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

Frequencell 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

June 21, 2021

# TEST REPORT
## Cell regenerative effects of the Anti-Aging Energy Cell

### Question of the present study

It is stated by Frequencell Inc. that the Anti-Aging Energy Cell is able to reduce the signs of skin aging energetically and to stimulate the tightness of connective tissue.
In the present *in vitro* study with connective tissue fibroblasts in culture, we used a current cell biological test method to find out whether the Anti-Aging Energy Cell actually has a direct beneficial effect on cell regeneration. Considering that cell regeneration is crucial for overall health and especially for improving most skin conditions, this is a highly important test.

### Testing parameters

The tests were conducted with connective tissue fibroblasts (cell line L-929, ACC-2, Leibniz Institute DSMZ, Braunschweig, Germany). Cells were routinely cultured in RPMI 1640 medium containing 10 % growth mixture and 0.5 % gentamycin in a gassed incubator at 37 °C in an atmosphere of 95 % air, 5 % $CO_2$ and nearly 100 % humidity.
The connective tissue fibroblasts were seeded at a density of 100,000 cells/ml in the four compartments of a silicone frame (4 well-culture inserts; ibidi, Gräfelfing, Germany). The individual compartments were separated from each other by 500 µm thick silicone strips. Because of the special adhesion area of the silicone frame, it adhered firmly to the bottom of a culture dish, creating a cell-free area that cells could colonize by proliferation and migration after removing the frame.
Post reaching confluency (= cells are dense and dense) within 48 hours after cell seeding, the silicone frames were carefully removed with tweezers and the cell cultures were placed on an Anti-Aging Energy Cell and covered with several layers of aluminum foil. Untreated

control cells from the same initial culture without the Energy Cell were cultured under identical conditions and also covered with multilayer aluminum foil. After staying in the incubator for 24 hours, the cells were washed with phosphate buffer saline, fixed with methanol, stained with Giemsa methylene blue solution, were air-dried and the width of the remaining cell-free area was measured with microscope at a minimum of 6 fields of view. A total of three independent test series (n = 3) was conducted. Finally, the relative cell regeneration due to the influence of the Energy Cell in comparison to the untreated control was calculated.

## Result

The microscopical examination of the fixed and stained samples showed a significant actively narrowing of the cell-free area due to an exposure to the Anti-Aging Energy Cell when compared to the untreated control (Fig. 1). Quantification revealed a statistically significant improvement of the cell culture colonization after exposure to the Anti-Aging Energy Cell by 18.7 ± 5.0 % in relation to the untreated control cell cultures (mean value ± standard deviation; $p \leq 0.05$, n = 3; nonparametric two-tailed Wilcoxon-Mann-Whitney test).

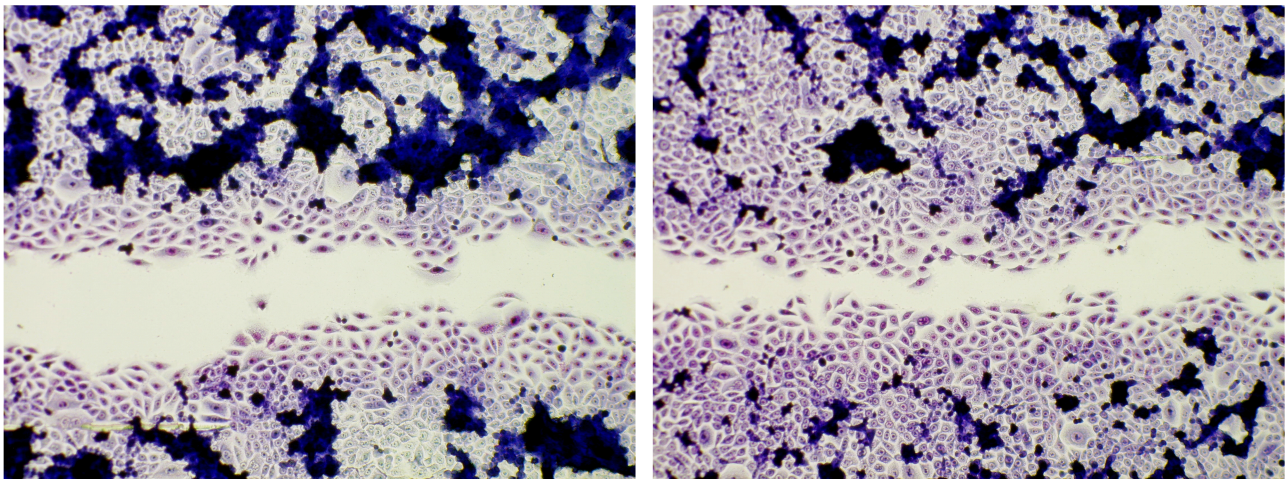

**Fig. 1:** Micrographs after 24 hours of regeneration time of fixed and stained connective tissue cell cultures. Left image: Untreated control. Right image: Cell-free area after exposure to the Anti-Aging Energy Cell. Note the increased closure of the cell-free area after Energy Cell treatment. Olympus IX 50 inverted microscope with Olympus Planachromate 10x and Olympus E-10 digital camera at 4 megapixel resolution and bright field illumination.

dartschSCIENTIFIC
INSTITUT FÜR ZELLBIOLOGISCHE TESTSYSTEME

## Conclusions

The result obtained in the present *in vitro* study with the Anti-Aging Energy Cell confirms the observations of users and clearly demonstrates that the regenerative properties of connective tissue cells are improved. Although the aging process of the skin is a multi-faceted and complex process that is still not completely explained today, the improvement in cell regeneration can fight skin aging. According to our studies, the Anti-Aging Energy Cell has a positive effect on the healing processes of connective tissue and, therefore on skin aging and overall well-being.


Prof. Dr. Peter C. Dartsch
Certified Biochemist