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# The effect of mret treatment on human astrocyte cells post exposed to ionizing radiation of 2gy and 8 gy

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***Abstract—The anomalous viscosity and electrodynamic characteristics (dielectric permittivity and electrical conductivity) of MRET water was discovered during the experiments conducted at Moscow State University, Russia. It confirms the high level of long-range dynamic structuring of water molecules in polarized-oriented multilayer formations in activated water produced with the help of MRET activation process. The similarity of molecular formations of cell water and MRET activated water contributes to their compatibility, easy bio-availability and assimilation of MRET activated water in biological systems. The introduction of MRET water to biological systems can contribute to the enhancement of the cellular transduction mechanism and the proper function of cells in biological systems. In theory the enhanced cellular transduction mechanism may help living cells to survive impact of harsh environment such as exposure to high level nuclear radiation. To verify the feasibility of proposed hypothesis the effect of MRET treated cell media on human Astrocyte cells post exposed to ionizing radiation of 2Gy and 8Gy was studied at Molecular Diagnostic Services Inc., San Diego.***

***Keywords—MRET Activated Water, Dielectric Permittivity, Electrical Conductivity, Ionizing radiation, Astrocyte cells.***

## I. OBJECTIVES

The objective of this article is to demonstrate the enhanced viability of human Astrocyte cells introduced to MRET treated cell media after the exposure to high level of ionizing radiation of 2Gy and 8Gy. Such two doses of ionizing radiation were selected specifically based on the following reason: 2 Gy of radiation is a standard fractionated (one time) dose for radiation therapy. The total dose is fractionated (spread out over time) for several important reasons. Fractionation allows normal cells time to recover, while tumor cells are generally less efficient in repair between fractions. Fractionation also allows tumor cells that were in a relatively radio-resistant phase of the cell cycle during one treatment to cycle into a sensitive phase of the cycle before the next fraction is given. Similarly, tumor cells that were chronically or acutely hypoxic (and therefore more radioresistant) may reoxygenate between fractions, improving the tumor cell kill. In North America, Australia, and Europe, the typical fractionation schedule for adults is 1.8 to 2 Gy. The dose of 8Gy is considered to be a high level radiation threashold for the development of Neurovascular syndrom. This syndrome typically occurs at absorbed doses greater than 30 Gy, though it may occur at 10 Gy. It presents with neurological symptoms such as dizzines, headache, or deacreased level of consiosness, occurring within minutes to a few hours, and with an absence of vomiting. It is invariably fatal (Wikepidia, en.wikipedia.org/wiki/Ionizing_radiation).

MRET Activated Water is produced with the help of patented in the USA Molecular Resonance Effect Technology (MRET). MRET water activator is the stationary source of subtle, low-frequency, resonant electromagnetic field with composite structure. The origin of the low-frequency composite electromagnetic field is the intensive electrical activity inside the nano-circles formed by linear molecular groups of MRET polymer compound (volumetric fractal geometry matrix) when polymeric body is exposed to the external electromagnetic fields of specific frequency and wavelength [Vysotskii, Smirnov 2005]. The anomalous viscosity and electrodynamic characteristics (dielectric permittivity and electrical conductivity) of MRET water provide some evidence regarding polarized-oriented multilayer structuring of MRET activated water and the possible effect of MRET water on the proper function of cells in biological systems.

The fundamental biophysical theories revealed the scientific paradigm regarding polarized-oriented multilayer structuring of cell water in biological systems [Ling 2001, Drost-Hansen 1971, 1991]. The suggested model of polarized-oriented multilayer structuring of cell water due to the interaction of water dipoles with pervasive matrix of fully-extended proteins constitutes the basis for the cellular transduction mechanism [Ling 2003]. Based on this scientific approach the similarity of molecular formations of cell water and MRET activated water can contribute to their compatibility, easy bio-availability and assimilation of MRET activated

water, as well as to the enhancement of cellular functions in biological systems. A number of researches confirmed the ability of MRET water to enhance morphology of blood cells and to suppress mutated cells *in vitro*, high germicidal activity of MRET water, the inhibition of growth of c*allus* tissue (mutated cells of botanical origin) in MRET water [Smirnov 2010, 2009]. Significant positive effect of MRET activated water regarding the tumor resistance in animals was observed in the experiments conducted on 500 mice. This investigation was conducted at Kiev Institute of Experimental Pathology, Oncology and Radiobiology, Ukrainian Academy of Science [Vysotskii 2006].

## II. METHODS

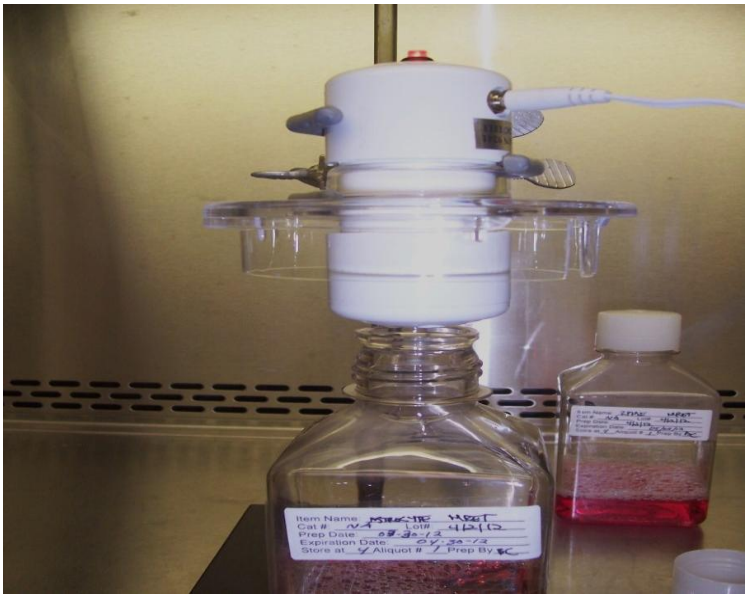
The effect of MRET treated cell media on human Astrocyte cells exposed to ionizing radiation of 2Gy and 8Gy was studied at Molecular Diagnostic Services Inc., San Diego.

Normal human Astrocytes were thawed, plated into poly-lysine coated flasks and cultured for several days prior to seeding of flasks for irradiation to obtain sufficient cell numbers. On the day before irradiation, cells were seeded in five poly-lysine T-25 coated flasks.

Flasks of Astrocytes were taken for irradiation. One flask received no irradiation, one flask each received a radiation dose of 2, and 8 Gy. Cells were irradiated in a Gammacell model 40 extractor (MDS Nordion Gammacell).

Following irradiation, the cells from each flask were harvested, split in half, and resuspended in either normal untreated media or MRET media. The cells were counted and plated at a density of 5000 cells/well. The Astrocyte cells were plated in poly-lysine coated 96 well plates. The cells were plated according to the plate map shown below. One plate was assayed at 24 hours post-irradiation and the remaining plate was assayed at 72 hours post irradiation.

An MTT assay was performed at 24 hours and 72 hours using Cell Titer 96 Aqueous reagent (Promega) according to the manufacturer's recommendation. 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 MTT reagent. After collection of the study data, optical densities were plotted as a function of radioactive dose response curve for each cell line. Average OD values for replicate wells of each dose/treatment were plotted along with standard deviations.



**Picture: 1 The cell media treatment with MRET activator for 30 minutes period of time.**

## III. RESULTS

Figures 1 and 2 contain radiation dose response graphs of the MTT assay data for the Astrocytes in regular media or MRET treated media while Tables 1 and 2 contain the raw data.

For the Astrocytes treated with normal cell media, little change in the MTT signal was observed in the 2Gy treated wells compared to the untreated cells at both 24 and 72 hours post irradiation incubation. However, the 8Gy radiation dose resulted in 7.8% and 24% drop in MTT signal at 24 hours and 72 hours of incubation respectively compared to the cells that received no radiation. When cell media was treated with MRET, little to

no loss in MTT signal was observed in the 8Gy treated wells at both 24 hours and 72 hours of incubation (14% increase and 3% increase respectively).

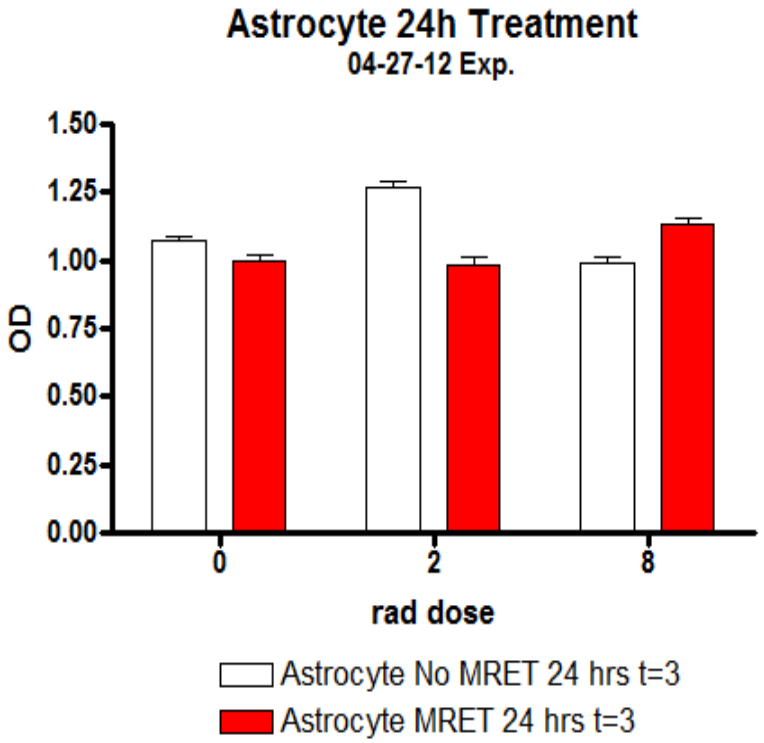



**Figure 1: Effect of MRET on Astrocyte cells with varying radiation dose - 24 hour treatment**

**Quintuplicate** wells of cells were exposed to no radiation, 2 Gy, and 8 Gy, treated for 24 hours with either MRET or normal media, and then assayed using MTT. The average values of quintuplicate wells are plotted. Error bars represent standard deviations.

| | No MRET | | | | | MRET | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 Gy | 2 Gy | 8 Gy | Media | | 0 Gy | 2 Gy | 8 Gy | Media |
| | 1.046 | 1.169 | 0.917 | 0.291 | | 0.941 | 0.876 | 1.074 | 0.285 |
| | 1.036 | 1.261 | 1.036 | 0.292 | | 0.999 | 0.991 | 1.122 | 0.285 |
| | 1.085 | 1.318 | 1.013 | 0.290 | | 0.998 | 0.987 | 1.13 | 0.285 |
| | 1.083 | 1.264 | 0.969 | 0.290 | | 0.995 | 1.036 | 1.193 | 0.285 |
| | 1.113 | 1.310 | 1.011 | 0.291 | | 1.068 | 1.026 | 1.156 | 0.282 |
| Ave | 1.073 | 1.264 | 0.989 | 0.291 | Ave | 1.000 | 0.983 | 1.135 | 0.284 |
| StDev | 0.031 | 0.059 | 0.047 | 0.001 | StDev | 0.045 | 0.064 | 0.044 | 0.001 |

**Table 1: Effect of MRET on Astrocyte cells with varying radiation dose -24 hour treatment**

**Quintuplicate** wells of cells were exposed to no radiation, 2 Gy, and 8 Gy, treated for 24 hours with either MRET or normal media, and then assayed using MTT. The average values and standard deviation of quadruplicate wells are shown.

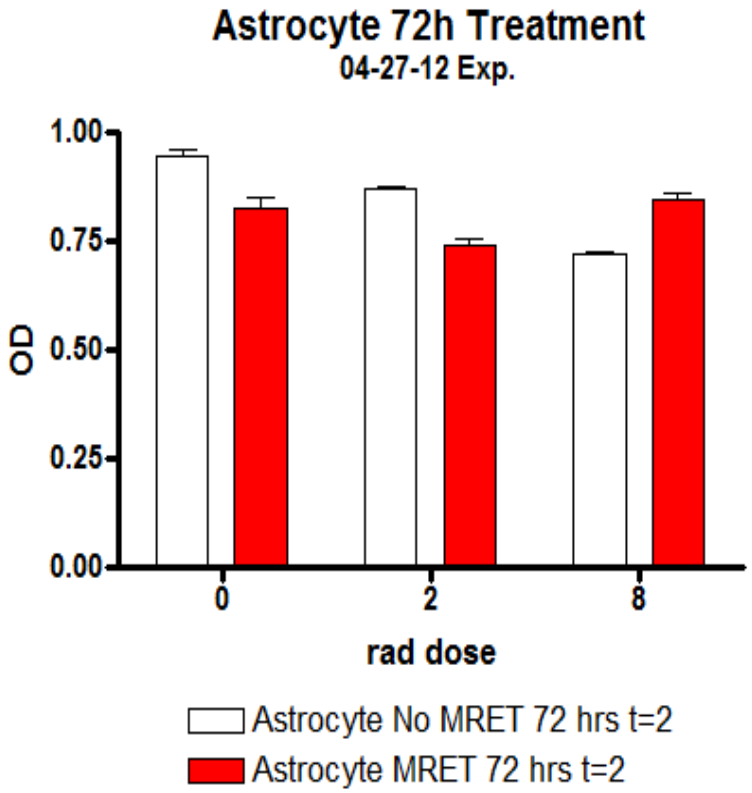



**Figure 2: Effect of MRET on Astrocyte cells with varying radiation dose - 72 hour treatment**

**Quintuplicate** wells of cells were exposed to no radiation, 2 Gy, and 8 Gy, treated for 72 hours with either MRET or normal media, and then assayed using MTT. The average values of quintuplicate wells are plotted. Error bars represent standard deviations.

| | No MRET | | | | | MRET | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 Gy | 2 Gy | 8 Gy | Media | | 0 Gy | 2 Gy | 8 Gy | 0.202 |
| | 0.938 | 0.847 | 0.730 | 0.211 | | 0.866 | 0.746 | 0.811 | 0.193 |
| | 0.934 | 0.869 | 0.709 | 0.208 | | 0.749 | 0.745 | 0.881 | 0.199 |
| | 1.005 | 0.877 | 0.726 | 0.204 | | 0.874 | 0.689 | 0.848 | 0.208 |
| | 0.911 | 0.860 | 0.706 | 0.204 | | 0.807 | 0.775 | 0.833 | 0.203 |
| | 0.943 | 0.892 | 0.736 | 0.198 | | 0.834 | 0.744 | 0.860 | 0.201 |
| Ave | 0.946 | 0.869 | 0.721 | 0.205 | Ave | 0.826 | 0.740 | 0.847 | 0.202 |
| StDev | 0.035 | 0.017 | 0.013 | 0.005 | StDev | 0.051 | 0.031 | 0.027 | 0.006 |

**Table 2: Effect of MRET on Astrocyte cells with varying radiation dose -72 hour treatment**

**Quintuplicate** wells of cells were exposed to no radiation, 2 Gy, and 8 Gy, treated for 72 hours with either MRET or normal media, and then assayed using MTT.The average values and standard deviation of quadruplicate wells are shown.

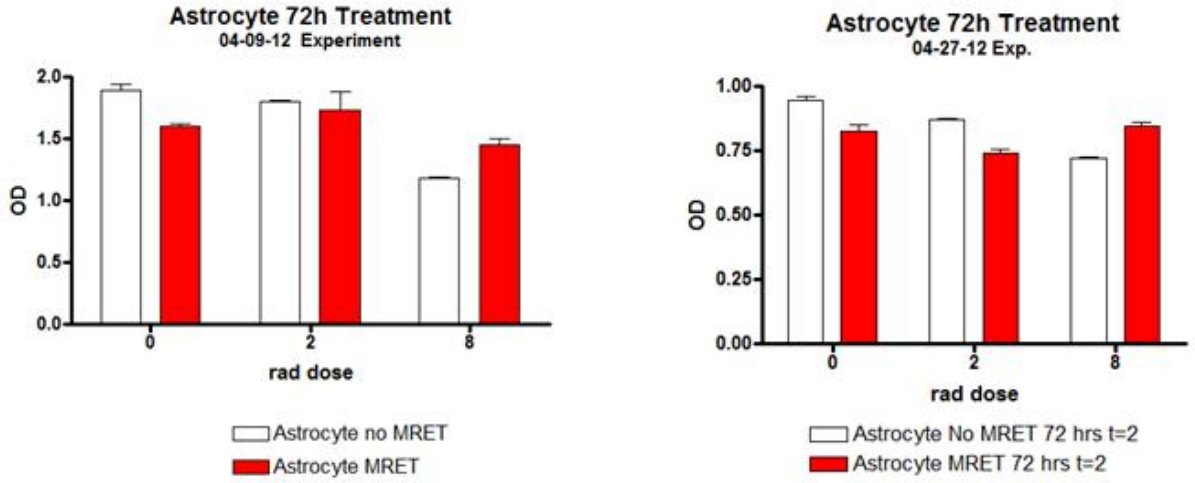



**Figure 3: Comparison of 04-09-12 and 04-27-12 experiment.**

Graphs of 72 hour data from 04-09-12 versus the same treatment and radiation dose from the 04-27-12 experiment showing similar response.

## IV. CONCLUSIONS

For the Astrocytes treated with normal media, little change in the MTT signal was observed in the 2Gy treated wells compared to the untreated cells at both 24 and 72 hours post irradiation. However, the 8Gy radiation dose resulted in a 7.8% and 24% drop in MTT signal at 24 hours and 72 hours of incubation respectively compared to the cells that received no radiation. The 24 and 72 hour data from this current experiment (04-27-12) is in agreement with the previous experiment (04-09-12) in that 1) MRET treatment resulting in a slight depression of the signal in the non-irradiated cells and 2) MRET treatment resulting in an advantageous/protective effect at 8Gy (see Fig. 1 and 2). A side by side comparison of the 72 hour of incubation results from this experiment (04-27-12) and the previous experiment (04-09-12) is presented in Figure 3. In both experiments, the treatment of cells with MRET media following exposure to 8Gy irradiation results in an increase in the MTT signal thus, it implies a protective effect (30% and 25% increase in signal for 04-09-12 and 04-27-12 experiments respectively). Thus, the MRET treatment appears to protect the Astrocytes cells against the ionizing radiation induced damage.

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