Dartsch Scientific GmbH · Auf der Voßhardt 25 · D-49419 Wagenfeld

VitalField Technologies USA Inc
9171 Wilshire Blvd.
Suite 500
Beverly Hills
CA 90210, USA

Auf der Voßhardt 25
D-49419 Wagenfeld, Germany

Fon: +49 5444 980 1322
Mobil: +49 151 2272 1294
Email: info@dartsch-scientific.com
Web: www.dartsch-scientific.com

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TEST REPORT

Investigations on the effects of the „VitalField Energy Cell“ chip by using cultured organ-specific cells

1 Background and question of the investigation

According to the homepage of VitalField Technologies USA Inc, the „VitalField Energy Cell“ chip „can transfer the informed/positive vibration to the organism and protect it from damaging influences or at least significantly influence them.“ The present study was undertaken to investigate the effects of this energy chip by using *in vitro* test systems with cultured organ-specific cells. The tests used here have been described in numerous previous peer-reviewed publications and are accepted by the international scientific community.

2 Preliminary tests with unlabeled anonymized chips

At the beginning of the experiments, two unlabeled anonymized chips were send to our laboratory in order to compare the efficacy of both chips with each other after having done some preliminary tests. One chip has been informed and the other one was a dummy chip without any information. The preliminary tests clearly showed a significant difference between the two chips. Finally, it was reassured that the informed chip was the one which showed a much higher efficacy when compared with untreated controls and the dummy chip. Thus, we decided to do some more testing with the „VitalField Energy Cell“ chip.
Even in the preliminary tests it became obvious that experiments with a duration of only some hours of energy chip application could not show a distinct effect. It took at least more than 12 to 24 hours of continuous cell exposure to get an unequivocal effect of the energy chip. This was taken into account in the following tests.

3 Investigations with connective tissue fibroblasts

The first set of the investigations presented here was done with connective tissue fibroblasts of cell line L-929 (ACC-2; Leibniz Institute DSMZ, Braunschweig, Germany) and

used in subcultivation stages 80 to 88. Cells were routinely grown in RPMI 1640 with 10 % growth supplement and 0.5 % of gentamycin and incubated at 37 °C in a humidified atmosphere of 5 % CO_2 and 95 % air.

3.1 Examination of basal cell metabolism

For the experiments, cells from subconfluent mass cultures were seeded at a density of 20,000 cells/well in 96-well plates (200 µl culture medium/well) and incubated for 24 hours to achieve cell attachment and normal metabolism. Then, cell cultures were incubated for another 24 hours ± chip in direct contact to the culture plate which was wrapped in several layers of aluminum foil to avoid any unwanted influences between the cell cultures. After 24 hours, culture medium was discarded and a reaction mixture of phosphate-buffered saline with calcium and magnesium, 5 mM glucose and a red water-soluble tetrazolium dye (WST-1; Roche Diagnostics, Mannheim, Germany) was added. The metabolic activities of the cells cleave the red tetrazolium dye and yield a yellow color which can be examined photometrically. The cleavage of the dye is directly related to the metabolic activity of the cells. The optical density (= color) of each well was measured at several time points as a differential wavelength measurement at ΔOD = 450 – 690 nm with an ELISA reader (Bio-TEK Elx 808 with Gen 5 V3.00). For evaluation, the increase of optical density at time points t = 80,140, 240 minutes in comparison to the primary value at t = 0 was calculated.
As shown in Fig. 1, pre-exposure of the cells for 24 hours to the “VitalField Energy Cell“ chip resulted in a significantly increased cell metabolism by 12.6 ± 4.0 % (mean value ± standard deviation of 3 parallel experiments) in comparison to untreated controls ($p < 0.05$, Wilcoxon-Mann-Whitney test).

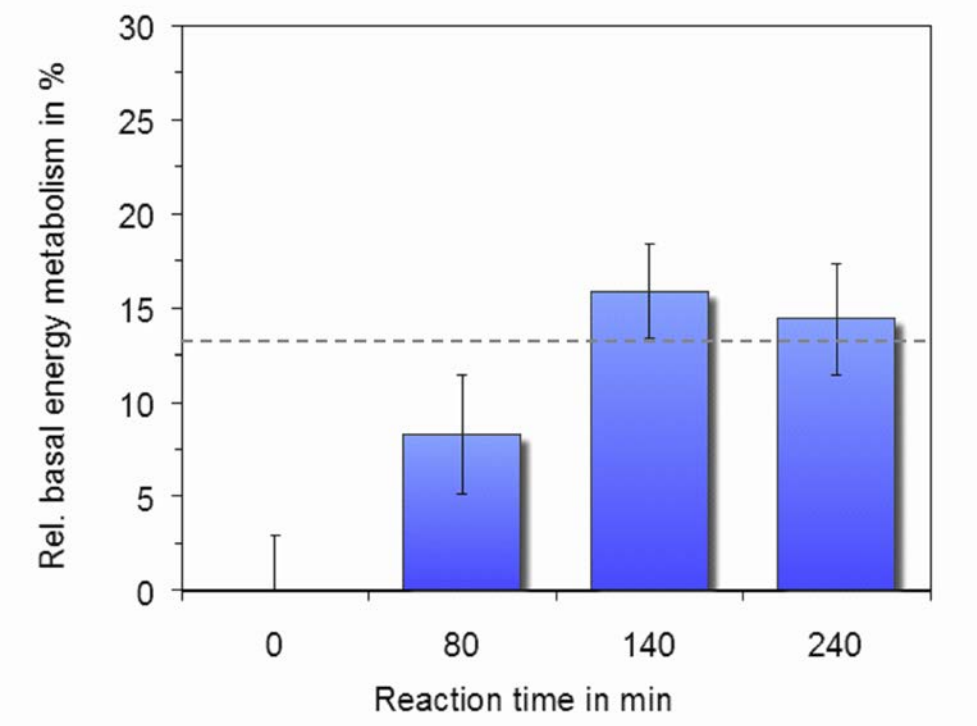

Fig. 1: Effect of the “VitalField Energy Cell“ chip on cell metabolism of cultured connective tissue fibroblasts after 24 hours of pre-exposure. The untreated control is set as “0”. The dashed line represents the average stimulation of 12.6 ± 4.0 %. Data represent mean value ± standard deviation of 3 parallel experiments.

3.2 Examination of cell vitality/proliferation in low density cultures

Cells from subconfluent mass cultures were seeded at a density of 200,000 cells/flask with a surface of 75 cm^2 and incubated for 24 hours to achieve cell attachment and normal metabolism. Then, cell cultures were incubated for another 6 days ± energy chip in direct contact to the culture flasks which were wrapped in several layers of aluminum foil to avoid any unwanted influences between the cell cultures. Medium was not exchanged during the incubation/exposure period. After 6 days, micrographs were taken representing the colonization of the surface and cells were completely detached by trypsin/EDTA treatment (30 minutes at 37 °C) and cell numbers and cell size distributions were examined using a CASY cell counter and analyzer system (OLS-OMNI Life Science, Bremen, Germany). This device quantifies cells and particles passing a measuring pore exposed to a low voltage electrical field. Based on cell size and conductivity, a resistance signal is generated and recorded.

As depicted in Fig. 2, the colonized surface of the cell culture flasks was much higher in the case of the cells treated with the energy chip for the total cultivation period of 6 days. The untreated control showed a lot of cell-free spaces, whereas the chip-treated cultures were confluent, i.e. with a dense cell layer all over the surface. When counting the cells, the untreated controls had a cell number of 8.3 ± 1,5 x 10^6 cells/flask (mean value ± standard deviation), whereas the cells treated with the energy chip had a cell number of 13.0 ± 1,8 x 10^6 cells/flask (mean value ± standard deviation). This represents a statistically significant increase in cell number by 56.6 ± 15.6 % ($p < 0.05$, Wilcoxon-Mann-Whitney test). Moreover, the use of the energy chip had no effect on the maximum and average cell size as depicted in Fig. 3. However, the Gaussian distribution becomes more prominent in the case of cells treated with the “VitalField Energy Cell“ chip.

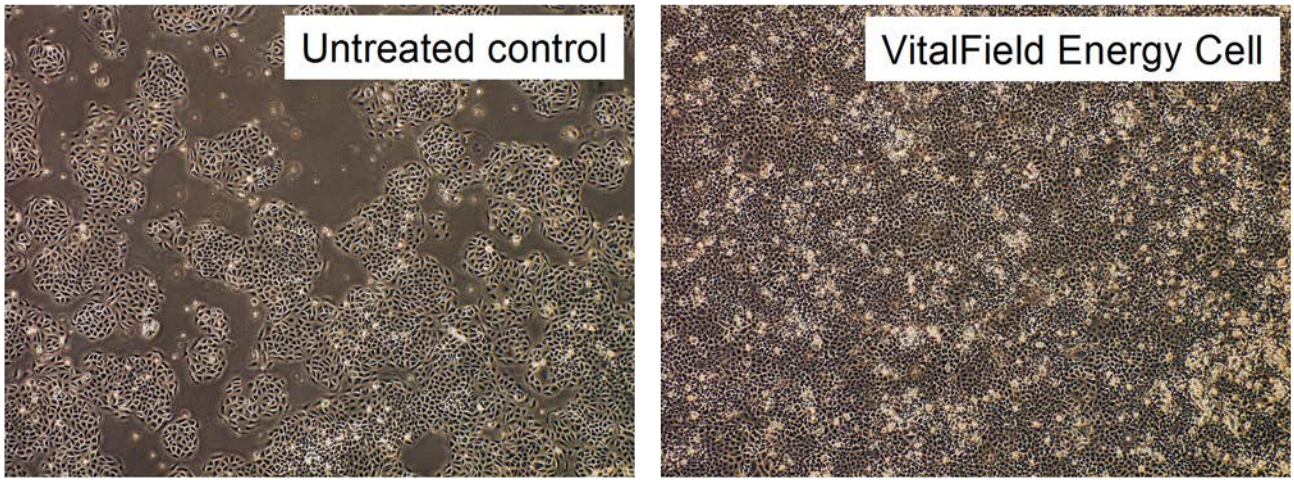

Fig. 2: Micrographs showing the surface colonization of the untreated control (left) in comparison to a culture treated with the energy chip for 6 days (right). Note the cell-free surface in the untreated control and the dense cell layer after treatment. Olympus IX-50 inverted microscope equipped with an Olympus Planachromate 10x and an Olympus E-10 digital camera with 4 megapixel resolution at phase contrast.

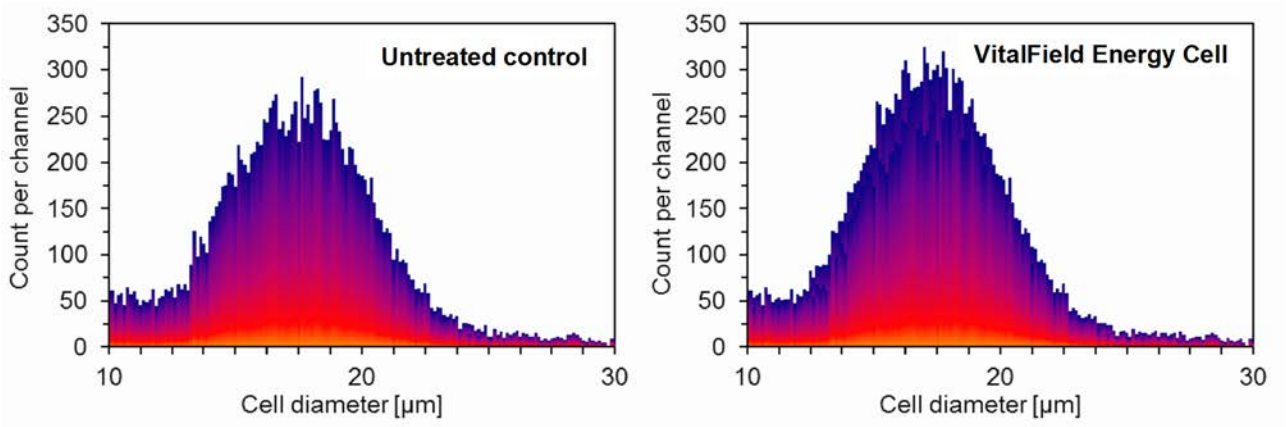

Fig. 3: Cell size distribution and cell numbers of untreated control cells (left) in comparison to chip-treated cells (right) as examined by a CASY cell counter and analyzer system. Note that the maximum diameter has not changed between both samples, but that the Gaussian distribution of the peak is more prominent in the chip-treated cell culture.

3.3. Examination of cell regeneration/wound healing

An improvement in cell metabolism and cell vitality is usually related to a stimulation of cell regeneration/wound healing. In this process, proliferation and migration of the cells is a key event at the cellular level. For this test, cells were seeded at a density of 50,000 cells/ml into the three cell culture compartments of silicone Culture-Insert 3 Wells (ibidi, Gräfelfing, Germany) which have been carefully placed on each well surface of a 12-well plate. The three compartments are separated by a 500 µm thick wall. Due to their especially designed bottom, the inserts stick to the surface firmly and completely prevent any cell growth under their silicone walls.

Connective tissue fibroblasts ± "VitalField Energy Cell" chip were pre-exposed for 24 hours after seeding as already described in detail in chapter 3.1 until a dense cell layer was achieved and the inserts were removed to leave a sharp cell-free space (artificial wound) without any cells. Cells began to migrate and proliferate into the cell-free space in order to close the wound. After another 20 hours ± chip exposure, cells were fixed with methanol and stained with a Giemsa's azur eosin methylene blue solution (Merck, Darmstadt, Germany), air-dried and the width of the remaining wound space was measured.

As depicted in Figure 4, the untreated cell culture still showed a cell-free space after 20 hours of incubation, whereas the chip-treated cell cultures had a partially closed wound at that time point. When the difference is quantified between both experimental series, the chip-treated cell cultures had a residual cell-free space of 28.5 ± 8.7 µm and the untreated cells of 80.0 ± 7.7 µm (mean value ± standard deviation, 3 parrallel test assays). This difference is statistically significant ($p < 0.05$, Wilcoxon-Mann-Whitney test) demonstrating the promotion of cell regeneration/wound healing by the "VitalField Energy Cell" chip.

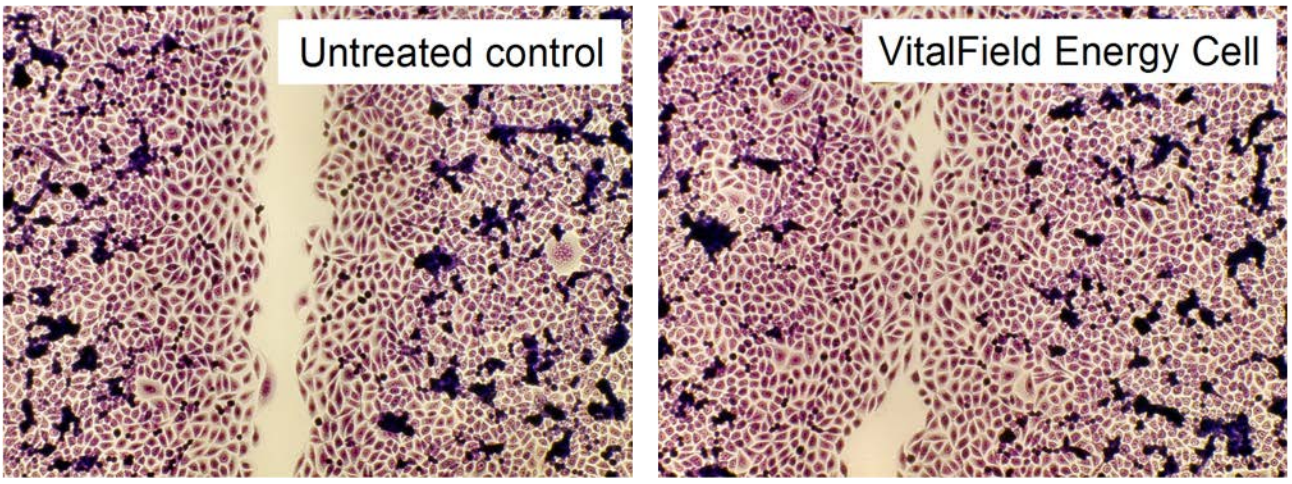

Fig. 4: Representative micrographs of stained cell cultures demonstrating regeneration and wound closure of the untreated control (left) in comparison to the chip-treated culture (right) after 20 hours. Note that the chip-treated culture has a nearly closed cell-free space, whereas the untreated control still shows a distinct space. Olympus IX-50 inverted microscope equipped with an Olympus planachromate 10x and an Olympus E-10 digital camera with 4 megapixel resolution at bright field.

4 Investigations with functional neutrophils

The second set of experiments presented here was done with human promyelocytes (cell line HL-60; ACC-3; ECACC 98070106; Leibniz Institute DSMZ - German Collection for Microorganisms and Cell Cultures, Braunschweig, Germany). Cells were routinely cultured as mass cultures in suspension in RPMI 1640 medium with 10 % growth supplement and 0.5 % gentamycin and incubated in an incubator at 37 °C and a humid atmosphere of 5 % CO_2 and 95 % air. By cultivation with 1.5 % dimethylsulfoxide for 5 to 7 days, HL-60 cells were differentiated to functional neutrophils which then possess the properties to act as a scavenger cell against microbial pathogens by an oxidative burst. An activation of the functional neutrophils might result in an increased primary defense in the blood *in vivo*.

HL-60 mass cultures were differentiated into functional neutrophils by treatment with dimethylsulfoxide and for the last 48 hours of differentiation ± “VitalField Energy Cell” chip. After several washing and centrifugation steps, the energy metabolism of the differentiated cells was monitored as already described in chapter 3.1 for connective tissue fibroblasts with the difference that suspended functional neutrophils react much faster than adherent fibroblasts. Thus, the examination time was only 30 minutes instead of hours.

The test resulted in a marked stimulation of chip-treated cells by 38.0 ± 4.8 % (mean value ± standard deviation; 4 parallel test assays) in comparison to untreated cells. This stimulation was statistically significant ($p < 0.05$, Wilcoxon-Mann-Whitney test) demonstrating an improvement of the primary defense against microbial pathogens.

5 Summary and conclusions

By use of different *in vitro* tests and cultivated organ-specific cells, the effects of the "VitalField Energy Cell" chip, distributed by VitalField Technologies USA Inc, USA, have been examined. The tests used here have been described in numerous previous peer-reviewed publications and are accepted by the international scientific community. Short-term experiments with a duration of only some hours of energy chip application did not show a distinct effect. It took at least more than 12 to 24 hours of continuous cell exposure to get an unequivocal effect of the energy chip.

In detail, the "VitalField Energy Cell"" chip had the following effects in the present *in vitro* study:

1 Pre-exposure of connective tissue fibroblasts for 24 hours to the "VitalField Energy Cell" chip resulted in an increased cell metabolism by 12.6 ± 4.0 % in comparison to untreated controls.

2 Long-term low density connective tissue cell cultures showed an increase in cell number by 56.6 ± 15.6 % after 6 days of continuous incubation with the "VitalField Energy Cell" chip when compared with untreated control cultures. The results of 1 and 2 show that the energy chip acts as a kind of cell energy booster, especially when applied for a longer period of time.

3 In the regeneration/wound healing assay with connective tissue fibroblasts, the "VitalField Energy Cell" chip-treated cell cultures had a residual cell-free space of 28.5 ± 8.7 µm and the untreated cell cultures of 80.0 ± 7.7 µm demonstrating that the wound closure is faster for the chip-treated cell cultures.

4 Pre-exposure of functional neutrophils for 48 hours to the "VitalField Energy Cell" chip resulted in a distinct stimulation of basal metabolism by 38.0 ± 4.8 % in comparison to untreated cells which represents an improvement of the primary defense against microbial pathogens.

The results from the present investigation suggest that the long-term continuous use of the "VitalField Energy Cell" chip can be recommended for a shortening of the body's regeneration time and vitality as well as for the improvement and maintenance of well-being.

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Prof. Dr. Peter C. Dartsch
Certified biochemist