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Research article

Effects of a Biophoton Triggering Device after Vitalisation of Organ-Specific Cell Cultures

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Abstract

The emission of biphotons from living matter has received great interest for the past 40 years. Human biophoton emission has been associated with high energy processes on cellular level and have also been suggested to reflect the organism's global state of health. Prompted by this background, the in vitro study presented here examined the effects of a specially constructed biophoton triggering device used for the vitalisation of organ-specific cell cultures. The vitalisation of the cells was done in two different approaches: (1) Tap water was vitalised by the photon triggering device and was then applied to the cell cultures and (2) the cell cultures were vitalised directly with the device. Although the water treated with the biophoton triggering device had no antioxidant properties, the addition of vitalised water to culture medium caused a concentration-dependent stimulation of the basal energy metabolism up to approximately 40 %. In contrast, a conventional red-light laser pointer did not show any stimulation of the cells with direct vitalisation. Moreover, the direct vitalisation of cell cultures as well as the addition of tap water, which was exposed to the biophoton triggering device, also stimulated cell regeneration/wound healing of connective tissue cells. In summary, the capability of the biophoton triggering device to influence (= stimulate) cultured organ-specific cells has been demonstrated in this study. The results also indicate that the role of biophotons in living matter might be much more essential than the actual scientific opinion suggests.

Keywords: Biophotons; beneficial effects; vitalisation; cell culture

Introduction

The emission of light from living matter has been researched for a long period of time [1,2] and has been described by Popp [3] as the emission of ultraweak packets of electromagnetic energy. As reviewed by Renger [4], living organisms are able to produce electromagnetic radiation either by enzymatic reactions resulting in bioluminescence [5] or by metabolic reactions, which cause the emission of ultraweak biophotons possessing unique properties [6-9].

Research on human biophoton emission has appeared in the literature since the 1970's and has been associated with high energy processes on the cellular level such as metabolism, growth, phagocytosis, neural activity and oxidative stress [10-14]. Moreover, biophoton emissions have also been suggested to reflect the organisms' global state of health and, thus, to be a non-invasive indicator for complementary and alternative medical interventions [15,16]. Therefore, coherent biophotons, i.e. biophotons which possess a consistent phase, have been suggested to transmit information and can be potentially capable to regulate and control metabolism and life processes [17,18].

The goal of this study was not to describe and shed light on the working principle of the biophoton triggering device used here, but to investigate whether the use of this device can cause any particular beneficial effects on the cellular level. For this purpose, actual cell culture methods have been used.

Biophoton Triggering Device

According to the distributor of the device (Vitarights Innovations GmbH, D-67657 Kaiserslautern, Germany), the team of Martin Becker has developed this unique biophoton triggering device based on many years of intensive research. It "emits a coherent light beam that supplies the organic matter onto which this light falls with an information impulse – this impulse stimulates biological regulation mechanisms (and is able to) vitalise the light energy management in the cells, re-inform the cells, and thus stimulate the self-healing powers of the body". Moreover, the device is described to possess a vitalising effect on the cluster structures of water.

In this particular study, the device was used in two approaches: (1) vitalised water, was applied directly to the cells and (2) the coherent light beam was directly pointed on the cultured cells.

Preparation of Water Samples and Concentrations in the Tests

Local tap water in a glass container was vitalised with the device from a distance of approximately 10 mm for 30 seconds. For later examinations on the effect of exposure time, the water was vitalised for up to 120 seconds. Local tap water of the same source was used as a reference without further treatment. The water samples were used in different dilutions in the tests ranging up to 50 vol%. The internal control used was the water sample test concentration of 0 vol%, i.e. pure culture medium. The results were always related to the corresponding sample without treatment with the device.

Antioxidative Effect of Vitalised Water

With this cell-free assay it was tested whether the water samples with and without vitalisation were able to inactivate free oxygen radicals and, thus, prevent oxidative stress. For the test, different concentrations of the water samples were pipetted to a potassium peroxide solution (1 mg/ml) and also a water-soluble tetrazolium dye WST-1 (Roche Diagnostics, Mannheim). With this test, the non-inactivated superoxide and still reactive superoxide anion radicals in the reaction mixture caused a change in the optical density (= colour) of the dye. The optical density was continuously recorded as a differential measurement ΔOD = 450 – 690 nm by an Elisa reader (BioTek SLx808 with software Gen 5 version 3.00) for the time interval up to 20 minutes. As a result, there was no significant difference in the antioxidative effect between tap water and vitalised water (not depicted).

Basal Energy Metabolism of Connective Tissue Fibroblasts

The investigations were performed with connective tissue fibroblasts of cell line L-929 (ACC-2, Leibniz Institute DSMZ, Braunschweig, Germany) and used in passages 66 to 68. Cells were routinely cultured in RPMI 1640 with 10% growth mixture and 0.5% gentamycin and cultivated in an incubator at 37°C with an atmosphere of 5% CO2 and 95% air and a humidity of approximately 100%.

For the experiments, cells were seeded from mass cultures at a cell density of 20,000 cells/well in 96-well plates (200 µl culture medium/well) and incubated for 24 h to achieve cell attachment and metabolism. Then, culture medium was removed and a reaction mixture consisting of phosphate-buffered saline with calcium and magnesium, 5 mM glucose, the different concentrations of the water samples and the tetrazolium dye WST-1 (Roche Diagnostics, Mannheim). The cleavage of the dye is directly related to the activity of the basal cellular energy metabolism, i.e. generation of adenosine triphosphate in mitochondria. The optical density was continuously recorded by an Elisareader (BioTek SLx808 with software Gen 5 Version 3.00) as a differential wavelength measurement ΔOD = 450 – 690 nm and analysed with Microsoft Excel for the time interval 0 to 120 minutes.

As depicted in Figure 1, both water samples reacted completely different in this assay. While the tap water caused a concentration-dependent decrease in basal energy metabolism of the cells when compared with untreated controls, the vitalised water showed a concentration-dependent increase in basal energy metabolism up to approximately 40% at a water concentration of 40 vol% present in the reaction mixture. At the highest test concentration of 50 vol%, basal energy metabolism decreased, but was still more than 25% higher in comparison to untreated control. When comparing the mean values of both water samples, the difference between tap water and vitalised water becomes even more prominent. For tap water, the decrease was statistically significant at water concentrations in the test ≥ 40 vol%. For vitalised water the increase was statistically significant at water concentrations in the test ≥ 10 vol% ($p < 0.05$; Wilcoxon-Mann-Whitney test).

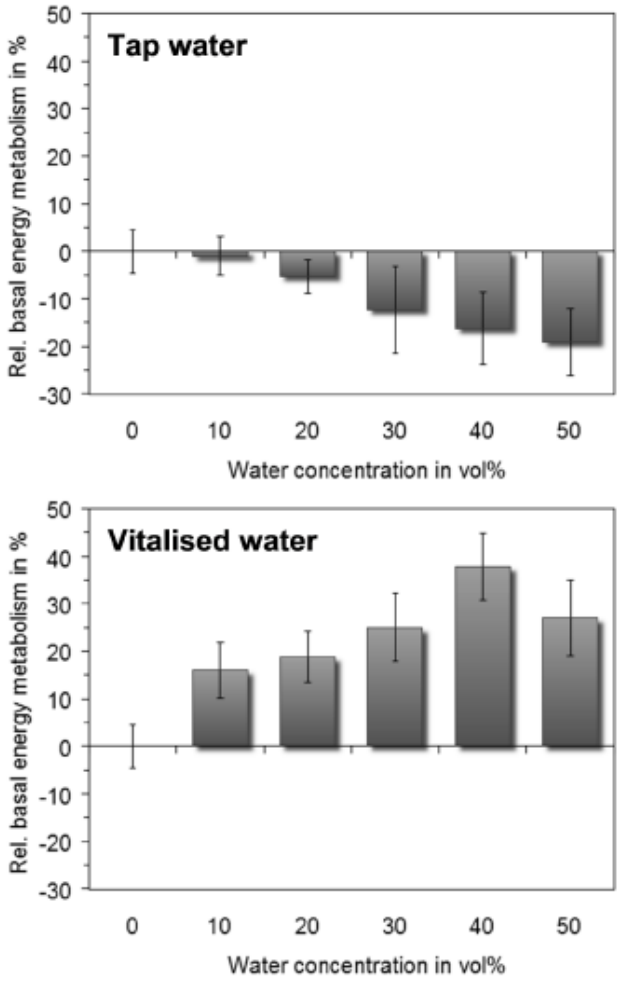

Figure 1. Effect of tap water and vitalised water on basal energy metabolism of cultured connective tissue fibroblasts. The values of pure culture medium (water concentration = 0 vol%) are set as 0. Note the decrease of tap water and the increase of vitalised water. Data represent mean value ± standard deviation of 3 independent experiments.

In addition, it was examined whether this stimulating effect of basal energy metabolism could be also achieved by direct vitalisation of the cells in comparison to a usual red-light laser pointer. For this purpose, cells were treated directly in the wells of a 96-well culture plate for 30 seconds with a laser pointer or the biophoton triggering device. Thereafter, basal energy metabolism was recorded for another 5 hours and examined as described above. As shown in Figure 2, the red-light laser pointer had no statistically significant effect when compared with the untreated control. In contrast, the device caused a stimulation of the basal energy metabolism by almost 15% in comparison to the untreated control. This value of the biophoton triggering device was statistically significant for both, the comparison to untreated

control and the comparison to the laser pointer ($p < 0.05$, Wilcoxon-Mann-Whitney test). This result clearly demonstrates that the emission of the red light by the biophoton triggering device, which seems to be comparable to the red-light laser pointer at first sight, might act only as a carrier and does not account for its stimulating effect by biophotons.

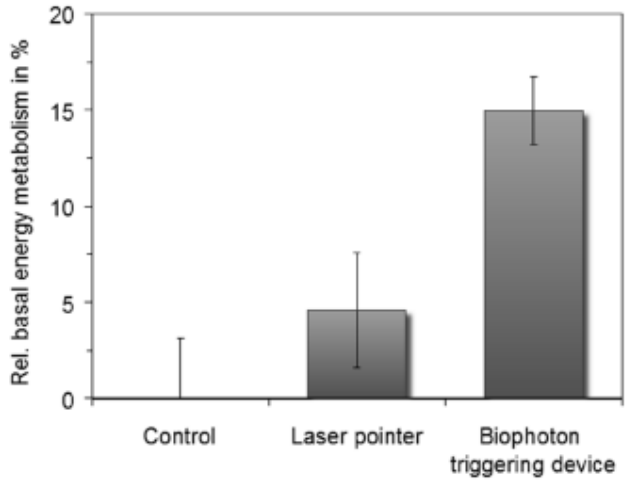

Figure 2. Effect of a conventional laser pointer vs. the biophoton triggering device on basal cell metabolism of connective tissue fibroblasts after direct vitalisation and a constant exposure time of 30 seconds. The values of the untreated control are set as 0. Note the significant increase after exposure to the photon triggering device ($p < 0.05$, Wilcoxon-Mann-Whitney test). Data represent mean value ± standard deviation of 3 independent experiments.

Cell Regeneration/Wound Healing of Connective Tissue Cells

A stimulation of the basal cellular energy metabolism is usually linked with an improvement of cell regeneration/wound healing. In vivo, the wound healing process can be divided in three distinct phases: cleaning phase, granulation phase and the differentiation phase. Especially the granulation phase, which is characterised by the occurrence of cell migration and cell proliferation of fibroblasts for defect filling, is simulated in the test performed here.

Cells were seeded at a density of 50,000 cells/ml into the three individual compartments of a silicone 3 well-culture insert made (ibidi, Gräfelfing, Germany). The single compartments of the inserts are separated by a 500 µm thick silicone bar with an outer silicone frame of 700 µm. Due to the special adhesion area, an insert adheres firmly to the bottom of a culture dish and forms a defined cell-free area (artificial wound), which the cells can colonise.

Upon reaching confluency within 48 hours after cell seeding, the silicone inserts were removed with tweezers to obtain sharp wound edges of cell-free areas between the compartments. By migration and proliferation the cell-free areas were then reduced subsequently. After 20 hours the cells were fixed with methanol, stained with a Giemsa's azur eosin methylene blue solution (Merck, Darmstadt, Germany), air-dried and the width of the remaining cell-free area was measured. Based on the results of the basal energy metabolism with a maximum stimulation at a test concentration of 40 vol%, the test concentration was also kept constant here at 40 vol% and the vitalisation time of the tap water was varied from 0 (= untreated tap water) to 120 seconds. In total, 4 measurements were made in two independent experiments for each vitalization time and the cell regeneration in comparison to the untreated tap water was calculated.

As shown in Figure 3, the biophoton triggering device produced a statistically significant improvement of cell regeneration/wound healing only with a tap water vitalisation time of 120 seconds ($p < 0.05$; Wilcoxon-Mann-Whitney test). In an additional test, we examined whether the direct vitalisation of the cell culture medium instead of tap water also improves cell regeneration/wound healing. The result is shown in Figure 4 as micrographs of the fixed and stained samples and clearly

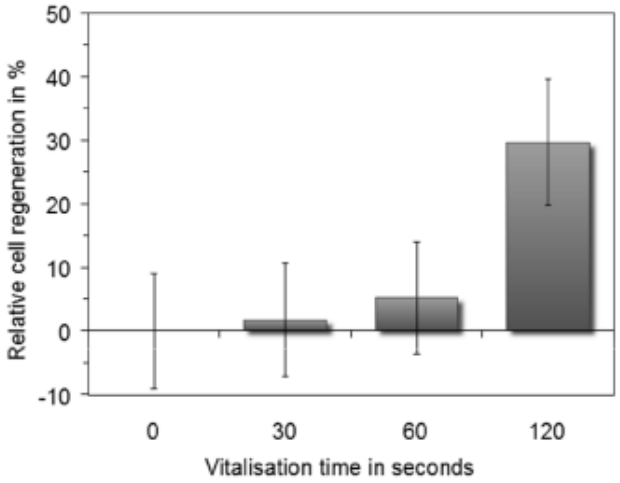

Figure 3. Effect of the vitalisation time by the biophoton triggering device on regeneration/wound healing process of connective tissue fibroblasts after addition of 40 vol% of vitalised water to the culture medium. Note the vitalisation time-dependent increase of the regeneration/wound healing process. The corresponding tap water without vitalisation is set as 0. Data represent mean value ± standard deviation of 2 independent experiments with 4 measurements each.

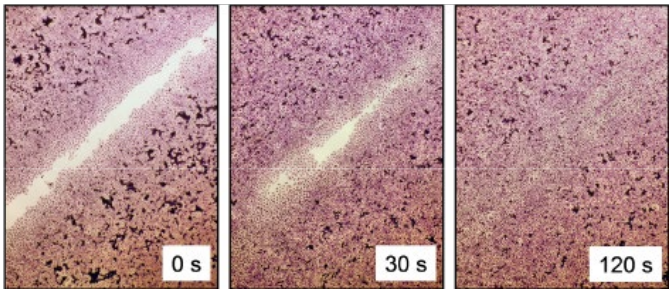

Figure 4. Effect of the vitalisation time by the photo triggering device on the regeneration/wound healing process of connective tissue fibroblasts after direct exposure of the cell cultures. The corresponding control without treatment still shows a prominent cell-free space, which disappears with increasing exposure times successively. Samples are stained with Giemsa's azur eosin methylene blue solution. Olympus IX-50 inverted microscope with an Olympus Planachromate 10x and an Olympus E-10 camera at 4 megapixel resolution at bright field.

demonstrates the time-dependent relationship of vitalisation and improvement of cell regeneration/wound healing. However, it is also evident that the increase of cell regeneration/wound healing is much more pronounced after only 30 seconds of vitalisation time while directly vitalising the cell culture medium. This seems to be feasible as the vitalised culture medium is present at 100 vol% in the cell cultures, whereas the vitalised tap water was only present at 40 vol%.

Discussion

When one looks at the current literature on biophotons, the vast majority of the publications deal with the emission of biophotons from living systems including humans, animals and plants or even parts of them. Biophoton emission has also been described for several cultivated cell lines [19-21]. To our knowledge, the effect of a radiation- or information-induced biophoton activation or modulation on the cellular or molecular level has rarely been described [for example, see 22]. This particular study we demonstrate that a biophoton triggering device is evidently able to influence cultured cells, either by direct interaction or via water which has been re-structured by the biophoton triggering device. Both effects are significant and at present be explained sufficiently yet. We assume the positive effect on the water cluster structures by water crystal formation, which can carry the information emitted by the device. That coherent laser light is able to carry information, was shown exactly 70 years ago [23]. It is also known that water is able to act as an information carrier [24]. When considering that the human body consists of approximately 70 % of water, one can assume that positive information can have an overall vitalising effect. The results of this study demonstrate that the vitalised water possesses new properties which are able to stimulate the energy metabolism and cell regeneration/wound healing of cells. Moreover, the direct application of the biophoton triggering device has the same stimulating effect which was even more prominent.

It has been stated that the role of radicals such as reactive oxygen species (ROS) in ultraweak photon emission might allow the monitoring of the oxidative metabolic processes and the oxidative stress reactions in biological systems [25]. However, further studies have to be performed to come to a closer understanding of the interactions between cells and biophotons and on the mechanisms of action.

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