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Research Article

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# Beneficial Effects of "Frequency Specific Microcurrent" on Regeneration of Cultured Connective Tissue Fibroblasts

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## ABSTRACT

Regeneration of complex structures after injury requires dramatic changes in cellular behaviour. One of the main functions of early signaling events after injury is the production of additional cells that are able to rebuild lost or damaged structures. This is mostly done by cell proliferation. Another fundamental cellular event is the migration of cells, especially in exogenous electrical fields. In this present *in vitro* study, we examined the effect of Frequency Specific Microcurrent (FSM) of the TimeWaver Frequency McMakin system on regeneration/wound healing process. By using a specific bioassay, the granulation phase, which is characterized by the occurrence of cell migration and proliferation, was reconstructed.

The study was conducted with cultured connective tissue fibroblasts (L-929) which were seeded into cell cultures plates containing silicon frames which represent a cell-free space (= artificial wound) after the cell layer has become confluent and the frame was removed. The cells were allowed to migrate and proliferate into the cell-free space for closure. After fixation and staining, the residual cell-free space was calculated by a specialized software. Untreated controls were compared with cells which have been exposed once for 5 to 6 min to two different programs of the Time Waver Frequency McMakin system. Three independent experiments with a total of 12 single measurements were conducted.

Morphological evaluation of cell regeneration/wound healing in comparison to the untreated control showed a significant improvement in the colonization of the cell-free space by the application of both programs with only a single short-term treatment of the TimeWaver Frequency McMakin system. Calculation of the residual spaces after 24 hours of fibroblast migration and proliferation into the cell-free space by a specialized software showed a residual cell-free space of 22.1 ± 3.8 % for the untreated control, whereas the residual cell-free space was only 9.6 ± 3.6 % for cells after exposure to program # 1 (= trauma/re-activation) and 15.7 ± 1.5 % for cells after exposure to programm # 2 (= cell regeneration/wound healing). All data represent mean value ± standard deviation. This means that the cells have been stimulated significantly for an improved regeneration process by migration and proliferation after only a single short-term exposure to the programs used in this study

The TimeWaver Frequency McMakin system has clearly demonstrated its beneficial effect in experimental test procedures at the cellular level. The application of both programs resulted in a significant improvement in regeneration/wound healing of connective tissue fibroblasts even after only a single short-term application. The present cellular results confirm previous findings from practical use of high-performance athletes during regenerative processes due to physical exercise or overload.

## Keywords

Microcurrent therapy
Cell regeneration
Wound healing
Connective tissue fibroblasts
L-929
Cell culture

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## INTRODUCTION

Regeneration of complex structures after injury requires dramatic changes in cellular behaviour. Regenerating tissues initiate a program that includes diverse processes such as wound healing, cell death, dedifferentiation. Moreover, newly regenerated tissues must integrate into preexisting cellular structures [1,2].

The skin is the largest organ system of the body. Therefore, it plays a pivotal role in the protection against numerous exogenous traumata such as mechanical forces, bacterial infections, fluid imbalance, thermal dysregulation and many others. The integrity of healthy skin plays a crucial role in maintaining physiological homeostasis of the human body [3]. *In vivo*, the cell regeneration/wound healing process can be divided into distinct phases: Inflammatory phase, granulation phase and differentiation phase [4-8].

According to the statements of TimeWaver Sport GmbH, the "team ... has been working continuously since 2016 to establish the added value of Frequency Specific Microcurrent (FSM) in the field of sports. In particular, FSM … is intended to shorten regeneration times, support sustainable rehabilitation and achieve performance goals more quickly ... Microcurrent therapy works with electricity that is comparable to that of the body's own cells ... By use of appopriate specific frequencies, the cell receptors should be activated to resume their natural function in order to support intracellular processes, metabolic processes, gene expression and the protein configuration of the cell." Such kind of electrical fields are important and represent fundamental components of development, cell regeneration and wound healing. Direct current electric fields mediate motility of many cell types. For example, in 3T3 fibroblasts electric fields increase the speed and cathodal directionality of motile cells [9]. The fields result in a polarized ion transport and current flow through electrically conductive pathways [10,11].

In this present *in vitro* study, we examined the effect of FSM on cell regeneration/wound healing process. By using a specific bioassay with cultured connective tissue fibroblasts, especially the granulation phase, which is characterized by the occurrence of migration and proliferation of the cells, was reconstructed. The bioassay has been already described in detail elsewhere [12,13].

## MATERIAL AND METHODS

### TimeWaver Frequency McMakin system-programs used

In this study, two different programs of the TimeWaver Frequency McMakin system were used. Programs were adapted after the first preliminary tests have been performed successfully:

- Program # 1: Trauma/re-activation consisting of two individual programs running one after the other: 1. Channel A: 294 Hz, Channel B: 77 Hz, signal strength: sharp, intensity: 500 µA, duration: 2 min 30 s; 2. Channel A: 321 Hz, Channel B: 77 Hz, signal strength: sharp, intensity: 500 µA, duration: 2 min 30 s. Total duration: 5 min.
- Program # 2: Cell regeneration/wound healing consisting of two individual programs running one after the other: 1. Channel A: 124 Hz, Channel B: 77 Hz, signal strength: sharp, intensity: 500 µA, duration: 3 min; 2. Channel A: 124 Hz, Channel B: 355 Hz, signal strength: sharp, intensity: 500 µA, duration: 3 min. Total duration: 6 min.

### Cell culture

The investigations were conducted with connective tissue fibroblasts (cell line L-929, ACC-2, Leibniz Institute DSMZ, Braunschweig, Germany). The cells were routinely cultured in RPMI 1640 medium with 10 % growth mixture and 0.5 % gentamycin in an incubator at 37 °C in an atmosphere of 5 % $CO_2$ and 95 % air at approximately 100 % humidity. All cell culture reagents were purchased from Pan-Biotech, Aidenbach, Germany.

### Experimental design

The connective tissue fibroblasts were seeded into the three compartments of a silicone frame (3 well culture inserts; ibidi, Gräfelfing, Germany) at a density of 100,000 cells/ml. The individual compartments are separated from one another by a 500 µm thick silicone bar. Because of the special adhesion area of the silicone frame, it sticks firmly to the bottom of a culture dish and thus forms a cell-free space after removing the frame. The cells can colonize this space by proliferation and migration. After reaching confluency (= cells are close together) within 48 hours after cell seeding, the silicone frames were carefully removed with tweezers so that a sharp cell edge was obtained between the three compartments of the frame.

Immediately after removing the silicone frame, the electrodes of the TimeWaver Frequency McMakin system were attached to the edge of the culture dish with clips so that the electrode pairs of channel A and channel B were diagonally opposite (Figure 1). At the same time, the electrodes were attached so that they were aligned parallel to the direction of migration of the cells into the cell-free space. The cells were exposed to the FSM for 5 min (program # 1) or 6 min (program # 2) and then incubated without any further treatment in the incubator for another 24 hours. Cells which had been treated in the same way, but without exposure to the FSM, served as corresponding controls.

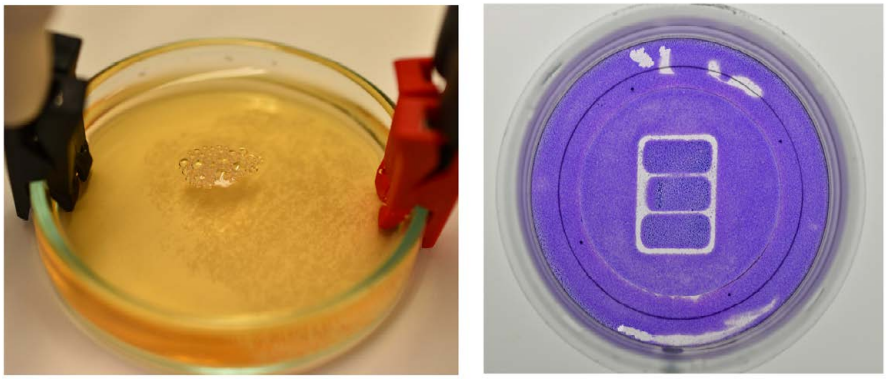

**Figure 1:** Left picture: Presentation of the experimental setup for exposure to the Frequency Specific Microcurrent. The air bubbles of the culture medium in the middle of the culture dish with the electrodes on both sides were left for better visualization and have no influence on the test result. Right picture: Stained culture dish after the end of the experiment, where one can see the residual cell-free spaces in the middle very well. In addition, the scratches of the clamped electrodes of the TimeWaver Frequency McMakin system at the upper and lower edge of the petri dish can be seen very clearly in the blue-violet colored cell layer.

Finally, cells were washed with phosphate-buffered saline, fixed with methanol, stained with Giemsa methylene blue solution (Sigma-Aldrich, Deisenhofen, Germany) and air-dried. Micrographs documenting the width of the remaining cell-free space were performed at different locations for each sample. A total of 4 measurements of the remaining cell-free space was performed for each independent test series (n=3). IKOSA AI software with artificial intelligence (KML Vision, Graz, Austria) was used to calculate the residual cell-free space for the treated samples in comparison to untreated controls.

## STATISTICAL ANALYSIS

Statistical analysis of the test results was done by using the parametric two-tailed Wilcoxon-Mann-Whitney test.

## RESULTS

As depicted in Figure 2, the morphological evaluation of cell regeneration/wound healing in comparison to the untreated control showed a significant improvement in the colonization of the cell-free space by the application of both programs with only a single short-term treatment of the TimeWaver Frequency McMakin system. However, there were differences between the results obtained by the two programs (Figure 3). For the untreated control a residual cell-free space of the total view of field was 22.1 ± 3.8 % (mean value ± standard deviation) as calculated from all 12 single measurements, whereas the residual cell-free space was only 9.6 ± 3.6 % (mean value ± standard deviation) for cells after exposure to program # 1 (= trauma/re-activation) and 15.7 ± 1.5 % (mean value ± standard deviation) for cells after exposure to programm # 2 (= cell regeneration/wound healing). This means that the cells have been stimulated significantly for an improved regeneration process by migration and proliferation after only a single short-term exposure to the programs used in this study (significance at $p \leq 0.01$ for program # 1 and $p \leq 0.05$ for program # 2).

## DISCUSSION

In most regenerating processes, the closure of a defect in a given structure requires the production of new cells. Therefore, one of the main functions of early signaling events after injury is to stimulate the production of additional cells that are able to rebuild lost or damaged structures. This is mostly done by cell proliferation, for example either proliferation of stem cells or of terminally differentiated cells [1].

In addition, the second fundamental cellular event during the granulation stage of regeneration/wound healing is the migration of cells [14-16]. *In vivo*, fibroblasts are typically found in connective tissue where they synthesize collagens, glycosaminoglycans and other important glycoproteins of the extracellular matrix.

Another aspect which should be mentioned in correlation to this study is the stimulation of a directed cell migration in electrical fields after injury in the course of a subsequent regeneration/wound healing process. As outlined by Messerli and Graham, cultured vertebrate cells commonly respond to exogenous weak direct current electrical fields by aligning, migrating or growing along a direction to the electrical field lines [11]. Besides numerous cell types, this phenomenon has also been described for fibroblasts [17]. Moreover, around 1990 the first reports appeared that described a significant increase in the rate of healing by electrical fields [18]. From then onward electrical stimulation became the term for the many different forms of applied electrical fields used to improve wound healing.

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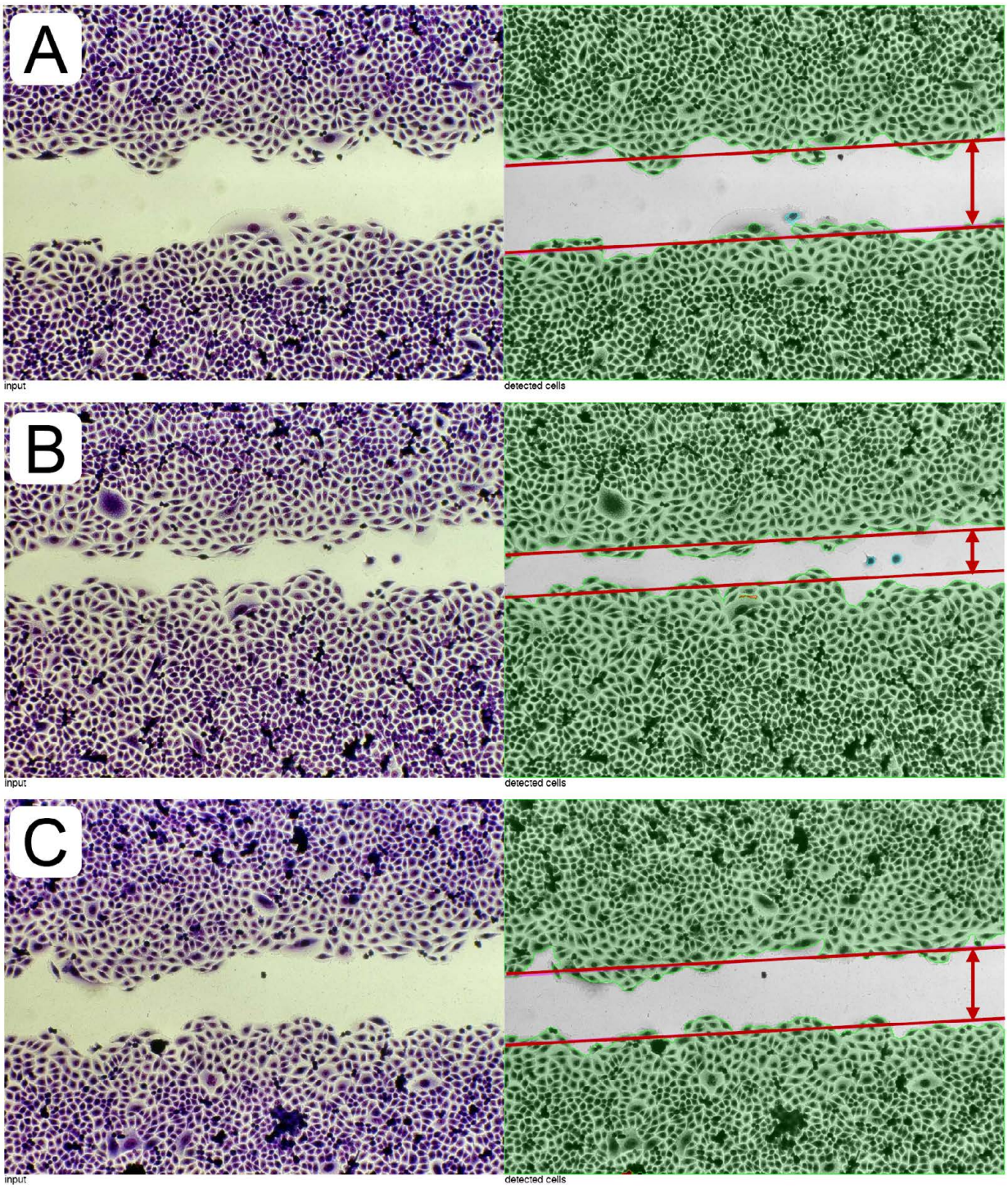


**Figure 2:** Microscopic presentation of the effect of the Frequency Specific Microcurrent of the TimeWaver Frequency McMakin system on regeneration/wound healing of cultivated connective tissue fibroblasts after 24 hours. Cells have been fixed and stained. (A) Untreated control; (B) Effect of a single exposure by program # 1 (trauma/re-activation) for 5 min; (C) Effect of a single exposure by program # 2 (cell regeneration/wound healing) for 6 min. Left column: Original micrographs documenting the width of the residual cell-free space. Right column: Micrographs after evaluation by IKOSA AI software showing the regression of the cell migration front (marked as red lines) and the resulting residual width of the cell-free space (marked by red arrows). Olympus IX-50 inverted microscope equipped with an Olympus 10x planachromate and an Olympus E-10 digital camera with 4 megapixel resolution at bright field illumination.

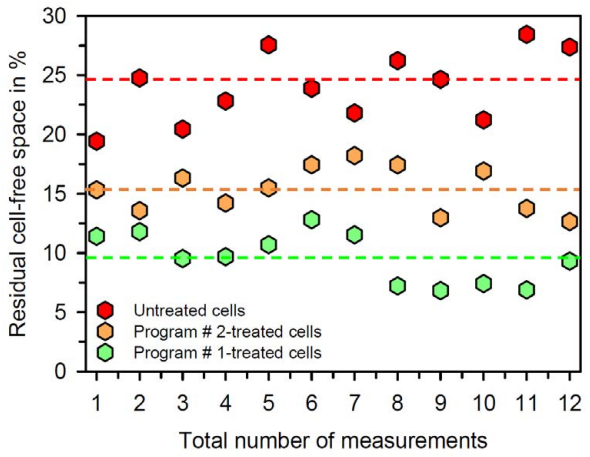


**Figure 3:** Graphical presentation of the effect of the Frequency Specific Microcurrent of the TimeWaver Frequency McMakin system on regeneration/wound healing of cultivated connective tissue fibroblasts after 24 hours. The data show the single measurement points of 3 independent experiments as well as the calculated means (dashed lines) of the relative residual cell-free areas of untreated cells (red colour), program # 1-treated cells (green colour) and program # 2-treated cells (orange colour). Note that both programs significantly improve the regeneration process by a stimulation of cell proliferation and migration resulting in a reduced residual cell-free space.

The present study also demonstrates the stimulation of the cell regeneration/wound healing process *in vitro* after application of FSM by the TimeWaver Frequency McMakin system. One of the most remarkable points is the fact that only a single application for 5 min (program # 1) or 6 min (program # 2) induced a significant improvement of connective fibroblast migration and proliferation causing a significant reduction of the residual cell-free space after 24 hours. However, many mechanisms have been proposed to describe how electrical fields promote wound closure [19,20]. If one of these mechanisms is acting during FSM application, is currently unknown. However, the interest of the present study was not focused on the study of directed cell migration into a cell-free space, but only on the possible improvement of cell regeneration/wound healing by FSM.

## CONCLUSIONS

Although the mechanism of action might be a point of speculation, the Time Waver Frequency McMakin system has clearly demonstrated its beneficial effect in experimental test procedures at the cellular level. The application of both programs resulted in a significant improvement in regeneration/wound healing of connective tissue fibroblasts even after only a single short-term application. The present cellular results confirm previous findings from practical use of high-performance athletes during regenerative processes due to physical exercise or overload.

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