Investigations on the Beneficial Effects of BICOM optima mobile Bioresonance Device On Cultured Connective Tissue Fibroblasts

Dartsch PC

Dartsch Scientific GmbH, Institut für zellbiologische Testsysteme, Auf der Voßhardt 25, D-49419 Wagenfeld, Germany

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Author's address: Prof. Dr. Peter C. Dartsch, Dartsch Scientific GmbH, Institut für zellbiologische Testsysteme, Auf der Voßhardt 25, D-49419 Wagenfeld, Germany
E-mail: pc.dartsch@dartsch-scientific.com

Abstract

Background. The holistic bioresonance method uses the electromagnetic waves it receives from the patient and alters the energy field of the organism. Thus, it can be used as a diagnostic and therapeutic method to improve well-being and the success in the treatment of various diseases.

Experimental Principle. In this preclinical and experimental study we used connective tissue fibroblasts to investigate whether the program chain “pathogene Ai” of the BICOM optima mobile bioresonance device has a positive impact on vitality and regeneration/wound healing process of these cells. Since both cell properties are directly related to each other, an established cell culture test system simulating the granulation stage of wound healing, which is characterized by an increased cell migration and proliferation, was used.

Results. The results show that treatment of cells with the BICOM optima mobile bioresonance device resulted in a significant improvement of cell vitality by 38.0 ± 14.5 % (mean value ± standard deviation; $p \leq 0.01$; two-tailed Wilcoxon-Mann-Whitney test) when compared with untreated controls. Moreover, in the cell regeneration/wound healing test system, a cell migration distance of 300 ± 34 µm (mean value ± standard deviation) was measured for bioresonance-treated cells. For untreated cells a migration distance of only 166 ± 38 µm (mean value ± standard deviation) was examined. When calculating the relative values in direct relation, bioresonance treated cells improved wound closure by 81.1 ± 9.2 % (mean value ± standard deviation; $p \leq 0.01$; two-tailed Wilcoxon-Mann-Whitney test).

Conclusions. In summary, the BICOM optima mobile bioresonance device has demonstrated its beneficial potential on the experimental cellular level. By use of cultivated connective tissue fibroblasts, the present preclinical investigation has shown that a significantly improved cell vitality and regeneration/wound healing process *in vitro* was caused by the bioresonance intervention due to an increased fibroblast migration and proliferation. The results confirm findings on the human body after the application of bioresonance intervention.

Introduction

Although conventional medicine has made lots of new diagnostic and therapeutic approaches in the last 100 years, a need of a complementary and alternative medicine in order to maintain and improve our well-being arises as a more individualized and holistic point of view.

It is well known that the cells of our body emit and receive electromagnetic signals which allow an intercellular electromagnetic communication [1,2]. In case of disorders or diseases, this kind of cellular communication is disturbed [3]. The holistic bioresonance method uses the electromagnetic waves it receives from the patient and alters the energy field of the organism. Thus, it can be used as a diagnostic and therapeutic method to improve well-being and the success in the treatment of various diseases [4-6]. For example, Karakos et al. [3] have conducted a recent clinical study with more than 300 patients with nasal, eye, respiratory, cutaneous and gastrointestinal symptoms. After bioresonance treatment, 90 % of the patients observed no more symptoms at all or showed a significant improvement of their symptoms after a bioresonance treatment period of 12 months.

Since preclinical and experimental studies with mammalian cell cultures on the effect of bioresonance are quite rare [7], we conducted this present investigation on cell vitality and regeneration/wound healing of normal connective tissue cells. Since both cell properties are directly related to each other, an established cell culture model simulating the granulation stage of wound healing [8-11] was used to examine whether the BICOM optima mobile bioresonance device has a positive impact on this complex process.

Material and Methods

BICOM Bioresonance Device

A BICOM optima mobile device (Fig. 1) equipped with the BICOM power applicator GST71 (Fig. 2) was kindly provided by REGUMED Regulative Medizintechnik GmbH, D-82152 Planegg, Germany, for the duration of the experiments for several months.

The program chain “pathogene Ai” was used as the basis. All three single programs of the programm chain were set to a duration of 30 minutes, so that a complete treatment cycle was 90 minutes. This cycle ran twice in succession when the cell cultures were treated. Thus, the cells were treated for a total of 180 minutes. The sample beaker at the top right (channel no. 1; see Fig. 1) was always filled with the same culture medium for the experiments as the cell samples.

Of course, the duration of an intervention in humans should not exceed one hour. However, the primary question of this study was to check whether the BICOM resonance device could actually achieve a reaction of the cultivated connective tissue fibroblasts.

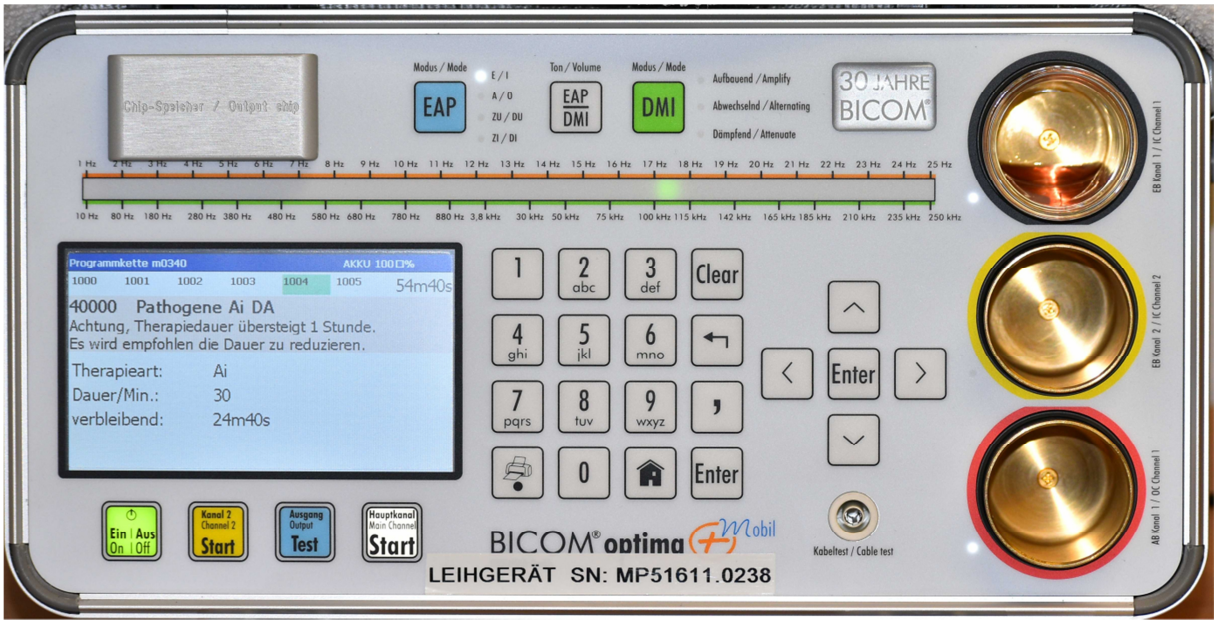

Fig. 1: Presentation of the BICOM optima mobile bioresonance device used with the program chain "pathogene Ai" for the experiments. Note that cell exposure was extended to 180 minutes and the information in the display that the therapy duration of one hour should not be exceeded. The device was connected with a BICOM power applicator GST71 as depicted in Fig. 2. Also note the red culture medium filled in the sample beaker at the top right position (channel no. 1).

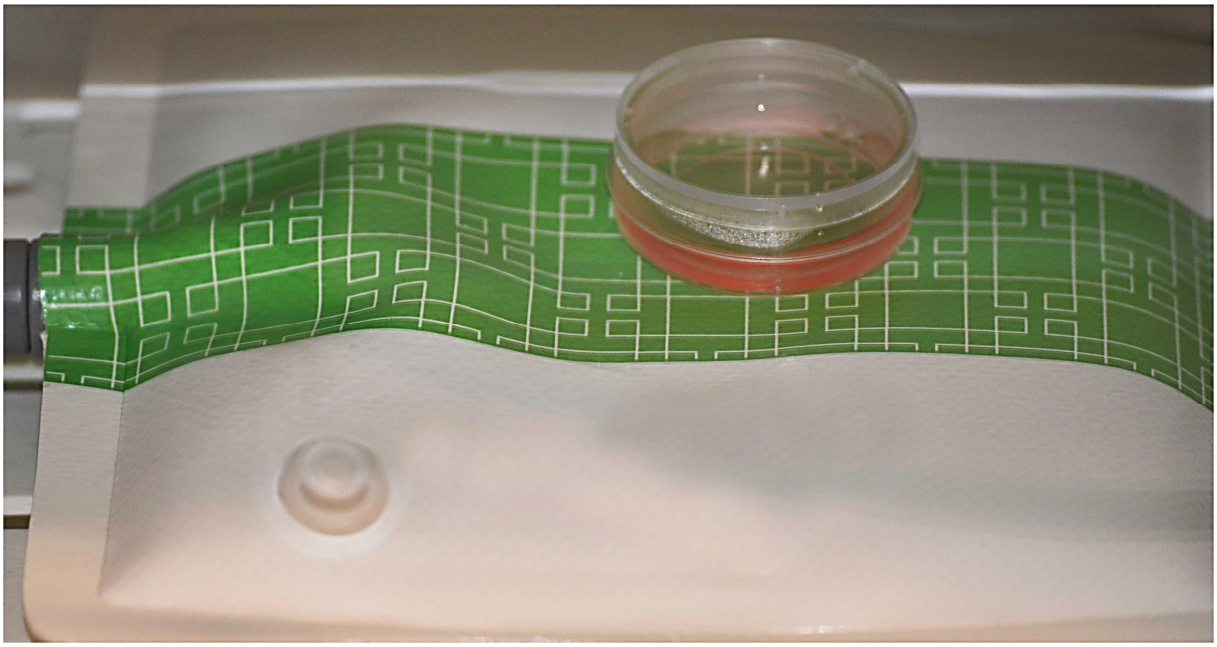

Fig. 2: Arrangement of the power applicator within the external mini-incubator and a cell culture dish in direct contact with the applicator during treatment to the BICOM optima mobile bioresonance device (Fig. 1).

Routine Cell culture

The investigations were conducted with connective tissue fibroblasts of the cell line L-929 (ACC-2; Leibniz Institute; DSMZ - German Collection for Microorganisms and Cell Cultures, Braunschweig) and used in the subcultivation stages (passages) 40 to 57 over a period of several months. Cells were routinely cultivated in RPMI 1640 with 10 % growth mixture and 0.5 % gentamycin and kept in an incubator at 37 °C with an atmosphere of 5 % CO_2 and 95 % air and a humidity of at least 98 %. All cell culture reagent were from PAN-Biotech, Aidenbach, Germany.

Examination of Cell Vitality

For each experiment, the cells from mass cultures were seeded at a cell density of 20,000 cells/well in 24 central wells of two 96-well culture plates (200 µl culture medium/well) and incubated for 24 hours until the cells were completely attached and spread. As reference cells for the sample beaker at the top right of the BICOM bioresonance device, the cells were seeded in the same density on round glass cover slips with a diameter of 16 mm and preincubated for 24 h.

The previous culture medium was then replaced by 200 µl of Leibowitz L-15 medium with 10 % growth mixture and 0.5 % gentamycin. The special medium ensured a constant pH value of 7.4 at normal atmosphere with its low CO_2 content. The first

culture plate was placed directly on the power applicator in a temperature-controlled external mini incubator (Cultura M; Almedica, Switzerland) and treated for a total of 180 min to the program chain “pathogene Ai”. As a reference, the cells on the round glass coverslip were placed into the sample beaker (channel 1) of the BICOM device. The second culture plate was incubated as the control in a mini-incubator, about 3 m away from the other one. Finally, both culture plates were incubated for another 22 h in the same external incubator until cell vitality was examined.

Finally, the culture medium was replaced by a reaction solution consisting of 180 µl of fresh culture medium and 20 µl of XTT (Xenometrix, Allschwil, Switzerland) which was added to each well. The optical density (= color change) was measured after 0 min and 120 min at 37 °C as a differential measurement at ΔOD = 450 – 690 nm with an Elisa reader (BioTEK Elx 808 with software Gen 5/3.00) and compared with one another.

XTT is the sodium salt of 2,3-bis [2-methoxy-4-nitro-5-sulfopheny]-2H-tetrazolium-5-carboxyanilide and has a yellowish color. Mitochondrial dehydrogenases of living cells cleave the tetrazolium ring of XTT and orange-colored, water-soluble formazan crystals are formed. The intensity of the orange color of the reaction solution is proportional to cell vitality/metabolic activity [12-14].

Examination of Cell Regeneration/Wound Healing

As previously described in detail elsewhere [15-17], connective tissue fibroblasts were seeded at a density of 50,000 cells/ml into the three individual compartments of a silicone 3 well-culture insert which was attached to the glass bottom of a µ dish (both from ibidi, Gräfelfing, Germany). The single compartments of the inserts are separated by a 500 µm thick silicone bar with an outer frame of 700 µm. Due to the special adhesion area, an insert adheres firmly to the bottom of a culture dish and forms a distinct cell-free space (artificial wound), which the cells can close by migration and proliferation.

Upon reaching confluency within 48 hours after cell seeding, the silicone inserts were carefully removed with tweezers to achieve a sharp edge of the cell-free space between the compartments. The standard culture medium was replaced by Leibowitz L-15 medium to keep the pH at 7.4 at normal atmospheric conditions. Treatment of cell cultures to the program chain “pathogene Ai” of the BICOM bioresonance device for 180 min was done as described. Control cell cultures were incubated in the second incubator under the same conditions.

After the treatment period was finished, L-15 medium was replaced by the standard culture medium and the cell cultures were incubated at standard conditions for another 21 hours to allow the cells to migrate and proliferate into the cell-free space. Then, cells were fixed with 100 % methanol, stained with Giemsa's azur eosin methylene blue solution (Merck, Darmstadt, Germany) and were air-dried. The width of the remaining cell-free space was measured by micrographical methods at 4 different cell layer edges with triplicate measurements at each edge for each cell culture. Therefore, for each experimental situation 24 data points were taken in a single experiment. The resulting mean values with and without BICOM bioresonance treatment were used for the final evaluation.

Statistical Analysis

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

Results

As shown in Fig. 3, treatment of the cells with the program chain “pathogene Ai” for 180 min resulted in a pronounced improvement of cell vitality when compared with untreated control cells. The difference between BICOM treated cells and untreated control cells becomes even more obvious as depicted in Fig. 4 when the relative stimulation was calculated. The stimulation of cell vitality by use of the BICOM optima mobie in comparison to untreated control cells resulted was 38.0 ± 14.5 % (mean value ± standard deviation). According to the Wilcoxon-Mann-Whitney test the stimulation is statistically significant ($p \leq 0.01$).

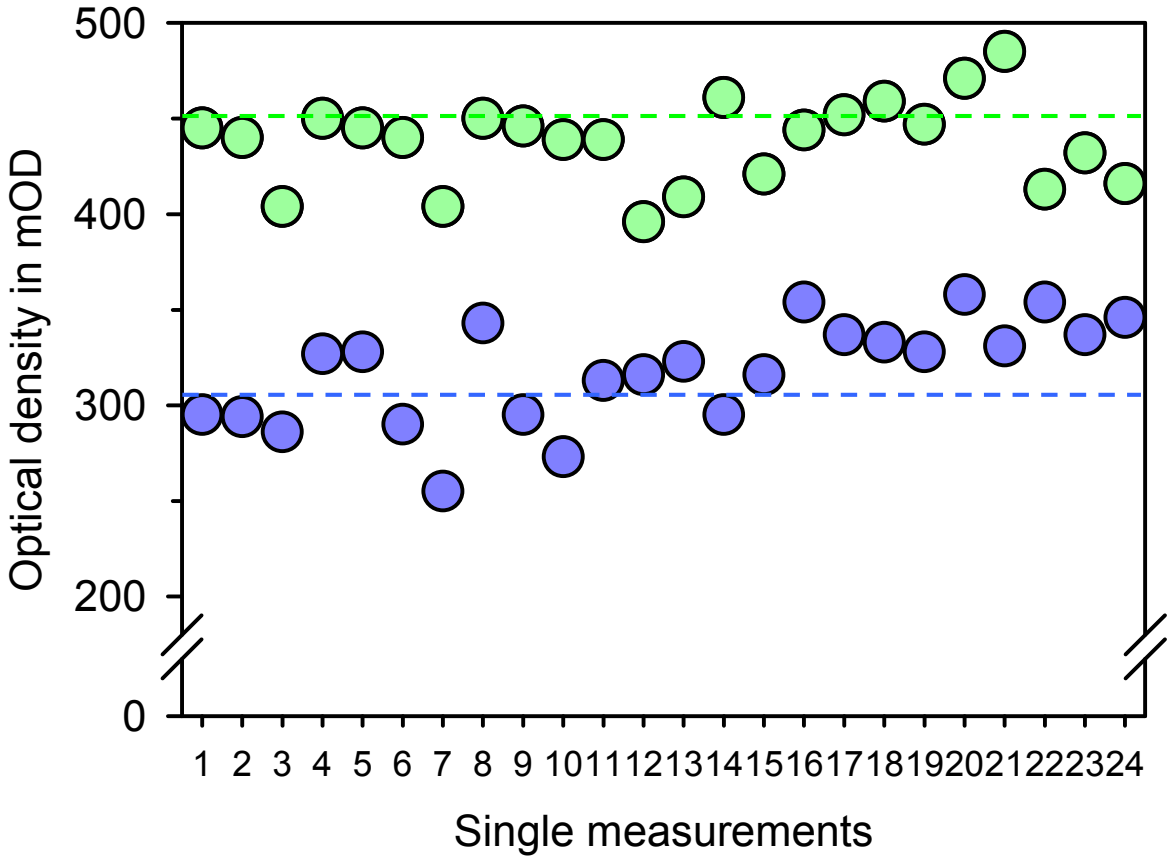

Fig. 3: Presentation of the absolute measuring values for cell vitality after treatment with the BICOM optima mobile bioresonance device and the program chain “pathogene Ai” for 180 minutes (green data points) in comparison to the untreated control (blue data points). The individual pairs of measured values from the same experiment in the two 96-well culture plates are plotted and the mean value for each experimental situation is given as a dashed line in the appropriate color. It can be clearly seen that, despite the variations of a biological system, the mean value for the treated cells is markedly higher than the one for the untreated cells.

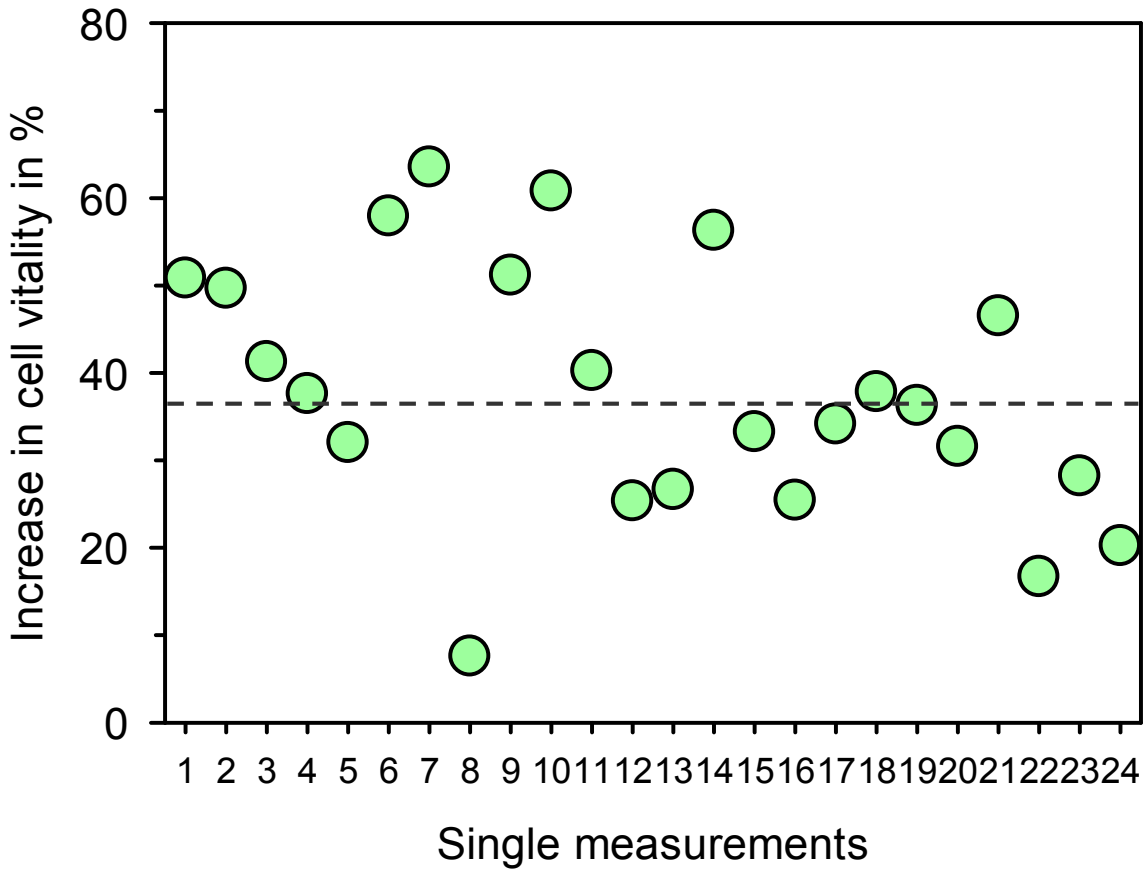

Fig. 4: Presentation of the relative increase in cell vitality after treatment with the BICOM optima mobile bioresonance device and the program chain “pathogene Ai” for 180 minutes in comparison to untreated control cell culture. The dashed line represents the mean value of the single measurements.

According to the stimulation of cell vitality, treatment of the cultivated connective tissue cells with the program chain “pathogene Ai” of the BICOM optima mobile bioresonance device also caused the cells to migrate and proliferate much faster. Consequently, the closure of the cell-free space was more apparent within 21 h when compared with the untreated control (Fig. 5). When the wound closure was evaluated by the migration distance of the connective tissue fibroblasts within 21 h after treatement with the bioresonance device, a cell migration distance of 300 ± 34 µm (mean value ± standard deviation) was measured. For the untreated control cells a value of 166 ± 38 µm (mean value ± standard deviation) was examined. Thus, when calculating the relative values in direct relation, the BICOM optima mobile bioresonance device improved wound closure by 81.1 ± 9.2 % (mean value ± standard deviation; $p \leq 0.01$). In several follow-up experiments conducted with both, µ-dishes with glass and plastic bottom, similar results were obtained.

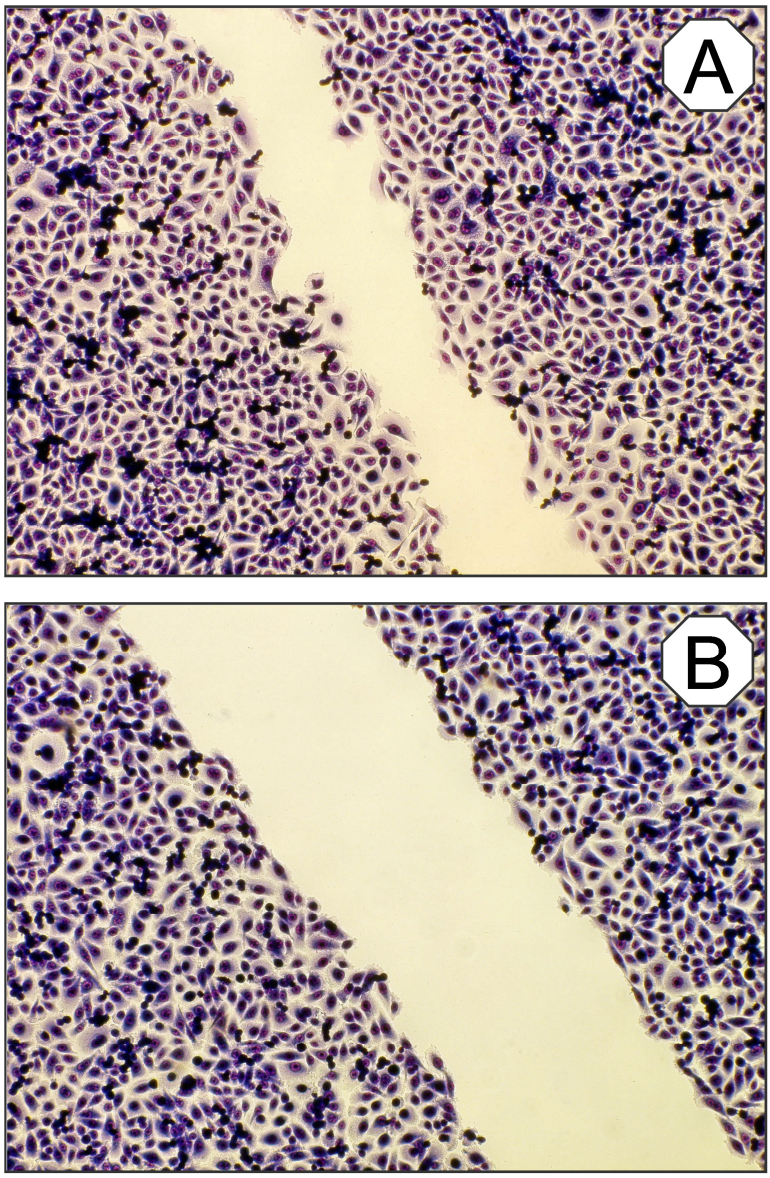

Fig. 5: Micrographs of cultured connective tissue cells after an incubation period of 21 hours and after treatment with the BICOM optima mobile bioresonance device and the program chain “pathogene Ai” for 180 minutes (A) in comparison to the untreated control culture (B). Olympus IX 50 inverted microscope with planachromate 10x and Olympus E-10 digital camera at 4 megapixel resolution at bright field illumination.

Discussion and Conclusion

Although the bioresonance principles are not really accepted in conventional medicine as a method for diagnosis and therapy, the present investigation has shown that bioresonance obviously has an effect on the cellular level. One might argue that cell cultures are not similar to the complex humans body, but it should be considered that cell cultures can focus on single aspects of living as done here. The investigation has two main topics, namly cell vitality which is represented by the metabolic activity

of a cell population which also includes mitotic activity (= proliferation) and migration. Based on this consideration, it makes sense to have a closer look on the cell regeneration/wound healing process, because this feature of cells is the result of both processes, migration and proliferation. However, the terms regeneration and wound healing are often used synonymously and describe one of the most complex biological processes that occur during human life [for review, see 18]. Complicated wound healing processes might also be associated with the occurrence of an excess of oxygen radicals which cause a local oxidative stress in the tissue [19,20].

In the investigation presented here, the BICOM optima mobile bioresonance device has demonstrated its potential to increase not only vitality of cultivated connective tissue fibroblasts, but also the regeneration/wound healing process. This is a new preclinical finding and an indication that the bioresonance intervention has its beneficial health effects not only in case studies [4], but also on an experimental cellular level. Although bioresonance principles and interventions are not really accepted in wound healing management in conventional medicine, the intervention might be useful in cases with complicated lesions and wound healing processes as a complement to conventional therapies.

In conclusion, the BICOM optima mobile bioresonance device has demonstrated its beneficial potential on the experimental cellular level. By use of cultivated connective tissue fibroblasts, the present preclinical investigation has shown that a significantly improved cell vitality and regeneration/wound healing process *in vitro* was caused by the bioresonance intervention due to an increased fibroblast migration and proliferation. The results confirm findings on the human body after the application of bioresonance intervention.

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