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April 9, 2014

– Test report and professional information –

Mini-Rayonex

In vitro-investigations with cultured connective tissue fibroblasts on the stimulation of wound healing process

Background & question of the study

The stages of wound healing are hemostasis, inflammation, proliferation or granulation, and remodeling or maturation. This test system used here simulates the phase of granulation which is characterised by the migration and proliferation of mainly connective tissue fibroblasts for closure of wound gap.

Numerous users all over the world have felt the positive resonance of Mini-Rayonex devices up to now. The present *in vitro*-investigation was performed to examine whether the application of the Mini-Rayonex device might be also beneficial by stimulating migration and proliferation of connective tissue fibroblasts for a faster closure of a wound gap.

This scratch wound healing assay has been widely adapted and modified by researchers to study the effects of a variety of experimental conditions on cell migration and proliferation. Its basic principle is that the wound gap (= cell-free space) in the cell monolayer is subsequently closed up towards the center of the gap.

Experimental design and data analysis

Mouse connective tissue fibroblasts, which are usually taken for the examination of biocompatibility of medical devices according to EN ISO 10993-5 (cell line L-929, ACC 173, passage P132) were taken for the investigations presented here. The cell line was purchased from Leibniz-Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, D-38124 Braunschweig. Cells were cultivated as mass cultures in a CO2

incubator at 37 °C with a moist atmosphere of 5 % CO_2 and 95 % air. Culture medium was RPMI 1640 supplemented with 5 % fetal bovine serum, 100 Units/ml of penicillin & 100 µg/ml of streptomycin. All cell culture reagents were from GE Healthcare Life Sciences, D-35091 Cölbe.

For the experiments, cells were taken from 80 to 90 % confluent mass cultures and were seeded into 12-well plates at a density of 50,000 cells/well (2 ml culture medium/well). The cells were incubated for 3 days in the incubator until confluency was obtained. Then, the cell monolayers in the wells were gently scratched with a new 5,000 µl pipette tip across the center of the well. While scratching across the surface of the well, the long-axial of the tip was always perpendicular to the bottom of the well. The resulting wound gap (= cell-free space) had a distance of 1,400 µm. A straight line in one direction was scratched and another scratched line perpendicular to the first one created a cross in each well. After scratching, the wells were washed with phosphate-buffered saline with calcium and magnesium to remove the detached cells. The wells were replenished with fresh pH-stable culture medium (2 ml/well) consisting of one part of RPMI 1640, one part of phosphate-buffered saline with calcium and magnesium, 5 mM glucose, 5 % fetal bovine serum, 100 Units/ml of penicillin & 100 µg/ml of streptomycin, and 15 mM HEPES buffer.

Culture plates were sealed with an adhesive tape to avoid drying-out of the wells during the 3 day incubation period and transfered to specially designed external incubators allowing temperature stability at 37.2 ± 0.2 °C. The incubators were placed in different rooms with a minimum distance of 4 m to avoid influence of bio-resonance of the Mini-Rayonex to untreated controls. The control wells were placed directly on the bottom of the external incubator, whereas the wells which were exposed to the resonance of the Mini-Rayonex were placed below and above the device in the other external incubator. Prior to use, the Mini-Rayonex devices were rinsed with running tap water and aligned in the incubator in the direction west – east with the lettering pointing to the upper and front side.

After 3 days of continuous exposure to the bio-resonance of the Mini-Rayonex device or without any bio-resonance (untreated controls), cells were fixed and stained according to Romanowsky-Giemsa yielding a blue-violet cytoplasm and red cell nuclei.

The distance of the gaps was measured for each well at two different positions after micrography. The evaluation was done in tabular and graphical form. A p-value ≤ 0.01 (Student's *t*-test) was used for calculation of statistical significance between exposed samples and untreated controls.

Results & conclusions

As depicted in Figure 1, the connective tissue fibroblasts migrated and proliferated in the cell-free space during the 3 days of incubation. In untreated controls, the wound gaps were closed from 1,400 µm to approximately 700 µm (Figure 2). The bio-resonance of the Mini-Rayonex device stimulated the wound healing process in the cultures which were placed

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below or above the device. Thus, the wound gap was significantly closed to 490 µm or 549 µm, respectively (Figure 2). When calculating the percentage stimulation, one gets a stimulation due to Mini-Rayonex by about 20 % vs. untreated controls. This stimulation was statistically significant when compared with controls ($p \leq 0.01$; Student's *t*-test). A significant difference between the multiplates above and below the device was not obtained.
In summary, the present *in vitro*-results with connective tissue fibroblasts confirm the positive effect of the Mini-Rayonex device as already described by numerous users all over the world. The degree of wound healing stimulation by approximately 20 % is very impressive, because this process includes stimulation of both, cell migration and proliferation. Therefore, the use of the Mini-Rayonex device can be recommended as an application to induce and stimulate wound healing processes.

Investigator and responsible for the correctness of the presented experiments and results.

Schongau – April 9, 2014

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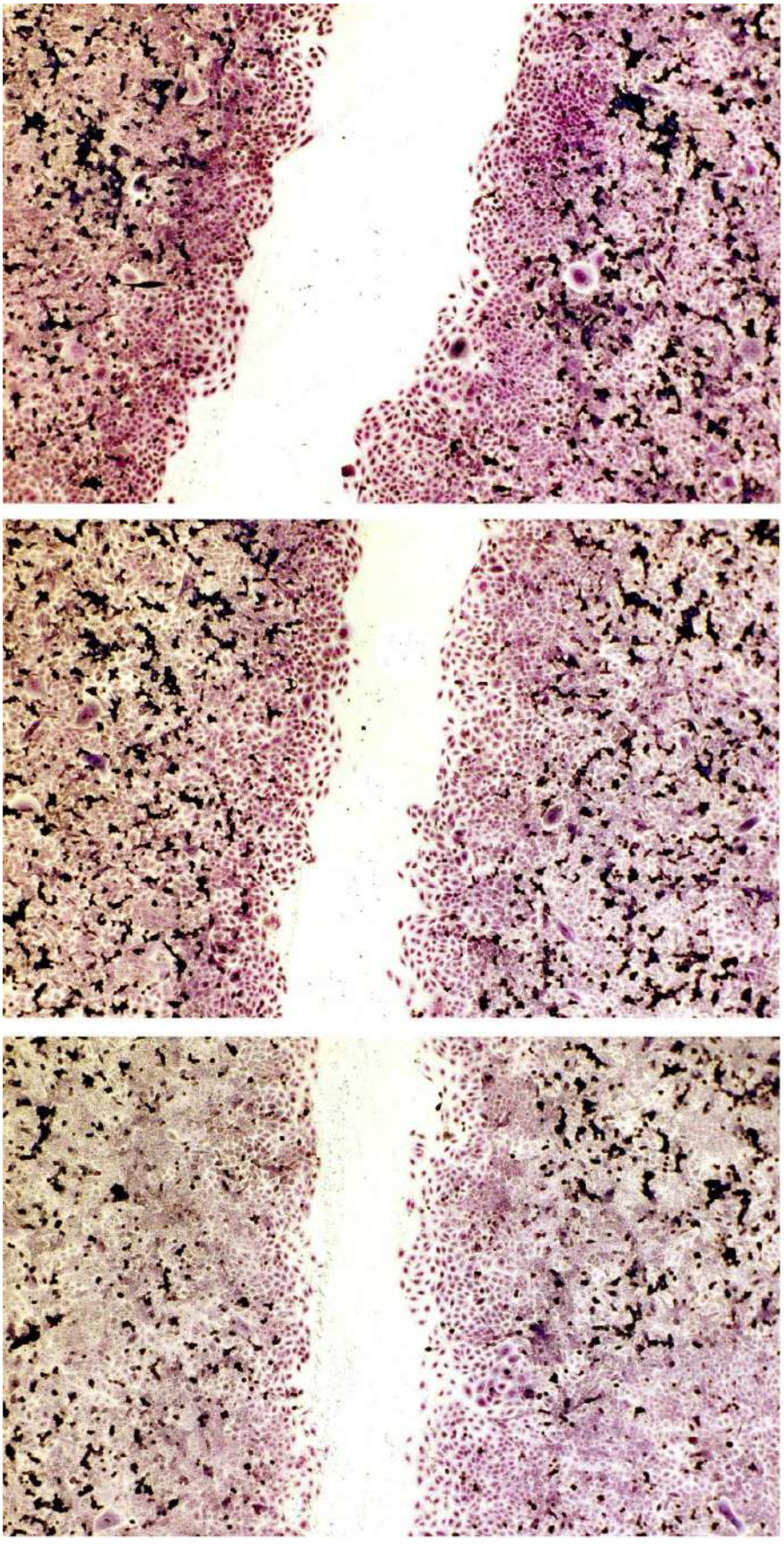

Figure 1: Micrographs of stained cell cultures using bright field after 3 days of wound healing. The cell-free area in which the cells have migrated and proliferated is good to be seen in all three micrographs. However, the wound gap is more prominent in the picture at the top. In addition, the loose wound edge due to cell migration can be easily distinguished from the densely packed cell layers covered with extracellular matrix and distant from the wound gap.
Top: Wound healing of untreated control.
Middle: Wound healing with the multiplate above the Mini-Rayonex device.
Bottom: Wound healing with the multiplate below the Mini-Rayonex device.

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Sample	Wound gap after 3 days (single values in µm)				Mean value (n = 3)	S.E.M. (n = 3)
Untreated control	591	682	819	573	697	71
	591	727	764	682		
	545	672	736	986		
Multiplate above Mini-Rayonex	709	682	467	610	490	83
	473	564	362	228		
	482	364	364	571		
Multiplate below Mini-Rayonex	410	500	672	546	532	57
	400	510	474	624		
	476	541	506	729		

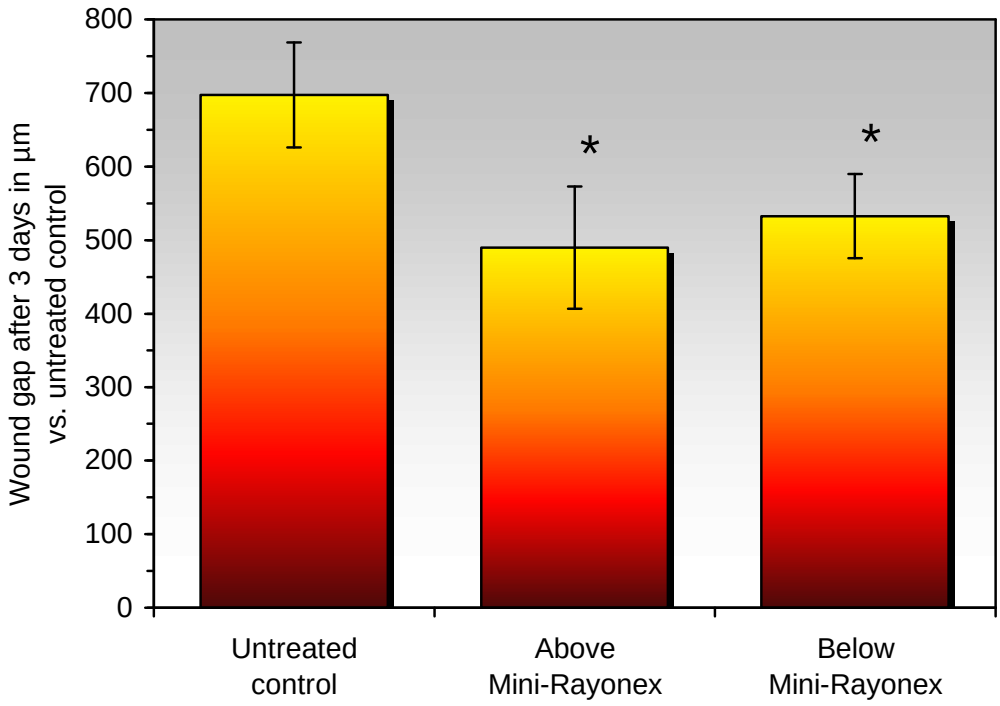

Figure 2: Presentation of measurement data in tabular and graphical form. The data show the results after 3 days of wound healing for untreated multiplates and multiplates which have been placed above and below the Mini-Rayonex device. Data represent mean value ± standard error of the mean (S.E.M.). A statistically significant stimulation of wound healing process (= smaller wound gap distance) is marked by the arrows ($p \leq 0{,}01$; student's *t*-test).