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Improvement of Antioxidative Defense of Cells Exposed to Radio Frequencies by a Nanotechnology Device

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Abstract: Transfer of an electromagnetic activity from a complex biological system, to another complex system is present both in current life and in quantum physics studies. Chlorophyll photosynthesis, is a typical model of an interaction between electromagnetic fields, deriving from solar energy and elements of a biochemical nature, the chlorophyll, responsible for the energy production, as a process deriving from chemical transformation. The following report presents a series of evidences, collected by means of various experimental approaches, aimed at demonstrating that by inducing the electromagnetic activity of an active substance on an electric field in stationary conditions, a quantum variation of the electric field can be obtained. Such electric field, is transferred to a support of fluorescent nanocrystals called "Quantum Dots", whose electronic structure is suitable to maintain the starting quantum characteristics stable. The application of patches containing the aforementioned nanocrystals, on two biological models: *Saccharomyces cerevisiae* colonies and *Pisum sativum* plants, exposed to the irradiation of specific routers, showed a protective activity of these patches, evidenced by a regular increase in antioxidative defense and cell proliferation. The results reported in this research suggest the possibility of application of patchs supporting fluorescent nanocrystals as an effective defense against the production of reactive oxygen species.

Keywords: Nanocrystals, Quantum Dots, Electromagnetic Field, *Pisum sativum*, *Saccharomyces cerevisiae*

1. Introduction

From the point of view of physics, the activity of biological systems are expressed in terms of non-linear excitations. Several forms of excitation have common features [1] and requires the supply of energy. In normal condition energy supply, is related to heating. However, In biological systems, metabolic energy supply is an essential step for biological organization [2]. Using model calculation, it was postulated that coherent excitations should play an important role in biological activity [3]. Living cells are able to channel their chemical energies into high frequency electrical and cooperative oscillations [4]. In particular, Frohlich (1969), predicted that there would be a very high frequency oscillation in the frequency range of 100 gigahertz, but left open the possibility that electrical oscillations of a large scale and of a cooperative nature at yet lower frequency ranges could be observed. Recently evidence, indicates that living cells, when metabolically active, produce oscillations, at the very high frequency range [5]. Evidence for natural electrical cellular oscillations of a lower frequency can be inferred by several sources. Indeed, long range interactions exist between live erythrocytes in plasma [6-7]. Natural electrical oscillations at lower frequencies by cells has been obtained [8]. According to authors two phenomena highlighted these evidences; dielectrophoresis (DEP), and cellular spin resonance (CSR). DEP, is the motion of neutral particles induced by the action of non-uniform electric fields [9-10]. DEP is useful to explore for the existence of non-uniform electric fields. Indeed, the motion of small polarizable particles around cells is a way to identify fields close to the cell surface. Generally, the motion of these polarizable neutral particles, will be towards the region of higher field strength, provided that their effective dielectric constant exceeds that of the suspending medium at that frequency. This phenomenon is called "micro-dielectrophoresis" (micro-DEP). Several experimental evidences, on micro-DEP investigations on cells, support the presence of natural radio frequency oscillations in a number of ways. Such observations, minimize the probability that the observed micro-DEP effects are due to chemically-related selective effects, and support the interpretation that they are due to dielectric factors and not to chemical ones. That living cells are the source of natural electrical radio frequency oscillations is shown by several experimental means. The causes and consequences of these natural resonances need clarification. The natural cellular resonances are detectable in a very wide range of cell types, including bacteria, yeasts, algae, and avian and mammalian cells, and hence are probably “universal.” But if such natural electrical oscillations are necessary in the metabolic activity of cell this implies that there must be an electrical aspect to cellular growth and its control. The experimental studies outlined in the following report, seek to demonstrate that by inducing the electromagnetic activity of an active substance on an electric field in stationary conditions, a quantum variation of the electric field can be obtained, visible through the effects, when compared with those of the active ingredient. The electric field, which was induced by the quantum state electromagnetic activity of the active ingredient, adopts the characteristics of the same principle. Such electric field transferred to a support (H. I. T. patches), posses a suitable electronic structure, to maintain the starting quantum characteristics stable.

2. Materials and Methods

2.1. Biological Samples

120 VV-patches were used for the *in vitro* experiments and 120 NFC-patches were integrated into electronic devices on router (H. I. T. srl. Italy). Two biological models were employed: *Saccharomyces cerevisiae* colonies *and Pisum sativum* plants. On *Saccharomyces cerevisiae* colonies, experiments were performed to assess the antioxidant effect of both VV-patches and NFC-patches applications. Oxidized organisms, were cultured in Petri capsule in a medium containing agarose and maltose. In order to perform experiments on the apical part of a stem and on the apical part of each root, of *Pisum sativum* plants, three series of hydroponic crops were created, each series consisted of 10 plants, each of them arranged in a container. The containers of the first set were placed in contact with a VV-patch. The containers of the second set were placed at a distance of 4 meters from the first set and the containers of the third set were placed 100 meters away from the other two sets. The radical apex of *Pisum sativum* plants, were chosen for the presence of an open meristem that can be more easily histologically analyzed than the closed meristem.

2.2. Yeast Growth

Stationary *Saccharomyces cerevisiae* cultures were obtained by inoculation of isolated haploid colony (By4147) in liquid complete medium (YPD) containing 1% yeast extract, 2% peptone, and 2% glucose. The yeast was kept overnight at 30 °C with shaking at 250 rpm up to reach the stationary growth phase.

2.3. Technological Parameters

All electromagnetic waves emission systems have been placed in a 30 m long and 8 m wide room, placed on one of the short sides, above an amagnetic support, equipped with a 230V power supply system. The measuring stations have been calibrated by means of a laser distance meter; the stations have been placed on fixed supports at the desired distances. In addition, a measurement on a fixed length mobile rail, was prepared. Measurements were made by means of a reading system to Electromagnetic Field Meter – CHMAG (accuracy 0.01 m / 0.001 mW).

2.4 Router Apparatus

Four type of routers were employed: FRITZ! Box 7490, Tp-Link TD-w9980, NETGEAR DGN2200 and Huawei E5730. FRITZ! Box 7490 represents the top of the range and is compatible with any DSL. Equipped with 4 gigabit ethernet ports and VoIP switchboard functions, it supports the latest Wireless AC standard capable of reaching speeds up to 1,300 Mbit / s. Two analog ports, one ISDN port and one DECT base to connect six cordless phones are available. The integrated switchboard is also suitable for VoIP traffic. To complete the device, 2 USB 3.0 ports for printers, mass storage and UMTS keys to share on the network. Tp-Link, the TD-W9980 is an all-in-one device that allows users to access the Internet via VDSL or ADSL, enabling wireless connectivity up to 600Mbps. On VDSL lines can reach 100Mbps, while maintaining compatibility with traditional ADSL lines. The 4 gigabit ports provide wired connectivity at maximum speed. The Ethernet LAN / WAN port allows connection to other modems / routers, improving the flexibility of use. The ADSL2 + NETGEAR Wireless-N 300 modem router represents a top-of-the-range solution for both private and corporate environments. It manages Wireless-N technology, allowing simultaneous downloads, streaming audio / video and online games. Equipped with integrated DSL modem, it is compatible with all major DSL Internet service providers. ReadySHARE ™ facilitates download storage and shared access to USB storage devices.

2.5. Statistical Analyses

The statistical analysis was performed using the Mann-Whitney U test for intergroup comparisons. Spearman’s rank correlation test and the linear regression analysis were performed to analyze correlations between groups and the duration of tests. Values were expressed as the mean ± SD. $P < 0.05$ was regarded as statistically significant

3. Results and Discussion

Saccharomyces cerevisiae colonies grow aerobically on glucose, maltose and trehalose and fail to grow on lactose and cellobiose. However, growth on other sugars is variable. It has been reported that reactive oxygen species are responsible of changes in cell morphology and permeability in *Saccharomyces cerevisiae* [11].

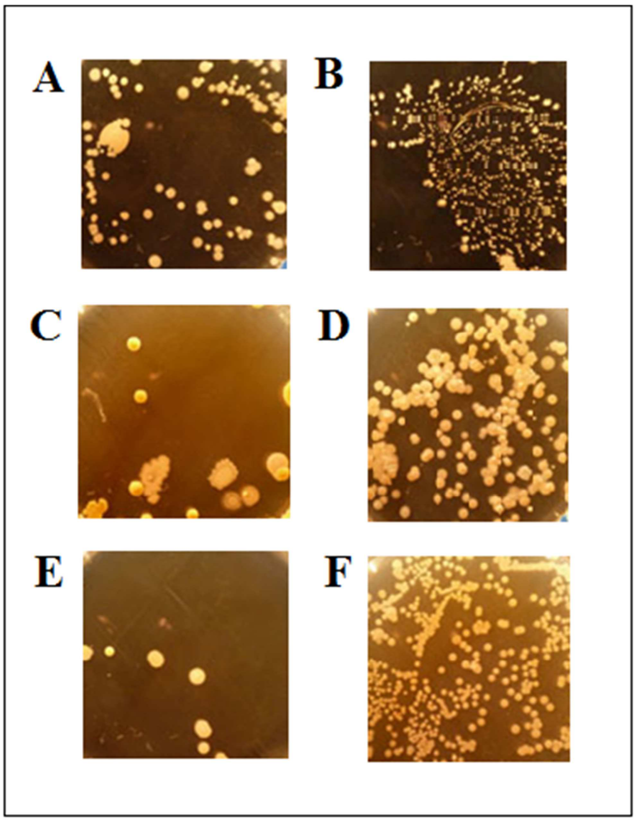

Figure 1. *Antioxidant activity of the application of NFC patches to three routers positioned at 3 meters from the Saccharomyces cerevisiae cultures. Control of the Fritz 4090 router without (A) and with NFC patch (B). Control of the Netgear router without (C) and with NFC patch (D). Control of the Tp-Link router without (E) and with NFC patch (F).*

Analyses of *Saccharomyces cerevisiae* colonies have been performed and the results evidenced a general antioxidant effect by the VVpatch system on average 72.6% of the treated cells. Keeping the three different routers (Fritz 4090, Netgear and Tp-Link), with the applied NFC pachts at a distance of 3 meters, the regular development of *Saccharomyces cerevisiae* colonies was highlighted. In Figure 1, it is possible to observe in the controls, the rarefaction and the irregular development of colonies with the formation of some agglomeration as a consequence of the oxidizing activity (Figure 1 A, B, C). On the contrary, samples treated with NFC patch, show a regular and abundant growth (Figure 1 D, E, F).

The cells of the plant meristem are not specialized but can become specialized to form the tissues of roots, leaves, and other plant parts. The growing tips of roots and stems and the tissue layer known as "*cambium*" are part of a plant's meristem. In our study, the radical droplets of *Pisum sativum* plants, whose containers were placed in contact with VVpatches (first set) developed an average of 250±42 meristematic cells within 24 hours. On the contrary, the radical root-drops of the components of the control series showed an average of 80±15 meristematic cells each within 24 hours (Figure 2 black dots). *Pisum sativum* roots have nodules, formed by the bacteria *Rhizobium leguminosarum*, which convert atmospheric nitrogen to ammonia. The stem, not very branched, of a variable length from 50 cm to 2 m, up to 3 m in the fodder varieties [12], is of undetermined growth.

It is hollow, with a cylindrical section, and climbs by clinging to the supports by means of the tendrils of the leaves. It is characterized by a certain number of vegetative nodules, or meshes, of which the former are purely vegetative (ie they emit only leaves or ramifications) and the subsequent reproductive ones, which produce flowers. The vegetative nodules of *Pisum sativum* plants, whose containers were placed in contact with VVpatches (first set) developed, an average of 18±6 foliage spreads equally spaced over space within 24 hours. On the contrary, vegetative nodules of the plant components of the control series showed an average of 5±2 leaf drafts each within 24 hours (Figure 2 red dots).

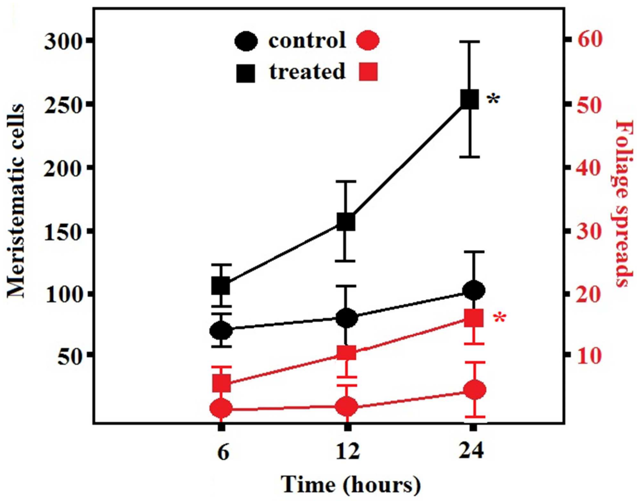

Figure 2. *Increase of meristematic cells and foliage spreads of Pisum sativum plants with and without VVpatches, exposed to routers at different times. The value is the average of five determinations ± S. D. P<0.05.*

The importance of cellular electrical phenomena is well known. Most investigations have been limited to static and quasi-static effects. Evidence arises from micro-DEP work that delivers information about the spatial distribution and temporal development of electric fields around biological cells. The increase in environmental contamination has led to a growing interest of biologists in understanding the mechanisms of plant resistance to stress factors [13-14]. One of these effects concern the generation of reactive oxygen species (ROS), such as superoxide anion ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^\cdot$), which results in the establishment of oxidative stress. ROS are generated in different compartments of the plant cell, including the cell wall (peroxidase and polyamine oxidase), the cytoplasm and the peroxisomes (xanthine oxidase), mitochondria and chloroplasts [15-16]. At high ROS concentrations, the greatest damage of cellular components occurs, such as proteins, lipids and nucleic acids. Plant cells can tolerate ROS through endogenous protective mechanisms involving various antioxidant molecules such as ascorbate, cysteine, glutathione, phytochelatins and α-tocopherol. Many enzymes are involved in this oxidation mechanism. Enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR), participate in the production and degradation of ROS in cells [17]. The results reported in this research suggest the possibility of application of H. I. T patchs as an effective defense against the production of reactive oxygen species, improving antioxidative defense and cell proliferation.

4. Conclusion

Overproduction of ROS, surpassing a cell's antioxidant capacity, can lead to oxidative stress and consequent DNA, lipid, or protein damages, inducing possible cell death. Indeed, generation of ROS and the oxidative stress are currently one of the most promising paradigms to assess and compare the toxicity of different contaminants in the environment. The significance of this study lies in its versatility that could be extended to new applications, in particular the deepening of the toxic action modalities of various environmental and food contaminants and above all the reduction of the use of biological and chemical materials in environmental defense.

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