Report "ATP Study", prepared by Dr. Robert Sheaff, 4-10-23

Overall Discussion & Conclusion

Written by:

Ian Mitchell, CSA
4-4-2023

After receiving the below report from Dr. Sheaff, my task as Chief Scientific Advisor for Leela Quantum Tech was to assess the data and see what true conclusions and implications, if any, could be derived.

All three of the below-mentioned experiments were conducted in a double-blind fashion.

Dr. Sheaff was unaware of which cells were and were not being charged via the Quantum Upgrade protocols with the advanced Leela Quantum Tech technology, as were the experimenters performing the quantum charging from over 500 miles away.

The data clearly shows that the expected stochastic distribution in ATP production rates is not observed consistently in all of the results. As the experiments progressed and variables and sources of potential complication were eliminated, a more clear picture became evident after analyzing the data sets.

By experiment #3, it can be clearly noted that the transient shift towards up-regulation in ATP production rates is a consistent function in all of the quantum-charged cells, which is in stark contrast to the uncharged cells.

ATP production rates jumped consistently in all treated groups by 20-25%.

This demonstrably shows from an evidentiary standpoint that the impact on cellular energy production via quantum charging has profound implications and, more broadly, that there is definitive non-local interaction occurring in biological systems.

An increase in cellular energy output in excess of 20% over baseline allows a person to have more resources at their disposal biologically across all domains. It can better help people heal from injuries, stave off diseases, allow for better mental processing, and greatly enhance athletic performance.

_Ian Mitchell

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Qualifications

I am a biochemist with over 30 yrs experience in the field. I received a BA from the University of North Carolina (Honors in Biology and Philosophy), a Ph.D. from the University of Colorado at Boulder (Chemistry), and did postdoctoral work at the Fred Hutchinson Cancer Research Center (Cell Biology). I am currently an Associate Professor of Biochemistry at The University of Tulsa. The clients contacted me to design and carry out a series of experiments to evaluate quantum effects on various cell parameters.

Disclaimer

This communication, the underlying work, and all opinions contained herein, are solely performed and made in my individual capacity as a consultant, and are not formal or informal statements or opinions made by or on behalf of The University of Tulsa.

Goal

Clients have hypothesized that two or more biological systems can influence each other despite physical separation in space via a quantum or aether effect. In physics, quantum entanglement is an unexpected, counterintuitive phenomenon that seeks to explain how two subatomic particles can be linked to each other even if separated by vast distances. As an alternative view, the aether model looks at the existence of a space-filling field as a transmission medium that's literally behind and within the matter. This field is everywhere, and it can be accessed, concentrated, and leveraged - for example, to influence (biological) systems over distance. Despite physical separation by distance, experimental evidence indicates that a change can be induced over distance via "entanglement" or simply "field-connection" despite being separated in space. The clients propose that a similar phenomenon exists at the biological level and that under certain conditions, a biological system (such as a cell) can be influenced from a distance.

Synopsis

This report describes the results of three initial experiments analyzing quantum or field effects on cellular ATP levels. ATP was selected as the biological read-out because it is the energy currency of the cell, playing an essential role in all aspects of cell function, activity, and overall health. Furthermore, ATP is continually being utilized and re-synthesized to maintain optimal levels, so its dynamic turnover means that anything (positive or negative) influencing either ATP use or production can have an observable and quantifiable impact on its levels. Extremely sensitive experimental techniques are available and routinely used in my lab to measure cellular ATP levels, typically to determine if and how they change in response to chemical agents that might affect biological processes (e.g., chemotherapeutic drugs or metabolic stimulants).

Experiment 1 (8262)

To test this hypothesis, the initial experiment involved generating ten identical plates (60mm) of A549 cells according to the scheme depicted in Figure 1. A549s are an adherent human non-small cell lung cancer cell line created in 1972 by D. J. Giard and colleagues by removing and culturing pulmonary carcinoma tissue from the tumor of a 58-year-old white male. The A549 cell line consists of hypotriploid alveolar basal epithelial cells, and is commonly used for both basic research and drug discovery.

Previous work in my lab has shown that A549 cells generate ATP using the same metabolic pathways evolutionarily conserved in other cell types and that these ATP levels can be manipulated by the addition of metabolic inhibitors. Thus, they are a suitable model system for the proposed experiments.

The cells were maintained in standard DMEM media supplemented with 10% FBS, and allowed to incubate overnight in a 37°C incubator with 5% CO_2 to allow attachment to the plate bottom. The next day, five plates were randomly labeled “A” and five plates were randomly labeled “B”. Both sets were then handed over to a colleague who carried out the quantum aspect of the experiment in conjunction with the clients in Germany. This part of the experiment—in which one set of cells was “treated” with the quantum effect while the other was not-- was carried out in my absence. Plates were returned to me within 5 minutes and placed in the incubator without my knowing which set had been treated. At the time points indicated in Figure 2, one plate from each set was removed from the incubator and the media was removed by aspiration. The plate was then washed with 2.5ml PBS, aspirated, and the attached cells removed from the plate bottom by treatment with 0.5ml trypsin at 37°C for 5min. Cells were pelleted by centrifugation and re-suspended in 500 microliters DMEM. ATP levels in 50 microliters of the cell suspension was then measured by the addition of CellTiterGlo, a commercially available reagent that measures ATP as a function of light emission by luciferase. Luminescence was measured on a Cystation plate reader with a luminometer. All time points were evaluated as duplicates so that the standard deviation could be determined. The identity of the treated set was not revealed until all data was collected but not yet evaluated.

Figure 2 compares the ATP levels in untreated vs treated cells over time. Note that ATP levels are already higher in the treated sample after 10 min and remain higher for at least 2hrs. This difference can be highlighted by re-graphing the same data as the % difference between treated and untreated cells (Figure 3). This difference could simply be explained by small variances in the number of cells plated, but if this were the case the difference in ATP should have been constant at each time point. Note that by 3 hr. ATP levels are back to levels of the untreated control, suggesting the transient increase is real.

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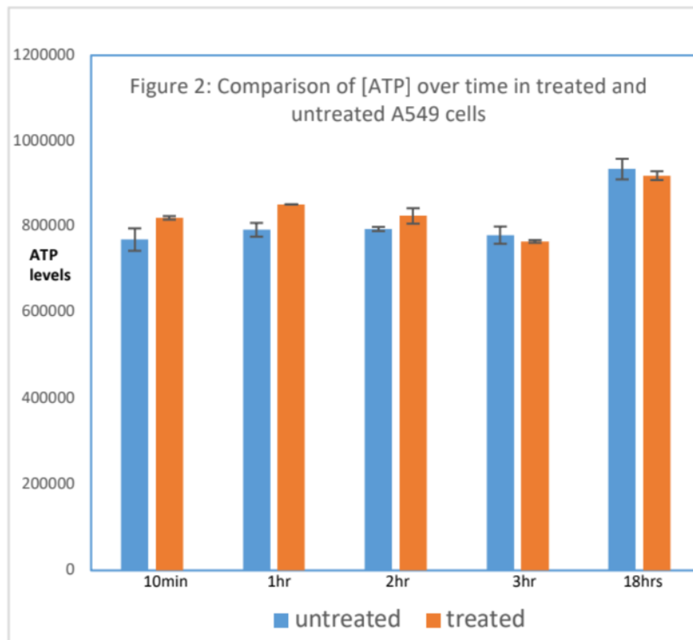

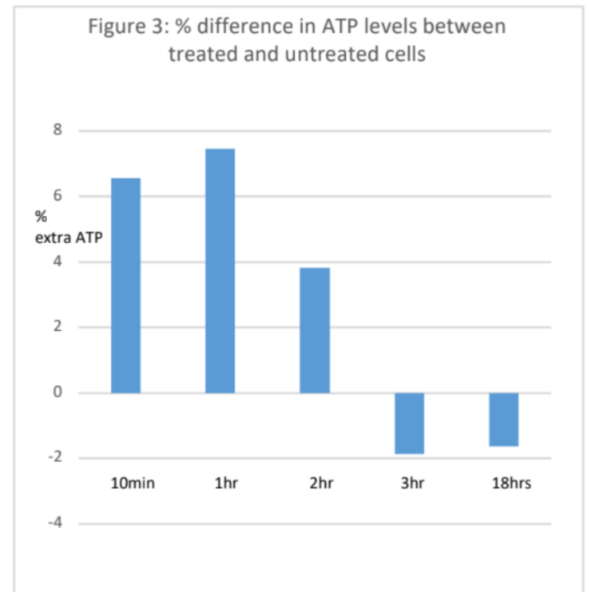

Nevertheless, to rule out this possibility (and to reduce or remove other potential complications with the initial experimental set-up) experiment 2 was performed.

Experiment 2 (8311)

The quantum effect on cellular ATP levels was re-evaluated with the following modifications designed to reduce potential sources of variability that could affect experimental results.

1. In addition to A549 cells, Human Diploid Fibroblasts (HDFs) were also analyzed. This is an immortalized but untransformed human cell line derived from male foreskin and immortalized by telomerase overexpression. It is commonly used as a more “normal” cell for comparative purposes with cancer cell lines.
2. The previous experiment utilized individual plates of cells. This introduced the possibility of variabilities in cell number at the time of plating. In addition, cells were allowed to attach and grow overnight, which could lead to stochastic differences in cell number over time (e.g., due to small variations in growth rate). Finally, the cells had to be removed from the plate for analysis, which is another possible source of error. To address these concerns cells were removed from stock plates with trypsin as described above, then distributed into two 96 well plates. Cells can now be analyzed directly (without removal from a plate). Furthermore, a no –treated control can be determined for each cell set.
3. This experimental scheme allows the media in which cells are maintained to be varied, which in turn influences which metabolic pathways are being utilized to generate ATP. This methodology is commonly used by my lab to determine which metabolic pathways are being targeted by a drug. DMEM minimal lacks nutrients, L15 has amino acids but lacks glucose, DMEM contains both amino acids and glucose, and DMEMF is the same as DMEM but also contains 10% FBS.

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A459 and HDF cells were removed from a 100mm plate with trypsin, re-suspended in a minimal DMEM media (lacking nutrients), and then aliquoted into individual tubes containing various media compositions. Each tube was then distributed into 12 wells of two 96 well plates (one of which will bereated and the other is the control), which ensures each well has the same number of cells (see Figure 4). Cells were incubated at 37°C + CO_2 for 1hr to allow them to equilibrate to the media composition and adjust their metabolic pathways accordingly. Note that cells are not given time to replicate so this variable is removed.

After 1hr ATP levels were measured in two wells of both plates. This is important because it provides the ATP levels in untreated cells for both plates (to be treated and untreated). As above, the plates were randomly labeled “A” and “B”. Both plates were then handed over to a colleague who carried out the quantum aspect of the experiment in conjunction with the clients in Germany. This part of the experiment—in which one set of cells was “treated” with the quantum effect while the other was not--was carried out in my absence. Plates were returned to me within 5 minutes and placed in the incubator without my knowing which set had been treated. At the time points indicated in Figure 5 ATP was analyzed by adding CTG directly to each well (in duplicate). This modification is important because it avoids possible complications of having to remove the cells from a plate.

Data in Figure 5 with A549s are qualitatively consistent with that presented in Figure 2, showing a transient increase in ATP levels in cells exposed to the quantum effect. The media variations provide additional information. Note that in DMEM minimal, there is not a significant difference in sets A and B, and that ATP levels actually decrease over time. This is to be expected because minimal media lacks the nutrients required for ATP production. In contrast, L15, DMEM, and DMEMF—which contain various nutrients required for ATP production-- all show a transient increase in ATP levels relative to the control. However, this effect does not seem to be persistent as long as in experiment 2, and there is a bit more scatter in some of the data sets. This latter issue likely reflects the attempt to take shorter time points, which is technically more challenging). In contrast, data with HDF cells does not show an increase in ATP levels of treated cells; in some cases (e.g. L15 and DMEM) ATP levels actually decrease in the treated cell set. A priori, it is unclear why the quantum effect might be cell type specific and/or have opposite effects.

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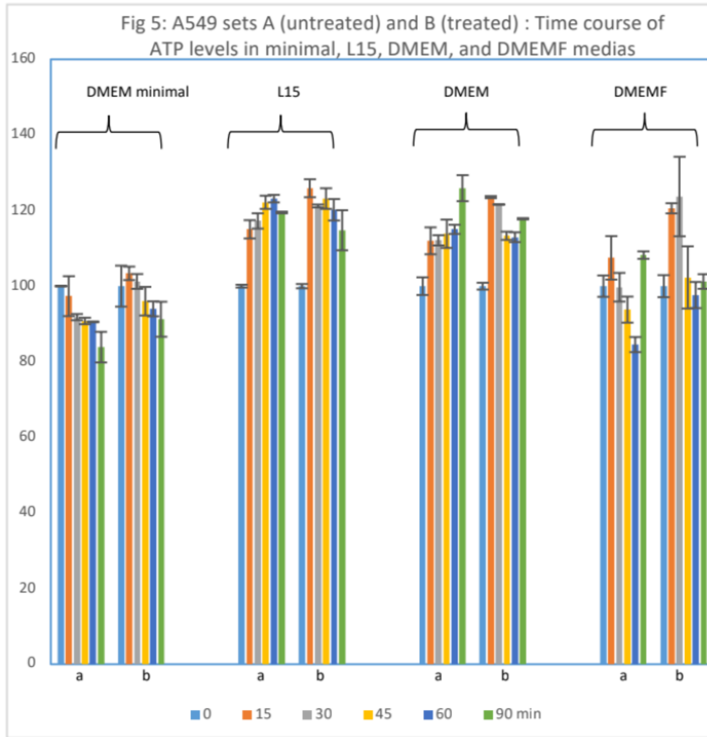

Figure 5: ATP levels over time in A549 cells exposed to quantum effects (set B) and untreated (set A). Cells were incubated in different medias to alter metabolic pathways involving ATP synthesis and/or use. Time zero is ATP levels in untreated cells before initiating the experiment. After treatment ATP levels were determined at the times indicated. Data are plotted as a % of the t=0 control.

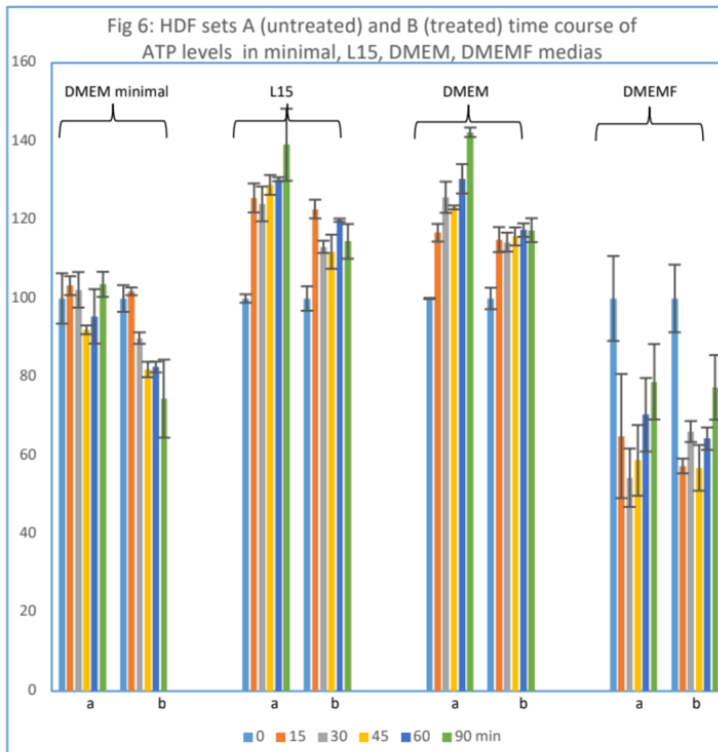

Figure 6: ATP levels over time in HDF cells exposed to quantum effects (set B) and untreated (set A). Cells were incubated in different medias to alter metabolic pathways involving ATP synthesis and/or use. Time zero is ATP levels in untreated cells before initiating the experiment. After treatment ATP levels were determined at the times indicated. Data are plotted as a % of the t=0 control.

In considering the experimental setup for experiment 2, we realized that the attempt to reduce variables had introduced another. In the experiment, cells were plated and allowed to attach to the plate surface overnight. Thus, the quantum effect was evaluated on attached cells. However, in experiment 2 cells were distributed directly from a plate to 96 well plates that were not designed to allow cell attachment. Furthermore, the experiment was performed immediately after cell distribution, ensuring that we were performing the experiment on unattached cells. This could be an issue, especially for HDFs. As a reminder, HDFs are Human Diploid Fibroblasts that are immortalized but not transformed. Thus, their natural state for proper functioning is to be attached to a surface. In light of this potential complication, experiment three was performed.

Experiment 3 (8549)

This experiment combines aspects of both previous set-ups. A549 and HDF cells were again distributed into two separate 96 well plates, but this time we used plates specifically designed to allow cell attachment. In addition, we decided to switch incubation conditions from the 37°C incubator +CO_2 to a 37°C incubator in the absence of CO_2. The rationale for this decision was based on previous work in the lab showing that repeated removal of cell plates from a CO_2 incubator could affect the pH, which in turn can influence ATP levels and/or metabolic pathways being utilized. For these conditions, we utilized L15 media -/+ glucose, which is specifically designed for culturing cells in the absence of CO_2. After plating, cells were allowed to incubate at 37°C (no CO_2) for 7 hrs., which is sufficient time to allow attachment to the plate (confirmed by microscopy), but not enough time to allow cell replication. After incubation, plates were again randomly labeled “A” or “B” and handed over to a colleague who carried out the quantum aspect of the experiment in conjunction with the clients from over 500 miles away. As previously, this part of the experiment—in which one set of cells was “treated” with quantum energy over distance while the other was not-- was carried out in my absence to establish a double-blind foundation. Plates were returned to me within 5 minutes and placed in the incubator without my knowing which set had been treated. At the time points indicated in Figure 7, ATP was analyzed by adding CTG directly to each well. In order to generate higher-quality data, time points were now taken in quadruplicate rather than duplicates.

Once again, a transient but statistically significant increase in ATP levels was observed in the treated cells. This increase was present in both the absence and presence of glucose, suggesting the effect was not confined to a particular metabolic pathway. Figure 8 is the identical experiment using HDF cells. While the data are not quite as clean as with A549 cells, a transient increase in ATP levels in the treated set can clearly be observed, especially in the set with glucose. Another important aspect to mention is that for experiment 3, the clients removed the spill-over effect that happened in experiments 1 and 2 of the charged cells to the non-charged cells. If that is the case, it makes sense why the first experiments "only" showed an up to 8% increase in ATP production (still significant) vs. the 20-25% in the third experiment in which the non-charged cells were truly non-charged.

Quantum Upgrade with the advanced Leela Quantum Tech technology ATP Production Rate Experiments 1-3

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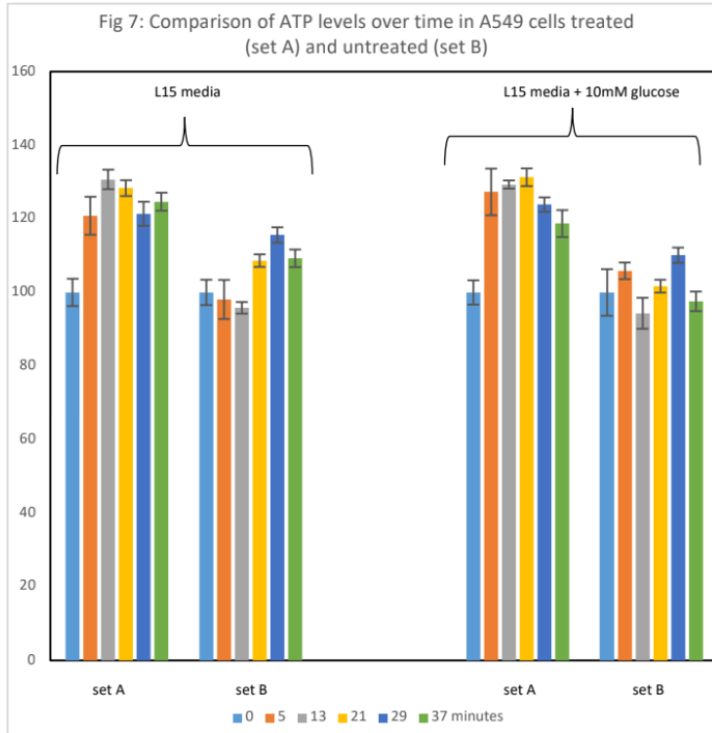

Figure 7: ATP levels over time in A549 cells exposed to quantum effects (set A) and untreated (set B). Cells were incubated in different medias to alter metabolic pathways involving ATP synthesis and/or use. Time zero is ATP levels in untreated cells before initiating the experiment. After treatment ATP levels were determined at the times indicated. Data are plotted as a % of the t=0 control.

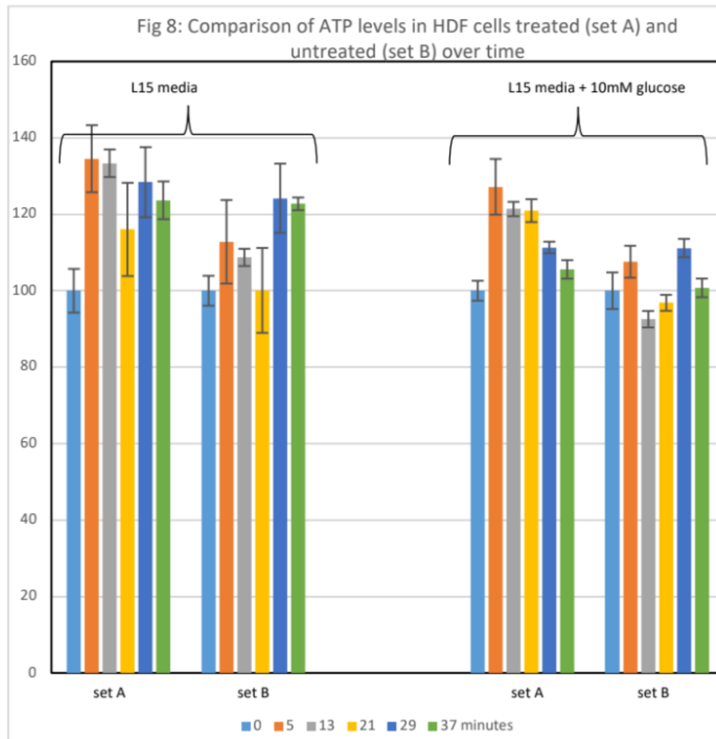

Figure 8: ATP levels over time in HDF cells exposed to quantum effects (set A) and untreated (set B). Cells were incubated in different medias to alter metabolic pathways involving ATP synthesis and/or use. Time zero is ATP levels in untreated cells before initiating the experiment. After treatment ATP levels were determined at the times indicated. Data are plotted as a % of the t=0 control.