Poster Number P288

Next Generation Genotoxicity Assessment in Human Hepatocyte Models: CometChip and Micronucleus Assay in Metabolically Competent HepaRG™ Cells

Abstract Number 2822

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HepaRG™ CometChip® enables medium throughput Comet Assay in a metabolically competent system, and pairs readily with flow cytometry-based Micronucleus Assay

Abstract

We are developing medium throughput genotoxicity assays using human-relevant metabolically competent hepatocytes to reduce, replace and refine the use of animals in the practice of genetic toxicology. We are combining HepaRG™ cells with CometChip® technology, a single cell array platform developed at MIT, and flow cytometry-based micronucleus (MN) assay to develop a New Alternative Methodology (NAM) aimed at reducing reliance on the in vivo comet and micronucleus assay. CometChip® facilitates rapid processing of 96 samples with unbiased-automated image-based scoring of the comet assay that can replace 30 yr old slide-by-slide one cell at a time scoring. Each imaged well of the 96 well plates have ≥ 200 scorable comets with the entire plate scored in less than 45 minutes compared to days needed to score 96 samples using traditional comet assay scoring. The in vitro and in vivo MN assay are part of the ICH S2R1 genetic toxicology test battery and we have adapted the flow cytometry-based micronucleus (MN) assay for use in HepaRG™ cells. To qualify this approach as a NAM we have: developed an initial basic protocol using a 3-day repeat exposure regimen, established qRT-PCR assays for functional assessment of HepaRG™ metabolic competency, conducted "power" studies to determine optimal number of comets scored per each sample, trained external collaborators at ILS, completed testing of an initial "training set" of negative and positive control test articles for use in the qualifying the HepaRG™CometChip® assay, and integrated HepaRG™CometChip® assay with MN assay to follow up in vitro MN positive responses. Multiple endpoint genotoxicity assessments in human hepatocyte models can serve as alternatives to animals for equivalent or better human-relevant safety and risk assessments than relying solely on rodent models. This work is funded by NIEHS SBIR 4R44ES024698-02.

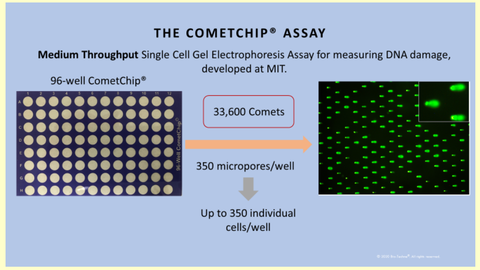
Introduction

- The in vivo Comet Assay, following OECD:489, is a common follow-up test to a compound testing positive in *in vitro* systems, and can use >45 animals per test article
- HepaRG™ cells contain both Phase I and Phase II metabolism enzymes
- CometChip® technology uses 96-well plate format to allow for rapid sample processing, combined with automated imaging technology. This allows for processing of an entire 96-well plate in less than 45 minutes.
- HepaRG™ cells can be readily used in the Micronucleus Assay with the addition of epidermal growth factor.

Study Objectives

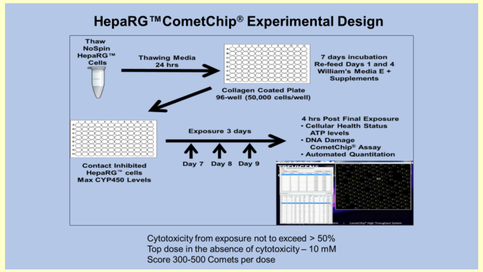
1. Establish an initial protocol for HepaRG™ CometChip®.
2. Conduct a "power" study to assess optimal number of comets to score per dose level.
3. Test known negative and positive control test articles for use in qualifying the HepaRG™ CometChip® Assay.
4. Integrate the HepaRG™ CometChip® Assay with the Micronucleus Assay.

CometChip® Technology



The CometChip® System uses standard 96-well format.

HepaRG™ CometChip® treatment Regimine



Cytotoxicity from exposure not to exceed > 50%
Top dose in the absence of cytotoxicity – 10 mM
Score 300-500 Comets per dose

Power study using Ethyl methanesulfonate (EMS)

EMS Power Study

EMS mM	1 Chip Cells scored	%DNA in Tail	P Value	% Survivability	2 Chips Cells scored	%DNA in Tail	P Value	% Survivability
0.0	83	6.09±3.94	1	100	317	6.39±2.17	1	100
0.3	399	6.41±2.14	1	93.74	520	8.70±2.04	1	92.57
0.5	276	6.88±1.89	1	89.27	516	11.63±1.91	0.9806	75.2
1.0	222	8.55±1.63	0.9962	84.78	460	23.94±7.39	0.1443	86.42
2.0	279	18.28±9.86	0.0767	87.13	480	27.60±12.13	0.0527	83.89
4.0	338	35.83±9.39	<.0001*	95.61	430	37.30±12.60	0.0023*	70.08
6.0	232	42.76±7.57	<.0001*	78.08	337	46.98±8.93	<.0001*	79.29
8.0	204	47.09±4.32	<.0001*	65.16	312	50.50±16.03	<.0001*	51.81
10.0	105	36.09±9.83	<.0001*	37.36	124	32.02±17.70	0.0135*	23.11

EMS mM	3 Chips Cells scored	%DNA in Tail	P Value	% Survivability	4 Chips Cells scored	%DNA in Tail	P Value	% Survivability
0.0	512	6.43±1.57	1	100	632	6.83±1.75	1	100
0.3	647	6.94±1.23	1	92.13	778	6.88±0.94	1	96.94
0.5	605	11.96±2.30	0.8575	79.29	718	12.49±1.62	0.6857	82.43
1.0	558	22.38±4.28	0.0193*	87.2	750	25.53±0.38	0.0015*	88.38
2.0	575	28.74±8.85	0.0017*	85.8	774	36.03±5.39	<.0001*	89.88
4.0	526	36.96±9.68	<.0001*	77.8	739	41.64±10.64	<.0001*	80.58
6.0	432	44.93±7.26	<.0001*	78.88	560	47.15±6.00	<.0001*	80.89
8.0	378	50.04±15.08	<.0001*	55.17	446	47.41±13.23	<.0001*	59.8
10.0	186	36.865±4.93	<.0001*	25.2	233	38.44±4.86	<.0001*	32.87

Reduced impact of increased comets once ~500 comets reached

Testing of known negative and positive control test articles

Chemicals Tested as Part of the Interlaboratory Validation Trial of HepaRG™ CometChip®

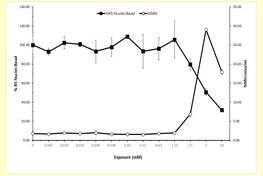
Chemical Name	Dose	Avg. ATP Concentration (µM)	Percent Cell Survival/ Viability	Mean of Well Medians	Standard Deviation of Well Medians	Statistical Significance against Vehicle Group (p-Value)	Trend Test (p-Value)	Comet Count
Vehicle (DMSO)	0 mM	8.61	100	4.62	1.21	-	0.2209	667
Anisicin	0.0781 mM	7.59	88.17	7.01	2.72	0.3238		388
Anisicin	0.1563 mM	6.55	76.11	7.69	3.62	0.2538		366
Anisicin	0.3125mM	7.23	84.01	6.44	2.41	0.5170		259
Anisicin	0.625 mM	5.91	68.67	6.03	1.53	0.2792		272
Anisicin	1.25 mM	7.18	83.46	5.79	1.50	0.8788		198
Anisicin	2.5 mM	6.39	74.25	5.28	1.81	0.9870		333
Anisicin	5 mM	6.42	74.64	8.54	7.61	0.0317*		409
Anisicin	10mM	7.37	85.69	6.54	3.01	0.5279		564
Vehicle (DMSO)	0 mM	3.24	100	5.20	1.65	-	0.2002	1072
Phenobarbital	0.0156 mM	2.03	62.82	4.70	0.56	0.3657		1042
Phenobarbital	0.0313 mM	1.77	54.69	4.88	0.86	0.9987		978
Phenobarbital	0.0625 mM	1.00	30.93	5.04	0.97	1.0000		764
Phenobarbital	0.125 mM	1.73	53.63	4.94	0.90	1.0000		645
Phenobarbital	0.25 mM	1.23	38.06	4.68	0.86	0.9019		679
Phenobarbital	0.5 mM	1.91	58.95	4.55	0.51	0.7947		908
Phenobarbital	1 mM	1.47	45.32	4.51	1.09	0.5234		858
Phenobarbital	2 mM	1.92	59.43	4.07	0.82	0.0538		991

Sample of known negative and positive control test articles. Depending on desired umber of comets, multiple test articles may be run on a single plate. ATP Assay was used in parallel with the CometChip® to assess cytotoxicity.

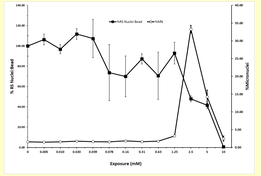
Chemicals Tested as Part of the Interlaboratory Validation Trial of HepaRG™ CometChip®

Chemical Name	Dose	Avg. ATP Concentration (µM)	Percent Cell Survival/ Viability	Mean of Well Medians	Standard Deviation of Well Medians	Statistical Significance against Vehicle Group (p-Value)	Trend Test (p-Value)	Comet Count
Vehicle (DMSO)	0 mM	9.61	100	4.62	1.21	-	< 0.0001*	667
Ethyl Methanesulfonate	0.0781 mM	7.19	83.51	7.29	2.34	0.8853		464
Ethyl Methanesulfonate	0.1563 mM	6.52	75.71	11.16	3.55	0.1348		220
Ethyl Methanesulfonate	0.3125 mM	7.54	87.65	18.47	4.65	< .0001*		193
Ethyl Methanesulfonate	0.625 mM	6.89	77.71	28.36	3.38	< .0001*		333
Ethyl Methanesulfonate	1.25 mM	7.67	89.17	36.72	5.87	< .0001*		332
Ethyl Methanesulfonate	2.5 mM	6.23	72.37	45.51	4.02	< .0001*		400
Ethyl Methanesulfonate	5 mM	5.71	66.40	47.37	6.73	< .0001*		464
Ethyl Methanesulfonate	10 mM	2.51	29.15	45.31	17.20	< .0001*		220
Benzo(a)pyrene (DMSO)	0 µM	3.36	100	4.83	1.00	-	< .0001*	769
Benzo(a)pyrene	0.195 µM	3.09	91.98	5.15	0.81	0.9090		677
Benzo(a)pyrene	0.391 µM	2.61	77.71	8.00	1.24	0.0573		378
Benzo(a)pyrene	0.781 µM	2.49	74.22	5.24	0.66	0.7917		359
Benzo(a)pyrene	1.563 µM	2.45	72.96	5.51	0.57	0.3459		305
Benzo(a)pyrene	3.125 µM	2.66	79.10	6.13	1.14	0.0231*		335
Benzo(a)pyrene	6.25 µM	1.98	58.80	7.79	1.80	< .0001*		396
Benzo(a)pyrene	12.5 µM	1.25	37.29	13.93	3.02	< .0001*		411
Benzo(a)pyrene	25 µM	0.76	22.19	16.11	4.31	< .0001*		477

Micronucleus Assay using HepaRG™

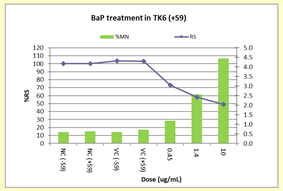


24 hour EMS treatment in HepaRG™



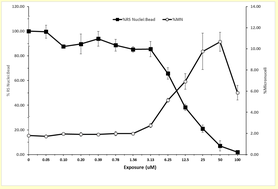
3 day EMS treatment in HepaRG™

Micronucleus Assay using HepaRG™ cont.

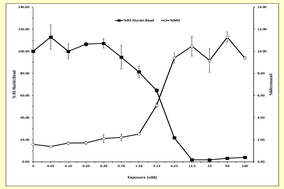


4 hour (+S9) BaP Treatment in TK6

- Comparison of TK6 and HepaRG™ Micronucleus data
- Robust response found in both HepaRG™ and S9 treated TK6 cells



24 hour BaP Treatment in HepaRG™



3 day BaP Treatment in HepaRG™

Summary and Conclusions

- Combining metabolically competent HepaRG™ cells and CometChip® technology provides the potential to develop a human-relevant New Alternative Methodology to reduce reliance on the *in vivo* Comet Assay.
- The throughput of CometChip® technology enables the conduct of experiment not possible using the 30+ year old one-at-a-time manual scoring. This is enabled through increased throughput, precision, and use of unbiased automated scoring.
- A possible extension of this is the use of CometChip® to score tissues collected from *in vivo* Comet Assay.
- The HepaRG™ CometChip® assay may be readily combined with the Micronucleus assay to further reduce reliance on *in vivo* testing.

Acknowledgements

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