

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](mailto:info@dartsch-scientific.com)  
Web: [www.dartsch-scientific.com](http://www.dartsch-scientific.com)

July 5, 2020

## TEST REPORT

### Effects of the „VitalField RESISTANCE Energy Cell“ on cultured cells representing part of the innate immune system

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#### ***1 Background and question of the investigation***

Neutrophils are the most abundant type of granulocytes in most mammals. They possess a dual role as phagocytes and as proinflammatory cells [1]. By being present in the bloodstream, they form the first line of cellular defense against invading microbial pathogens as an essential part of the innate immune system. Neutrophils are also the first responders of inflammatory cells which migrate towards the site of inflammation [2,3].

Neutrophils attack microbial pathogens by reactive oxygen species which are generated in the course of a so-called respiratory or oxidative burst. This burst is independent from energy-producing metabolic processes and involves the catalytic conversion of dimolecular oxygen into superoxide anion radicals by the NADPH oxidase complex [4].

Prompted by this background we investigated the effect of “VitalField RESISTANCE Energy Cell”, a novel immune chip, on the growth characteristics and the oxidative burst of cultivated human promyelocytes which have been differentiated to functional neutrophils in corresponding cultures without and with exposure to the chip.

1. Witko-Sarsat V, Rieu P, Descamps-Latscha B, Lesavre P, Halbwachs-Mecarelli L (2000). Neutrophils: molecules, functions and pathophysiological aspects. *Lab Invest* 80:617-653.
2. Ward PA (1999). The acute inflammatory response and its regulation. *Arch Surg* 134:666–669.
3. Nathan C (2002). Points of control in inflammation. *Nature* 420:846-852.
4. Lambeth JD. 2004. NOX enzymes and the biology of reactive oxygen. *Nature Rev Immunol* 4:181-189.

## **2 Description of the “VitalField RESISTANCE Energy Cell”**

Basically, the “VitalField RESISTANCE Energy Cell” (also named ENERGY CELL) is influenced by frequency spectra that are generated by special frequency generators. With the so-called vital field technology, electromagnetic fields (up to 120 GHz), microcurrent frequencies (up to 1 GHz) and different magnetic fields are generated via special antennas. The ENERGY CELL is placed on these antennas and exposed to the specific frequencies for a definite time period. Due to its storage properties, the chip is able to record this information and release it over several months. In the case of the ENERGY CELL, the stored frequencies of specific immune cells were used and the chip was exposed to this pattern in order to achieve the desired result.

This patented information store is manufactured using a special process after years of development work. A precisely defined proportion of high-quality storage media is poured into a particularly skin-friendly silicone base. This makes it possible to conserve the effect of these frequency spectra as long as possible. Experience to actual data show that the chip remains fully effective for at least 6 months and should be replaced afterwards.

## **3 Summary of the experimental design**

The investigations were conducted with human promyelocytes (cell line HL-60; DSMZ, Braunschweig). The cells were cultivated as suspension cultures in a nutrient solution in an incubator at 37°C in a specific atmosphere with almost 100% humidity. By adding 1.5% dimethyl sulfoxide for 6 days, cells were differentiated into functional neutrophils. During the differentiation process, the chip was in direct contact with the cell culture flasks. Untreated control cell were incubated separately at the same culture conditions.

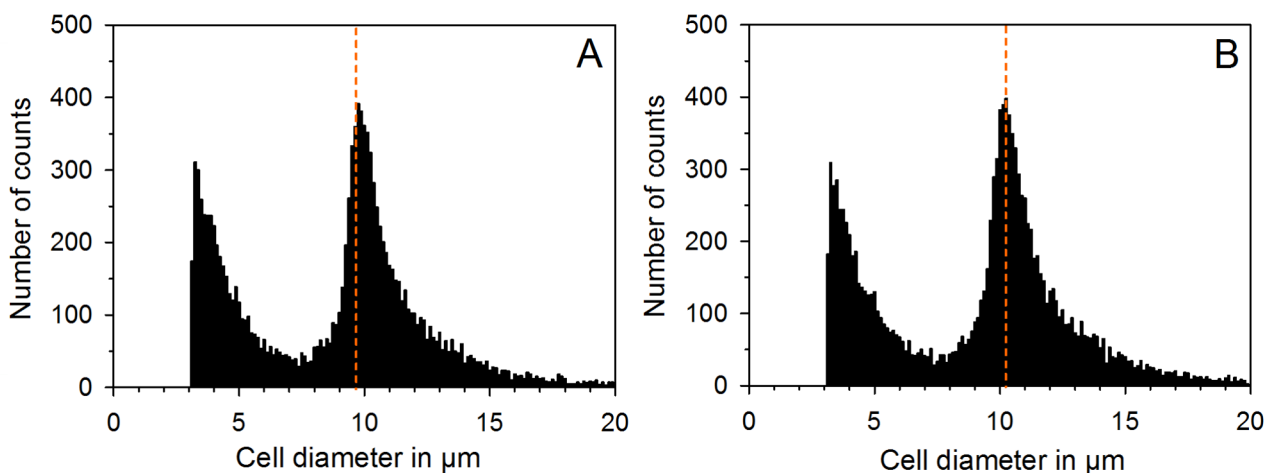
Finally, cells were prepared by centrifugation and repeated washing and pipetted to a reaction mixture which was able to trigger an oxidative burst in the cells [5,6]. The response of the cells was measured by changing of the color of the reaction mixture. By using a reaction mixture which did not cause an oxidative burst, the basal cell metabolism was also examined.

In addition, cell numbers and cell size distributions were determined using a cell analyzer system to estimate the homogeneity of the cell population. A total of 5 independent experiments were conducted over a test period of 4 weeks.

5. Tan AS, Berridge MV. 2000. Superoxide produced by activated neutrophils efficiently reduces the tetrazolium salt WST-1 to produce a soluble formazan: a simple colorimetric assay for measuring respiratory burst activation and for screening anti-inflammatory agents. *J Immunol Meth* 238:59-68.
6. Dartsch PC. 2006. TILOS – a sensitive and cell-based test assay for the screening of biologically active substances for their antioxidant potential. *Innov Food Technol* 32:72-75.

#### 4 Results

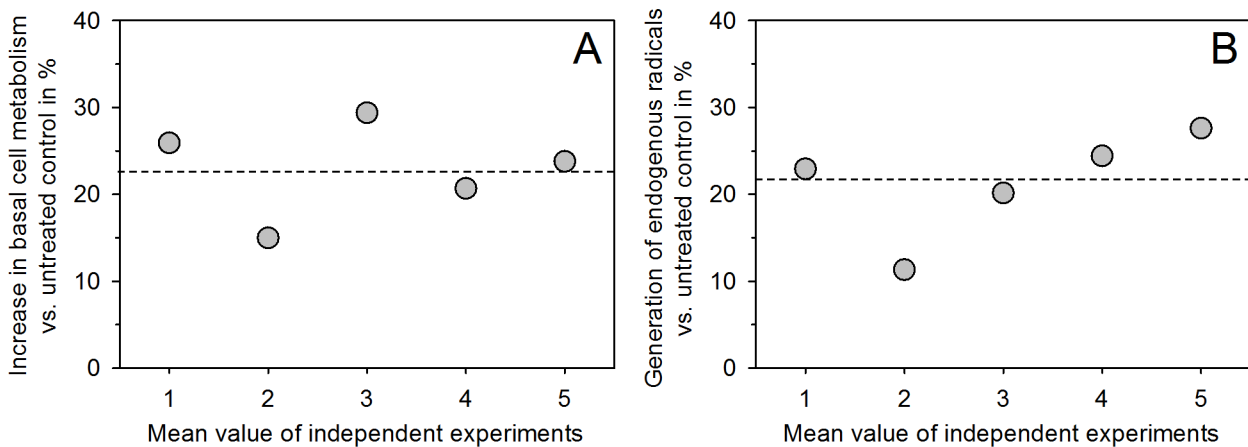
In all experiments, cell numbers were increased by the “VitalField RESISTANCE Energy Cell” in comparison to untreated controls by  $17.2 \pm 3.8\%$  (mean value  $\pm$  standard deviation). This increase in mitotic activity of the cells was statistically significant ( $p \leq 0.05$ ; Wilcoxon-Mann-Whitney-Test). Independent from the cell numbers achieved after the differentiation process, the peak cell diameter was also increased by the ENERGY CELL in all experiments from  $9.5 \pm 0.2 \mu\text{m}$  to  $10.4 \pm 0.3 \mu\text{m}$  ((mean value  $\pm$  standard deviation). This is equivalent to a relative increase of  $9.5 \pm 0.2\%$  and is also statistically significant ( $p \leq 0.05$ ; Wilcoxon-Mann-Whitney-Test). This shift in peak cell diameter can be clearly seen in Fig-



ure 1.

**Fig. 1:** Cell size distribution and cell numbers of untreated control cells (A) in comparison to ENERGY CELL-treated cells (B) as examined by a cell counter and analyzer system. Note that the maximum diameter has changed between both samples which show a very homogenous cell size distribution. The dashed lines mark the peak cell diameter of each sample. The left peak in both images represent small particles such as dell debris, cell fragments or dead cells and have not been used for evaluation

As shown in Figure 2A, the use of the ENERGY CELL resulted in an increased cell metabolism of the functional neutrophils in all 5 independent experiments when compared to the untreated controls. The overall increase in basal cell metabolism was  $23.0 \pm 5.5\%$  (mean  $\pm$  standard deviation), which was statistically significant ( $p \leq 0.05$ ; Wilcoxon-Mann-Whitney test). In accordance with the increased cell metabolism caused by exposure to the chip, the generation of superoxide anion radicals in the exposed cells was also increased by  $21.3 \pm 6.2\%$  (mean  $\pm$  standard deviation;  $p \leq 0.05$ ; Wilcoxon- Mann-Whitney test; Figure 2B), which was similar to the increased cell metabolism.



**Fig. 2:** (A) Increased cell metabolism of functional neutrophils after 6 days of exposure with the ENERGY CELL during the differentiation process. The data represent the means of each independent experiment (n = 5). The dashed line indicates the mean increase for all experiments. (B) Increased generation of superoxide anion radicals by functional neutrophils after 6 days of exposure to the chip during the differentiation process. The data represent the means of each independent experiment (n = 5). The dashed line indicates the mean increase for all experiments. In both diagrams, the controls are set as 0%.

## 5 Conclusions

In summary, the present results show that “VitalField RESISTANCE Energy Cell” is able to stimulate the mitotic activity as well as the basal metabolism and the generation of reactive oxygen species of functional neutrophils. Thus, the chip might be able to improve the innate immune system as a defense against microbial pathogens.




Prof. Dr. Peter C. Dartsch  
Certified biochemist