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TEST REPORT

Cell regeneration/wound healing in cultured connective tissue fibroblasts under the influence of the KLOUD device.

Background

This in vitro study investigated the possible beneficial effects of the KLOUD device from Centropix Global AG, FL-9491 Ruggell, on cell regeneration of cultured connective tissue fibroblasts. In vivo, the process of cell regeneration/wound healing can be divided into three defined stages: Purification phase, Granulation phase and Differentiation phase. In this study, the effect of the KLOUD device on the granulation phase was investigated, which is characterized by increased division and migration of connective tissue cells to colonize a cell-free space.

Brief description of the test product

According to Centropix Global AG, the KLOUD device is not a medical device, but belongs to the area of wellness and fitness products and is used for the "physical activation of physiological processes by means of defined, low-frequency, pulsating electromagnetic fields." According to the manufacturer, users report numerous positive effects, including an improvement in physical and mental performance and accelerated regeneration after exertion. These effects increase the quality of life and zest for life for users. A KLOUD device was kindly provided by the company Centropix Global AG for the duration of the investigations.

Used programs of the KLOUD device

Two different programs of the device were used in this in vitro study:

- ***Program 3 (Energizer):*** Signals that essentially change from a slow to a faster sequence of pulses and their intensity.
- ***Program 5 (Transformer):*** Signals designed to support self-healing and feel a refreshing sense of recovery

Experimental setup and execution of the tests

The studies were performed with connective tissue fibroblasts (cell line L-929, ACC-2, Leibniz Institute DSMZ, Braunschweig, Germany). Cells were routinely cultured in RPMI 1640 with 10% growth mixture and 0.5% gentamycin in a gassing incubator at 37 °C in an atmosphere of 5% CO2 and 95% air at approximately 100% humidity. The connective tissue fibroblasts were seeded at a density of 100,000 cells/ml into the four compartments of a silicone frame (4 well-culture inserts; ibidi, Gräfelfing, Germany). The individual compartments are separated from each other by a 500 µm thick silicone bar. Because of the special adhesion area of the silicone frame, it adheres firmly to the bottom of a culture dish, forming a cell-free space that cells can repopulate by division and migration after removal of the frame. After reaching confluence (= cells lie close to each other) within 48 hours after cell seeding, the silicone frames were carefully removed with tweezers. Thus, a sharp cell edge was obtained between the four compartments of the frame. Immediately after removing the silicone frame, the culture dishes were placed on the applicator of the KLOUD device. The cells were exposed to the signals of the KLOUD device for 5 and 15 min at intensity level 5 and then incubated undisturbed in the incubator for another 20 hours. Cells that had been treated in the same way, but without exposure to the KLOUD device, served as controls. After the 20 hours, the cells were washed with phosphate buffer, fixed with methanol p.a., stained with Giemsa methylene blue solution, air dried, and the width of the remaining cell-free area was measured on the microscope. A total of 12 measurements of the remaining cell-free space per culture dish were performed for each experimental set in a total of three independent experimental sets (n = 3). Cell regeneration was calculated in comparison to the untreated control. Statistical analysis of the experimental results was performed using the parameter-free two-sided Wilcoxon-Mann-Whitney test.

Results

Already the morphological presentation of the cultures in direct comparison showed that both programs of the KLOUD device were able to significantly improve the colonization of the cell-free space, and thus cell regeneration/wound healing, at 15-minute exposure time and intensity level 5 (Fig. 1). The quantitative evaluation showed this even more impressively, also demonstrating differences between the effectiveness of the two programs depending on the exposure time (Fig. 2). Program 3 caused a significant stimulation of cell regeneration by 23.8 ± 4.7 % (mean ± SEM; $p \leq 0.01$) compared to the untreated control exclusively after the 15-minute exposure time. In contrast, program 5 showed a significant stimulation of 19.9 ± 4.7 % already after 5 min, which further increased to 29.1 ± 4.9 % after 15 min (both mean values ± SEM; $p \leq 0.01$ compared to the control).

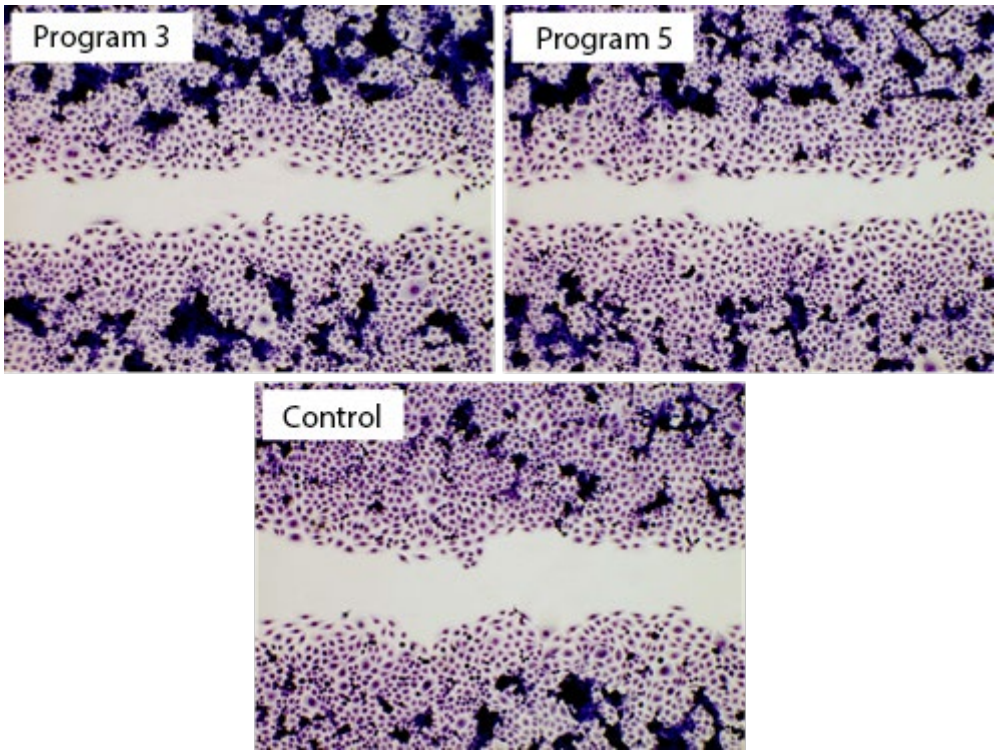

Fig. 1: Microscopic representation of the effect of the two programs of the KLOUD device used (15 min each, intensity level 5) on the subsequent 20-hour regeneration of cultured connective tissue fibroblasts. The significantly reduced cell-free space of the two treated cultures due to stimulated cell migration and division compared to the untreated control is clearly visible. Cell cultures after fixation and staining. Olympus IX-50 inverted microscope with 10x planachromat and Olympus E-10 at 4 megapixel resolution using transmitted light brightfield technique.

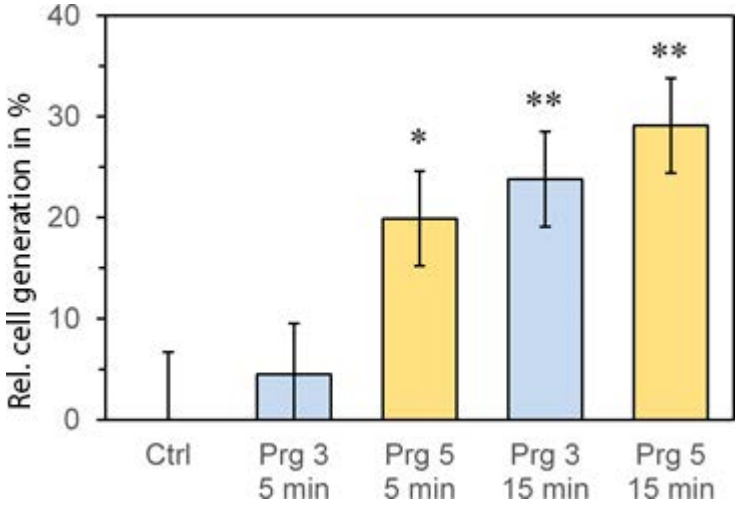

Fig. 2: Graphical representation of the stimulation of cell regeneration/wound healing with the two programs of the KLOUD device used at intensity level 5. The untreated control was set equal to "0". The columns represent the mean values ± SEM from 3 independent experiments. *p ≤ 0.05 and **p ≤ 0.01 versus the untreated control (two-sided Wilcoxon-Mann-Whitney test). No significant difference between the two programs at 15 min exposure time to the KLOUD device was observed.

Conclusions

The KLOUD device from Centropix Global AG, FL-9491 Ruggell, which was examined for beneficial effects in this experimental study, caused accelerated cell regeneration/wound healing in vitro with both programs used (Energizer and Transformer). This manifested itself in stimulated cell migration and cell division to colonize and close a cell-free space. Thus, also on the experimental cell biological level, the findings from the practical application can be confirmed and the use of the KLOUD device with these programs can be recommended.

CELL-BIOLOGICALLY TESTED PRODUCT
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PRODUKT ZELLBIOLOGISCH GEPRÜFT

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