Toxicity Assay Using OrganRX Plate for Liver and Heart Organs:

Validated by Celprogen Inc

The toxicity assays for major EPA toxins ((a) Cyclophosphamide (b) Aflatoxin (c) Tamoxifen) are well studied in the literature by Dr. Albert Li (Discovery Life Sciences) who studied in static culture. The toxicity studies were carried out by Celprogen Inc on Biopico's OrganRX system. Control experiments were carried out by seeding the cells on the corner wells and plate was incubated at 37℃ in a humidified 5% CO2 incubator. This provides direct oxygen access from the wells when the OrganRX plate is not in recirculation. For the recirculation experiments, the flow (0.1 dynes/cm^2) was established after one hour incubation with the drug concentrations and the recirculation was performed for 24 hours. Optimization for cell seeding density was carried out by seeding from 2,500 to 22,500 cells as in Fig. 1.

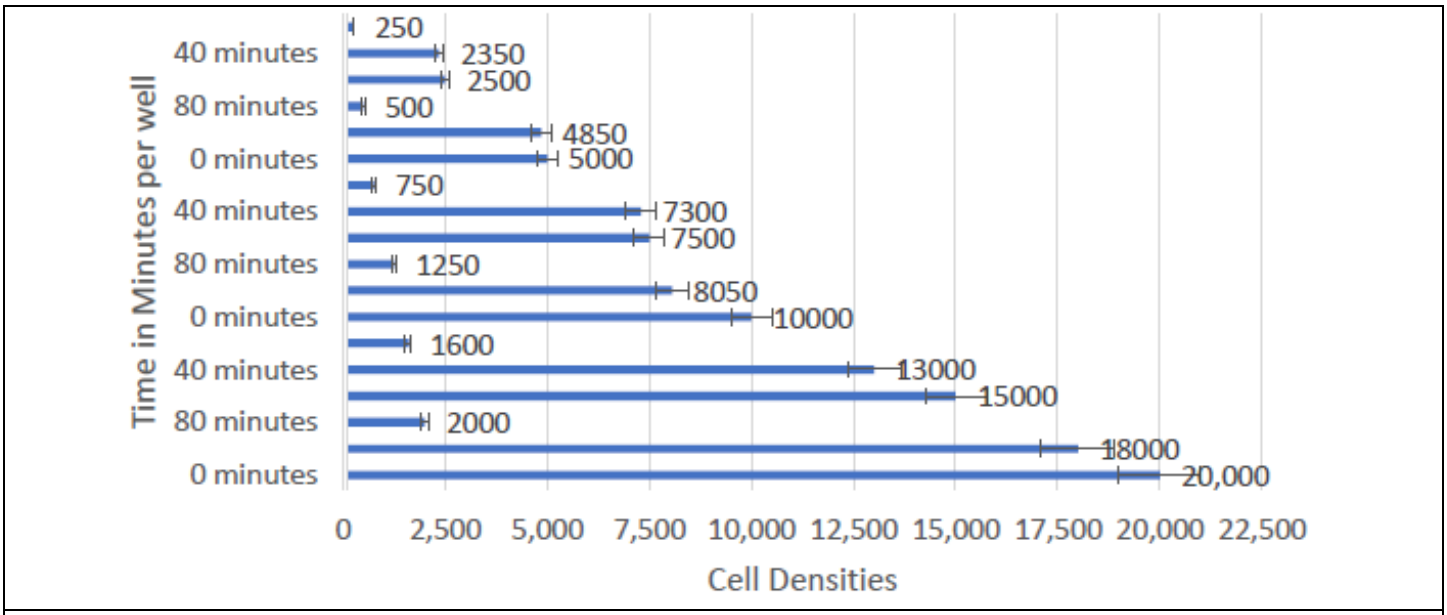
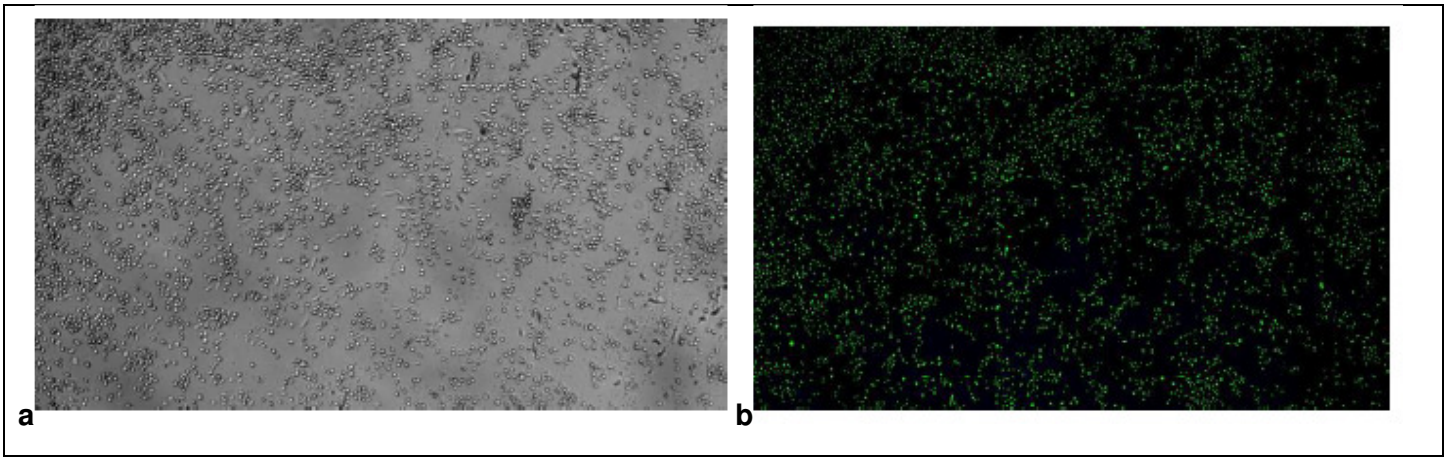


Fig. 1 Optimization of cell seeding using primary rat hepatocytes

At the end of 24 hours the cell viability was determined utilizing the IX71 inverted Olympus Microscope with Cell Dimension software to determine cell viability.



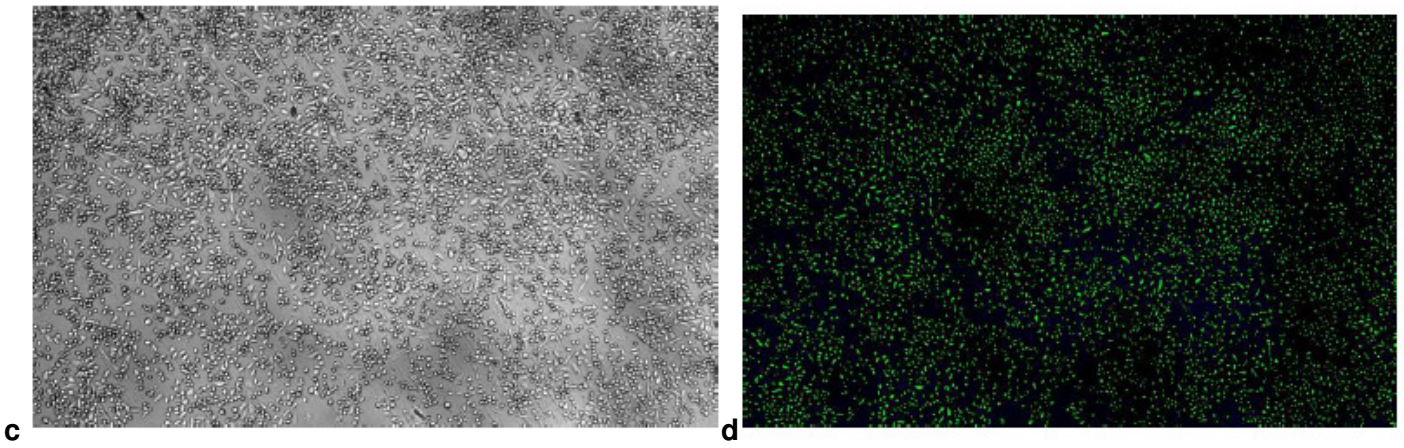


Fig. 2. Toxicological studies with the OrganRX Plate. (a) Rat heart organ (Cardiomyocyte) bright Field (b) Rat heart organ (Cardiomyocyte) (c) Rat liver organ (hepatocytes) bright Field (b) Rat liver organ (hepatocytes). 4X magnification. CellTracker™ CMF2HC dye was used for fluorescence.

Figure 2 shows representative images on the heart and liver organs for the toxicity assay. All measurements were performed in triplicates. The outer wells were seeded with 10K Hepatocytes per well and the inner wells were seeded with Cardiomyocytes 5K cells per well. Prior to seeding the cells into the chamber wells the wells and the channels coated Celprogen's 3D matrix. Toxicological studies on (a) Cyclophosphamide (b) Aflatoxin (c) Tamoxifen with the OrganRX Plate are presented in Fig. 3. The IC50 values were determined after 24 hours of drug incubated. The IC-50 values from the study are presented in Table 1. The IC-50 values are in agreement with the values reported in the literature.

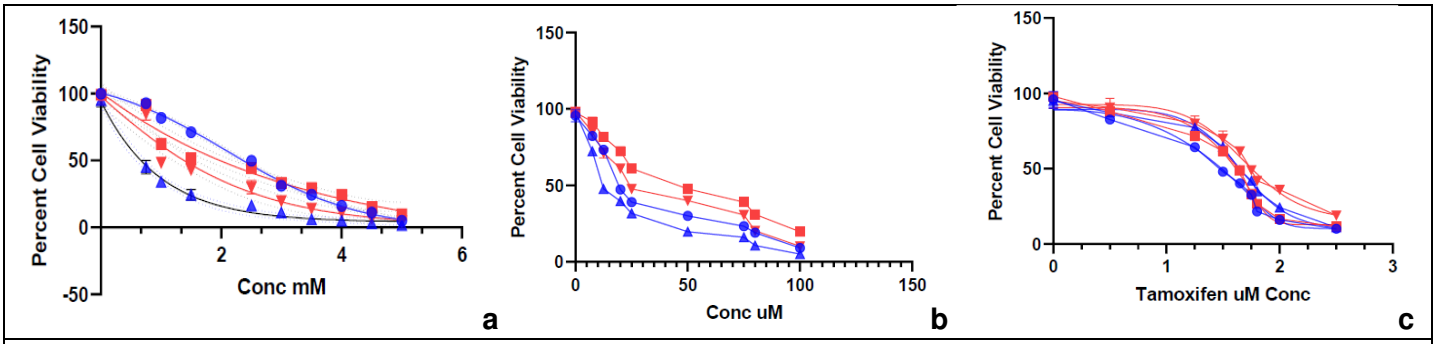


Fig. 3. Toxicological studies with the OrganRX Plate. (a) Cyclophosphamide (b) Aflatoxin (c) Tamoxifen

Heart percent viability (Static) Liver percent viabilty (Static)

Heart Percent viability (Flow) Liver percent viabilty (Flow)

Table 1: IC-50 values measured on OrganRX plate for rat heart and liver organs

	Cyclophosphamide (mM)		Aflatoxin (uM)		Tamoxifen (uM)	
	Static	Flow	Static	Flow	Static	Flow
Heart	2.50	0.75	20.0	12.5	1.50	1.65
Liver	1.50	1.00	0.5	25	1.65	1.75