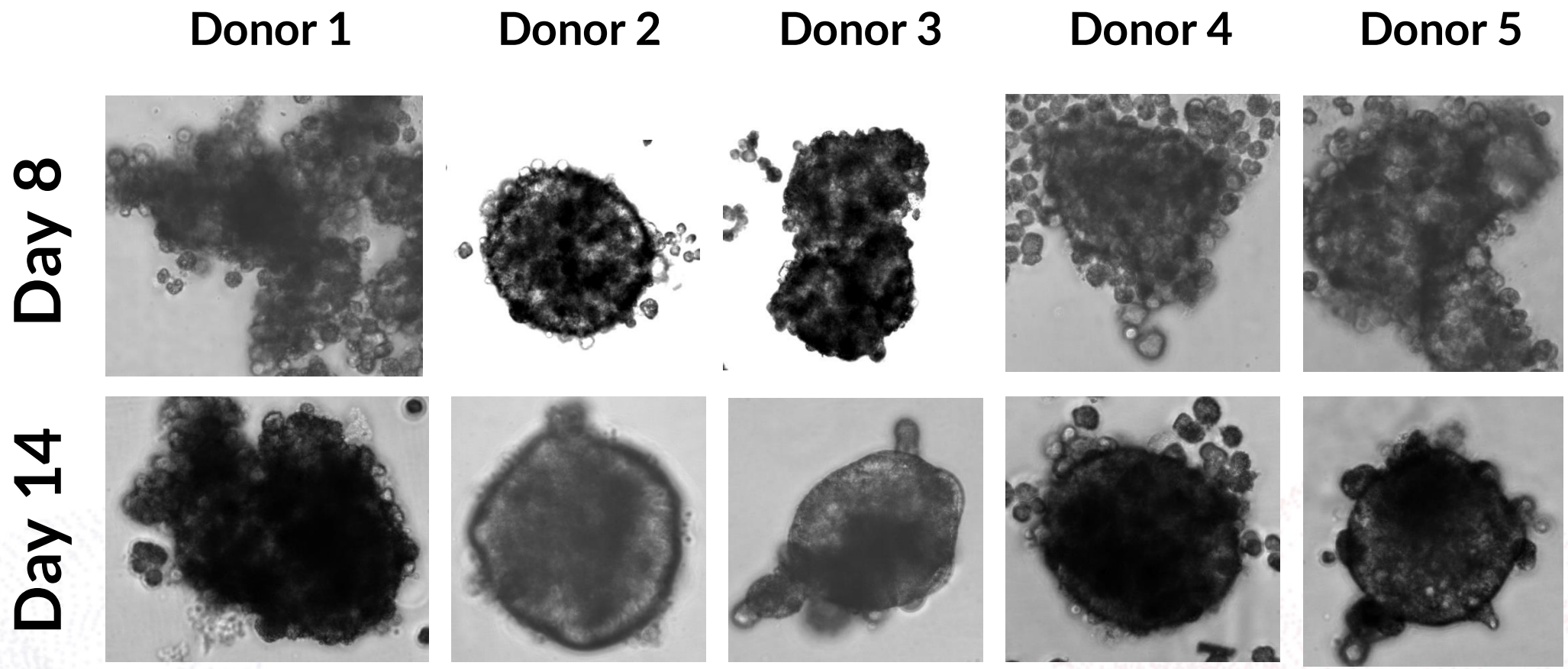

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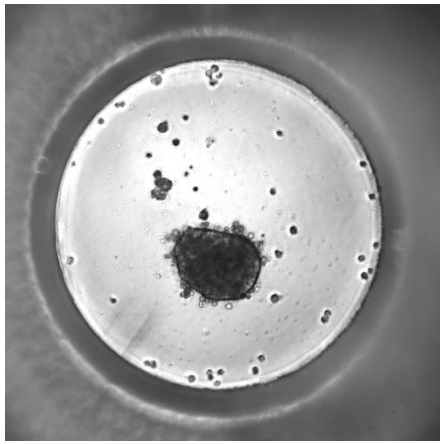
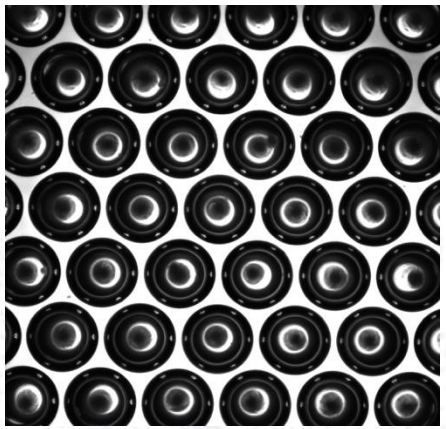
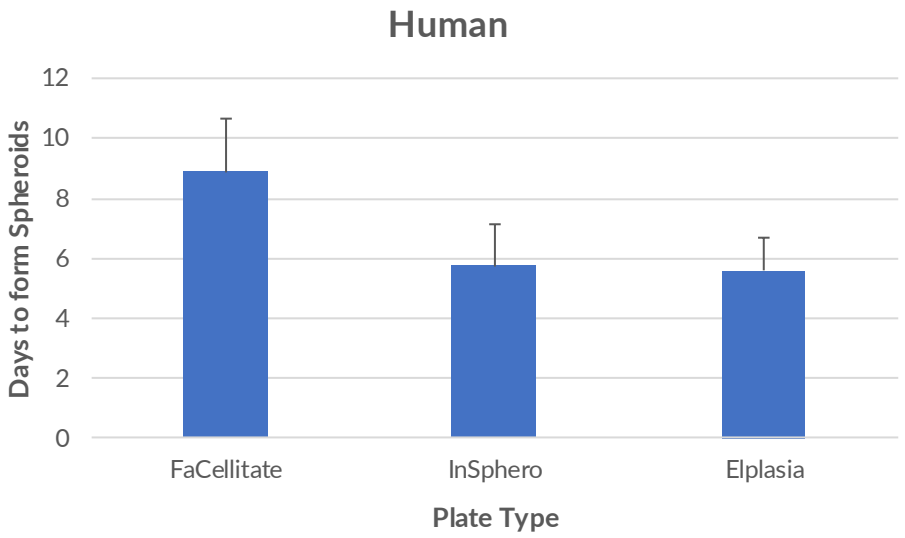
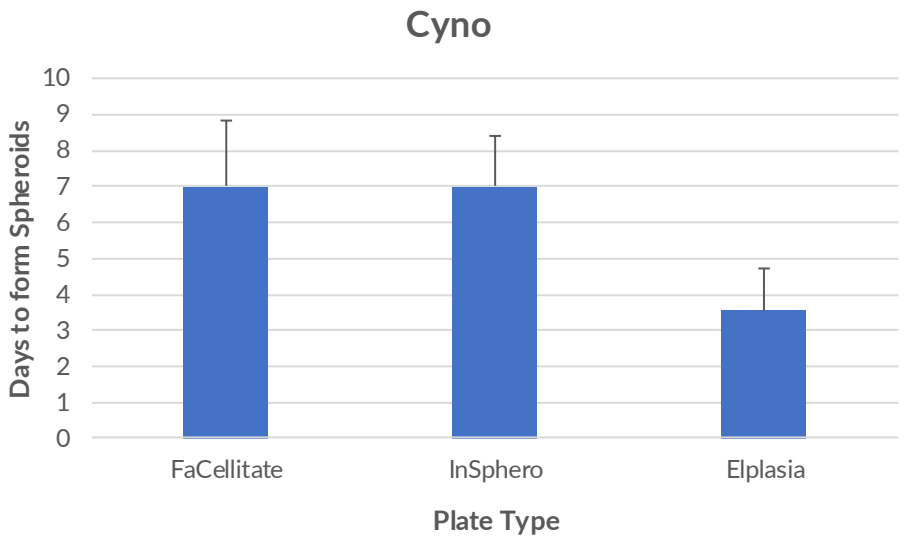
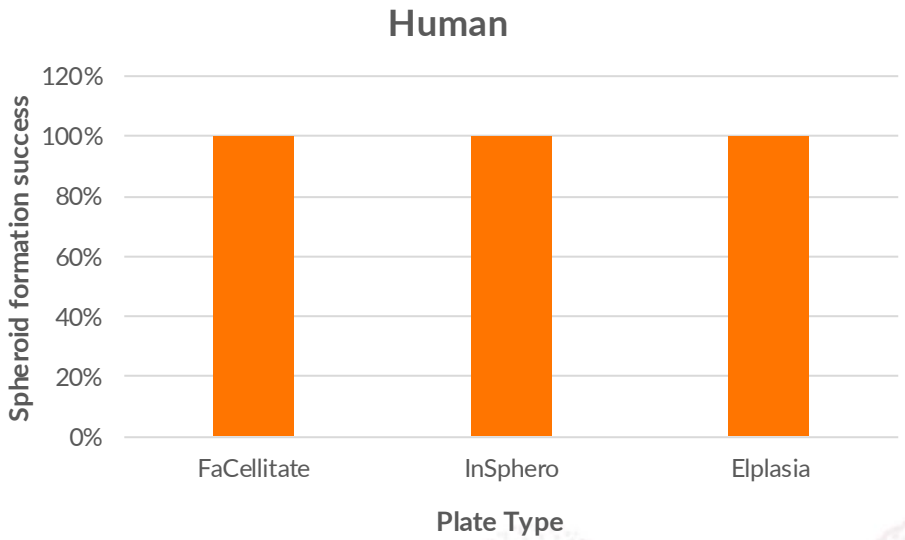
Plate Type Impacts Spheroid Formation

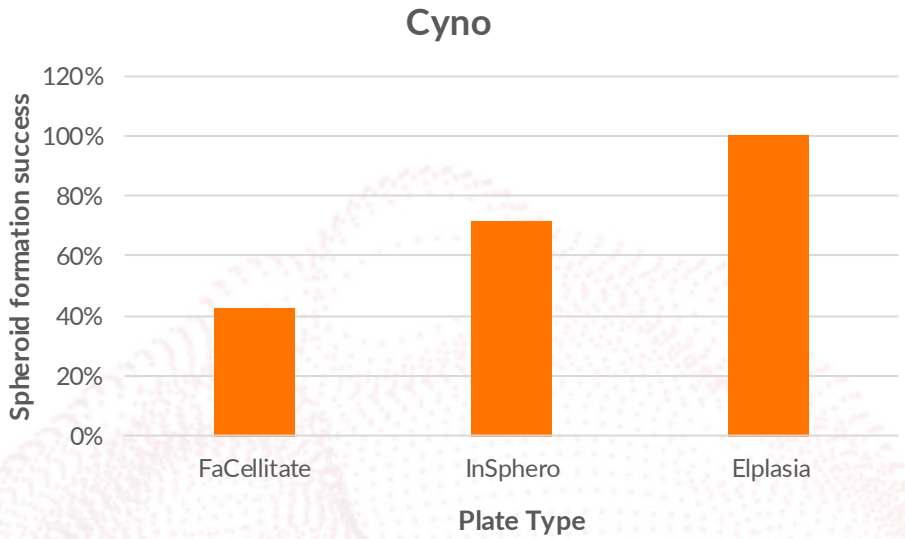












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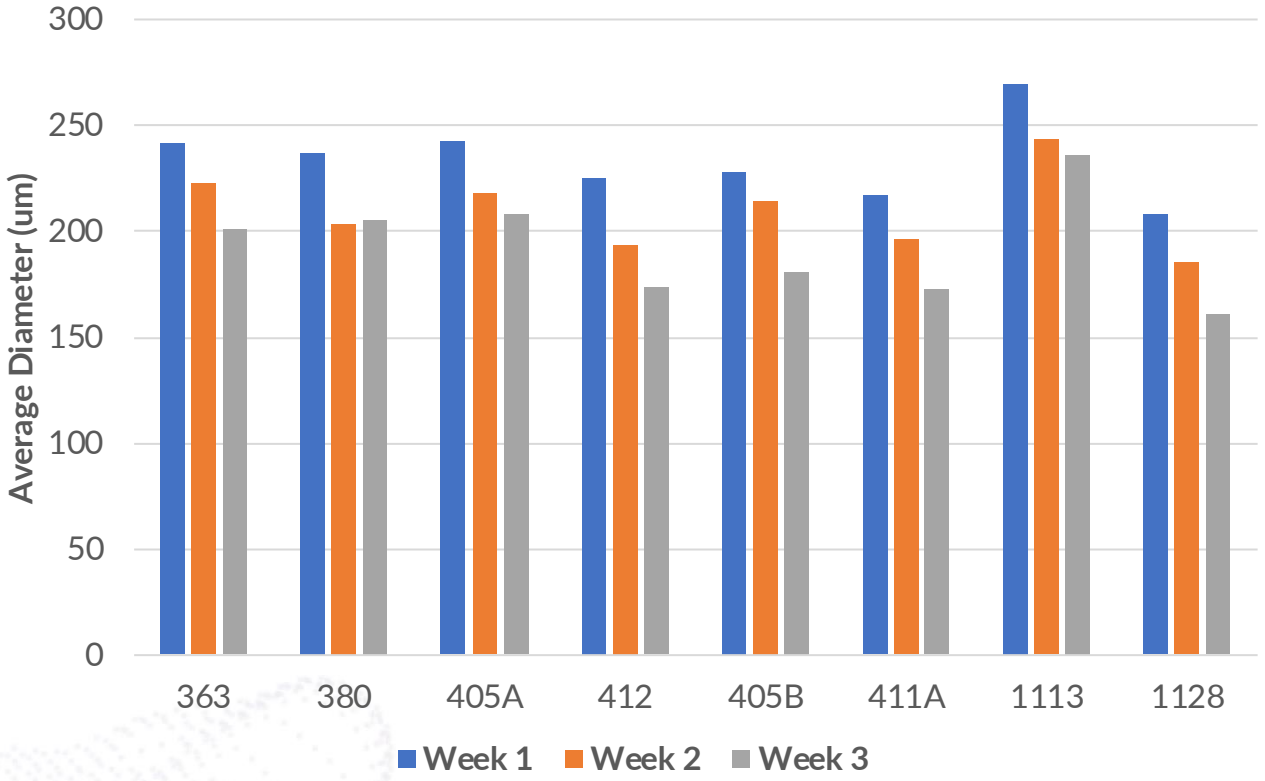
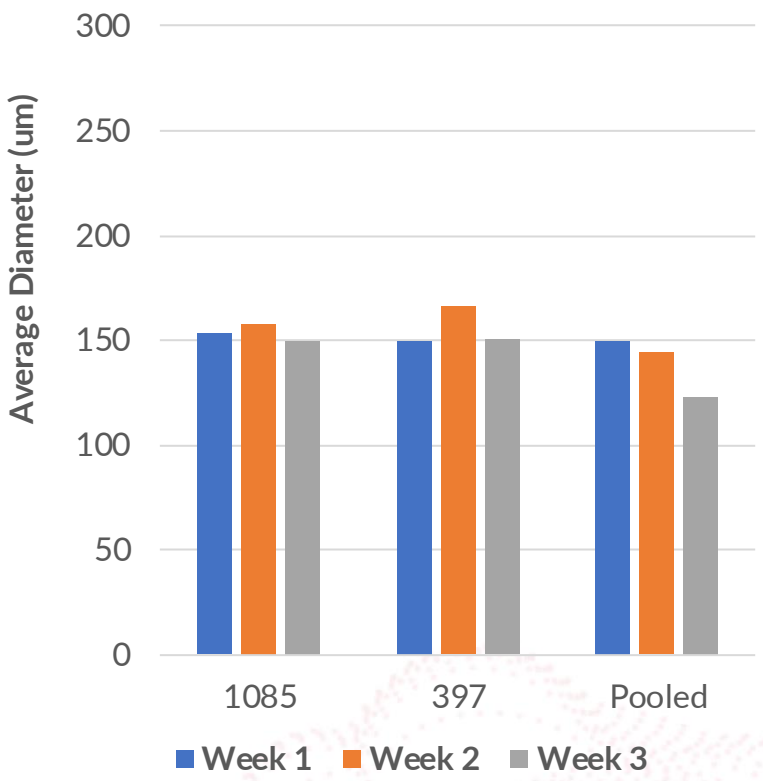
Maintained Health and Size for Over 3 Weeks



InSphero Plates



Elplasia Plates

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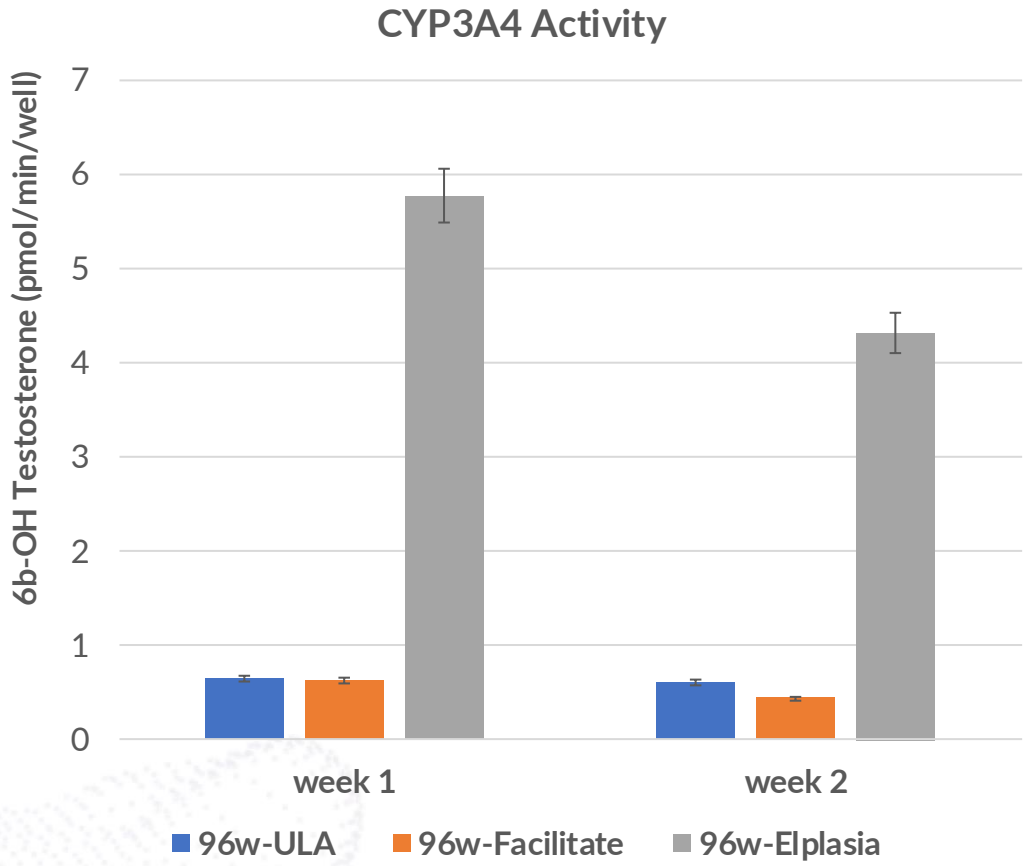
One vs Multiple Spheroids per Well



- Microcavity plates allow higher cell count per 96-well for spheroid formation (up to 40x more) compared to standard 96-well formats
- Higher activity per well generated, allowing higher detection sensitivity in metabolic assays

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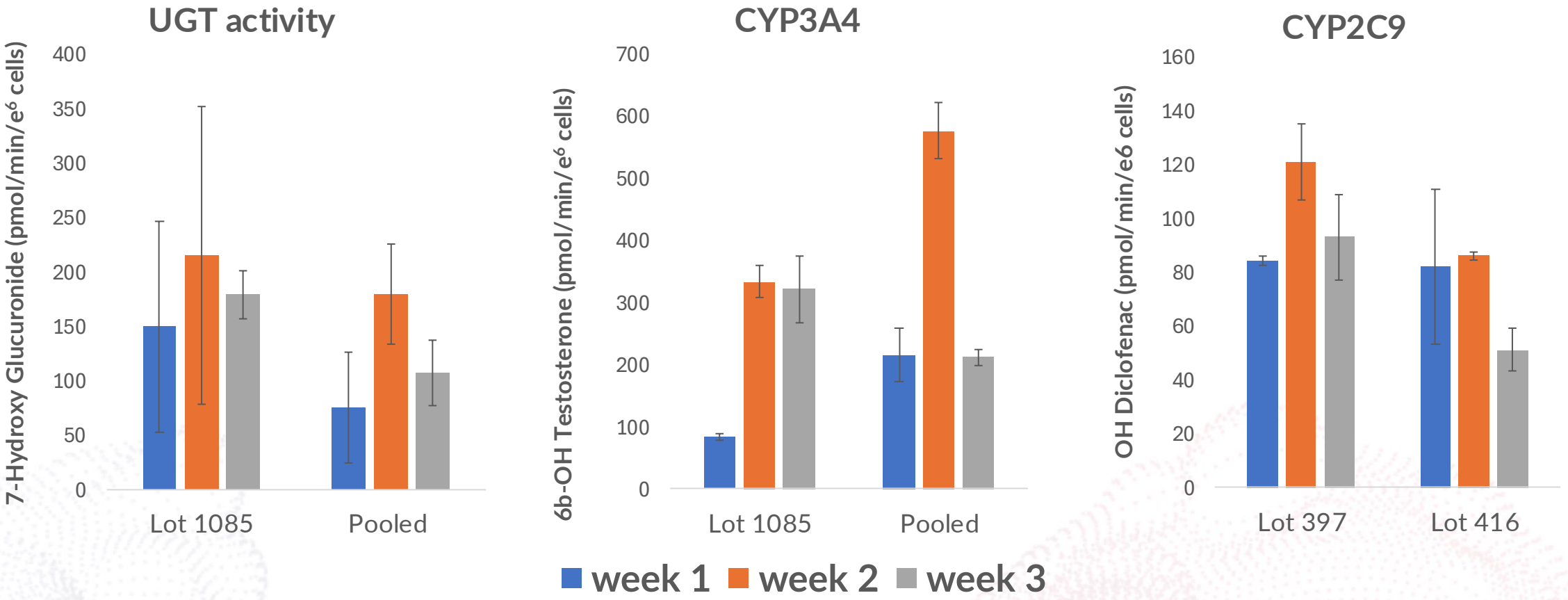
Maintained Enzyme Activity

UGT and CYP activity levels are stable over 3 weeks



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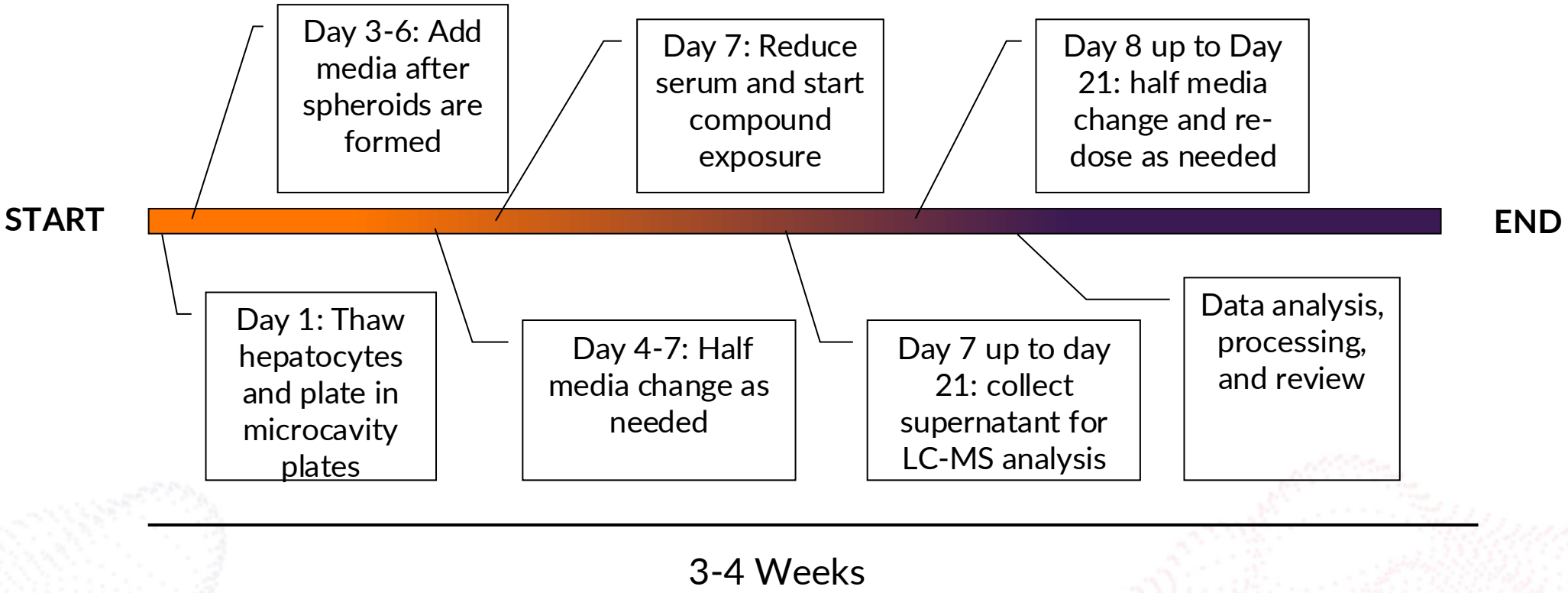
Hepatic Spheroids for Low Clearance Compounds

Experimental Setup



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Low Clearance Applications

Clearance Measured (µL/min/1e^6 cells)	Naloxone	Canagliflozin	Oxazepam	TFP	Warfarin	Tolbutamide	Risperidone	Midazolam	Diclofenac	Verapamil	Diazepam
	UGT	**U1A9, U2B4**	**UGT**	**U1A4, U2B10**	**2C9,3A4**	**2C9,2C19**	**CYP2D6**	**CYP3A4**	**CYP2C9, UGT**	**CYP2D6, CYP3A, UGT**	**2C19,3A4**
Lot 1085	7.51	3.65	1.81	7.29	1.08	0.56	3.60	5.18	2.10	4.34	1.80
Lot 397	7.90	4.19	2.62	6.75	n.d.	n.d.	n.d.	n.d.	2.52	4.58	1.78
Lot Pooled	7.52	2.65	1.18	7.59	0.81	0.54	1.90	6.00	n.d.	n.d.	n.d.
Kukla et al., 2024 (Spheroids)	9.27				0.14			2.22		2.02	0.4
Preiss et al., 2024 (Spheroids)	13.2				0.43	0.9			4.6	7.3	0.9
Preiss et al., 2024 (Hepatopac)	12.7				2.5	1.6			66.5	19.1	5.7
Preiss et al., 2024 (Hurel)	39.4				0.3	0.7			26.5	18.5	0.9

n.d., not determined

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Summary

Hepatic spheroids are useful for clearance assays

- Donor screening for spheroid formation is needed
- Plate type impacts spheroid formation and activity and needs to be selected for each application
- Human hepatocyte spheroids can be held in culture for 3 weeks without significant impact on viability and metabolic enzyme activity
- Hepatic spheroids can be use for low clearance assays

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Thank You

Questions?

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