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Project 121203-734

Thank you for selecting our services. This letter serves as our final report for continuation of project # 121203-734 testing the effect of cell phone radiation on Human Astrocytes cells as outlined below:

Work accomplished:

- The following primary cells were purchased as cryopreserved stocks: Normal human astrocyte cells samples.
- Astrocytes were thawed, plated into poly-lysine coated flasks and cultured for several days prior to seeding into 96 well plates. Cells were seeded in two poly-lysine 96 well plates (25,000 cells/well).
- Plates of astrocytes were treated with or without cell phone radiation daily for 6 hours/day (for a total of 5 days).
- Cell phone radiation was administered by placing the plate directly 1 inch above a Samsung Gusto^{TM2} cell phone at room temperature during which an active call was continuously ongoing for 6 hours. Phones were monitored every 10 minutes to ensure no interruption in the call occurred. The untreated plate was incubated at room temperature in a separate room. Room temperature was monitored throughout the experiment at each plate. Fluctuations in room temperature were minimal and deemed equivalent at each station.
- Three plates were included in the study. Plate one received no cell phone treatment, plate 2 received cell phone treatment alone, and plate 3 received cell phone treatment and was placed 30 ft from the Client provided MRET Noise Field Generator (NFG). Plate 3 was treated in the one room while plates 1 and 2 were kept in two separate rooms. Temperature in the rooms were monitored and controlled within 1 degree Celsius. On day 3, random temperature samples were taken on well in each plate and confirmed average temperature variations were a single degree Celsius or less from plate to plate.
- Following treatment, all plates were placed back into the 37°C incubator.
- At days 2, 3, 4 and 5, an MTT-like assay was performed using the Cell Titer 96 Aqueous reagent (Promega) according to the manufacturer's recommendation. MTT was added to wells and the plates were read on a 96 well plate reader (490 nm) (Molecular Devices Vmax kinetic microplate reader, Molecular Devices LLC) at various time-points after addition of the test reagent. Incubation time varied, as the number of cells increased over time. The same plate was used for all assay measurements and only a subset of wells treated with MTT on each assay date. To normalize data to account for the varying cell number and MTT incubation times, background (determined from average optical density value from wells containing media alone) was subtracted from individual data points and an average and standard deviation was calculated for the cell phone or no cell phone treated wells. The percentage of MTT signal in the cell phone treated wells relative to the no cell phone treated wells was calculated using the average and standard deviations from the two sample sets on each day of measurement. This percentage was then plotted as a function of days of treatment.
- The results of the MTT-like assay are presented in the Results section below.

- Images of the experimental setup are presented in Appendix I of this report

Results:

Figure 1 contains a dose response graph of the MTT assay data for the astrocytes with and without cell phone treatment and in the presence of the MRET Noise Field Generator (NFG). The no cell phone treatment signal on each day of measurement is set at 100% and the percentage of this signal obtained from the cell phone treated or cell phone plus NFG wells is graphed.

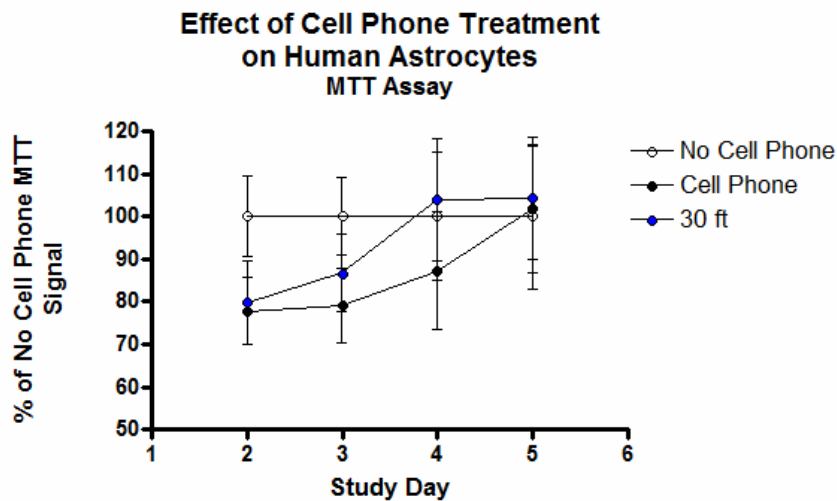


Figure 1: Effect of cell phone radiation on human astrocyte cells. Cells were treated with cell phone irradiation plus or minus the NFG (at 30 ft away) for 5 consecutive days. On days 2, 3, 4, and 5, an MTT Assay was performed. Data was normalized as indicated in the Work accomplished section above, and the percent signal relative to the no cell phone treated wells was plotted as a function of study day. Data points represent the average values for replicate wells. Error bars represent standard deviations. Standard deviations were calculated using standard methods for the propagation of errors.

Day 2	Raw Data		
position	NFG 30 feet	Phone only	No phone
	1.366	1.350	1.451
	1.341	1.375	1.539
	1.466	1.387	1.626
	1.403	1.407	1.570
	1.332	1.404	1.579
	1.323	1.324	1.491

Minus Background		
NFG 30 feet	Phone only	No phone
0.761	0.723	0.869
0.736	0.748	0.957
0.861	0.760	1.044
0.798	0.780	0.988
0.727	0.777	0.997
0.718	0.697	0.909

	Media			
	0.608	0.638	0.624	0.592
	0.602	0.623	0.630	0.572
Average	0.605	0.631	0.627	0.582

Average	0.767	0.748	0.961
StDev	0.054	0.032	0.063

	NFG 30 feet	Phone only	No phone
Average % of No Cell	79.8	77.8	100.0
StDev	9.69	7.89	9.33

Table 1: Effect of cell phone radiation +/- NFG on human astrocyte cells: Day 2 Data. Raw Data and Analyzed Data are presented for the MTT reading on cells treated with cell phone radiation for 2 days. Results are from an overnight read of the MTT data. After subtracting the average media alone signal, an average and standard deviation were calculated. The percentage of the Non-Cell Phone signal then calculated.

Day 3	Raw Data		
position	NFG 30 feet	Phone only	No phone
	1.336	1.247	1.493
	1.424	1.249	1.509
	1.326	1.340	1.500
	1.448	1.320	1.545
	1.414	1.333	1.519
	1.366	1.304	1.379

Minus Background		
NFG 30 feet	Phone only	No phone
0.729	0.659	0.901
0.817	0.661	0.917
0.719	0.752	0.908
0.841	0.732	0.953
0.807	0.745	0.927
0.759	0.716	0.787

	Media			
	0.602	0.589	0.588	0.594
	0.613	0.600	0.588	0.591
Average	0.608	0.595	0.588	0.593

Average	0.778	0.711	0.898
StDev	0.050	0.041	0.058

	NFG 30 feet	Phone only	No phone
Average % of No Cell	86.6	79.1	100.0
StDev	9.10	8.66	9.09

Table 2: Effect of cell phone radiation +/- NFG on human astrocyte cells: Day 3 Data. Raw Data and Analyzed Data are presented for the MTT reading on cells treated with cell phone radiation for 3 days. Results are from an overnight read of the MTT data. After subtracting the average media alone signal, an average and standard deviation were calculated. The percentage of the Non-Cell Phone signal then calculated.