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March 19, 2014

– Test report and professional information –

Mini-Rayonex

In vitro-investigations on the activation of cell metabolism in organ-specific cell cultures

Background

According to Rayonex Biomedical GmbH from D-57368 Lennestadt, Germany, „the most frequently detected resonance spot lies on the fundamental frequency value space 12.5. Within bio-resonance according to Paul Schmidt, this frequency value stands for energy. This is exactly what the organism needs to face disturbances of any kind ... Inside, the Mini-Rayonex is equipped with a dipole antenna system tuned to the fundamental frequency value 12.5, with a universal positive resonance. Using bio-resonance according to Paul Schmidt, you can detect it in an effective radius of 2 - 3 m around the Mini-Rayonex.
Tips for use: The writing on the Mini-Rayonex should always point upward or away from the body ... A Mini-Rayonex in stationary position is more effective if it is aligned in east-west direction in accord with the markings on the device. It works best if you rinse it with running cold water (tap water) for 20 seconds once or twice a week.“

Question behind the present *in vitro*-investigations

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 different organ-specific cell cultures are also able to respond to the positive resonance of the devices. The effect should be determined with objective and generally accepted experimental methods in the scientific world.

Experimental setup

Two different cell lines were taken for the investigations presented here: (1) 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 P128), and (2) adherent growing cells which have been differentiated to macrophages which are responsible for the first unspecific defense in the tissue of the body (cell line HL-60, ACC 3, passage P3). Both cell lines were purchased from Leibniz-Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, D-38124 Braunschweig.

Cells were cultivated as mass cultures in a Binder 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 in quintuplicate wells in a row for each cell density (96-well plates, 200 µl culture medium/well). The cell density varied in the single experiments from 5,000 to 20,000 cells/well. The seeded cells were incubated for 48 hours in the incubator to allow attachment, spreading and normalisation of metabolism. Then, culture medium was aspirated and replaced by a pH-stable exposure medium (180 µl/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.

The multiwell plates were transferred 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 (Figure 1). 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 2 hours (triplicate experiments) and 24 hours (quadruplicate experiments) of continuous exposure to the resonance of the Mini-Rayonex devices, the multiwell plates were taken from the external incubators, 20 µl of XTT was added per well and the multiwell plates were incubated for another hour at the same places as before within the incubators. Thereafter, the optical density of each well was examined by a difference measurement at 450 – 690 nm using a double-wavelength elisa reader (BioTEK Elx 808). XTT is the sodium salt of 2,3-bis[2-methoxy-4-nitro-5-sulfopheny]-2H-tetrazolium-5-carboxyanilide and has a yellowish colour. Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring of XTT yielding orange formazan crystals which are soluble in aqueous solutions. The intensity of the resulting orange solution is directly correlated with cell vitality and me-

tabolic activity. The results are expressed as absolute measurement values and percentage values in comparison to untreated controls.

Results and conclusions

As shown in Table 1 and 2 in detail for an application time of the Mini-Rayonex device of only 2 hours, the resonance of the device caused a remarkable stimulating effect on the cell metabolism of both cell types. The difference between the cells below and above the Mini-Rayonex device were statistically not significant (student's *t*-test). The percentage stimulation in comparison to untreated controls was between 32 % and 38 %. This means that application of the device increased metabolic activity of the cells by approximately one third. This stimulatory effect of the Mini-Rayonex could be increased further to a percentage value up to 45 % after an application time of 24 hours (Table 3 and 4). Again, a significant difference between the cells below and above the Mini-Rayonex device were not observed (student's *t*-test).

In summary, the present *in vitro*-results with two different cell types confirmed the positive effect of Mini-Rayonex devices as already described by numerous users all over the world. The degree of cell metabolism stimulation up to 45 % is very impressive and is obtained after only one day of continuous application. Therefore, the use of the Mini-Rayonex device can be recommended in specific life situations such as physical burden, mental disturbances, healing processes and others.

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

Schongau – March 19, 2014

Prof. Dr. Peter C. Dartsch
Diplom-Biochemiker

Figure 1: Alignment of 96-well plates below and above a Mini-Rayonex device with its lettering pointing upside and to the front. The multiplates were placed exactly in this way into the external incubator at 37.2 ± 0.2 °C. The lower part of the figure represents the wells with seeded and exposed cells which have cleaved the tetrazolium dye due to their vitality and activity of mitochondrial dehydrogenases (orange coloured wells).

Table 1: Presentation of single measurement values of all experiments obtained with connective tissue fibroblasts (cell line L-929) after an exposure time of two hours to the Mini-Rayonex device. The summary of the experiments presents the mean stimulation ± S.E.M. The difference between the cells below and above the Mini-Rayonex device is statistically not significant (student's *t*-test). S.E.M. = standard error of the mean.

Experiment # 1 - L-929 exposed for 2 h

Sample	Single measurement values of optical density					Mean value	±	S.E.M.	Stimulation in % vs. control	±	S.E.M. in %
Untreated control	142	124	173	121	109	134	±	11	0	±	8.3
Culture plate placed above Mini-Rayonex	184	161	181	190	175	178	±	5	33.2	±	2.8
Culture plate placed below Mini-Rayonex	195	228	168	135	187	183	±	15	36.5	±	8.4

Experiment # 2 - L-929 exposed for 2 h

Sample	Single measurement values of optical density					Mean value	±	S.E.M.	Stimulation in % vs. control	±	S.E.M. in %
Untreated control	110	123	119	118	107	115	±	3	0	±	2.6
Culture plate placed above Mini-Rayonex	206	180	153	128	119	157	±	16	36.2	±	10.3
Culture plate placed below Mini-Rayonex	136	163	159	182	174	163	±	8	41.1	±	4.8

Experiment # 3 - L-929 exposed for 2 h

Sample	Single measurement values of optical density					Mean value	±	S.E.M.	Stimulation in % vs. control	±	S.E.M. in %
Untreated control	167	128	179	164	143	156	±	9	0	±	5.8
Culture plate placed above Mini-Rayonex	208	198	209	187	206	202	±	4	29.1	±	2.0
Culture plate placed below Mini-Rayonex	188	201	254	158	174	195	±	16	24.8	±	8.4

Summary

Sample	Mean stimulation in % for V1-V3	±	S.E.M. in %
Culture plate placed above Mini-Rayonex	32.8	±	2.1
Culture plate placed below Mini-Rayonex	34.1	±	4.8

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Table 2: Presentation of single measurement values of all experiments obtained with HL-60 cells which have been differentiated to macrophages after an exposure time of two hours to the Mini-Rayonex device. The summary of the experiments presents the mean stimulation ± S.E.M. The difference between the cells below and above the Mini-Rayonex device is statistically not significant (student's *t*-test). S.E.M. = standard error of the mean.

Experiment # 1 - HL-60 adh. exposed for 2 h

Sample	Single measurement values of optical density					Mean value	±	S.E.M.	Stimulation in % vs. control	±	S.E.M. in %
Untreated control	96	123	178	145	131	135	±	13	0	±	10.0
Culture plate placed above Mini-Rayonex	246	198	148	187	152	186	±	18	38.3	±	9.6
Culture plate placed below Mini-Rayonex	166	206	211	165	175	185	±	10	37.1	±	5.4

Experiment # 2 - HL-60 adh. exposed for 2 h

Sample	Single measurement values of optical density					Mean value	±	S.E.M.	Stimulation in % vs. control	±	S.E.M. in %
Untreated control	110	123	119	118	107	115	±	3	0	±	2.6
Culture plate placed above Mini-Rayonex	180	166	205	132	145	166	±	13	43.5	±	7.8
Culture plate placed below Mini-Rayonex	176	144	161	200	177	172	±	9	48.7	±	5.4

Experiment # 3 - HL-60 adh. exposed for 2 h

Sample	Single measurement values of optical density					Mean value	±	S.E.M.	Stimulation in % vs. control	±	S.E.M. in %
Untreated control	136	147	159	133	154	146	±	5	0	±	3.4
Culture plate placed above Mini-Rayonex	181	177	189	203	206	191	±	6	31.1	±	3.0
Culture plate placed below Mini-Rayonex	166	213	223	169	175	189	±	12	29.8	±	6.3

Summary

Sample	Mean stimulation in % for V1-V3	±	S.E.M. in %
Culture plate placed above Mini-Rayonex	37.7	±	3.6
Culture plate placed below Mini-Rayonex	38.5	±	5.5

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Table 3: Presentation of single measurement values of all experiments obtained with connective tissue fibroblasts (cell line L-929) after an exposure time of 24 hours to the Mini-Rayonex device. The summary of the experiments presents the mean stimulation ± S.E.M. The difference between the cells below and above the Mini-Rayonex device is statistically not significant (student's *t*-test). S.E.M. = standard error of the mean.

Experiment # 1 - L-929 exposed for 24 h

Sample	Single measurement values of optical density					Mean value	±	S.E.M.	Stimulation in % vs. control	±	S.E.M. in %
Untreated control	92	155	173	98	112	126	±	16	0	±	12.8
Culture plate placed above Mini-Rayonex	219	136	153	192	132	166	±	17	32.1	±	10.2
Culture plate placed below Mini-Rayonex	143	219	275	182	144	193	±	25	52.9	±	12.9

Experiment # 2 - L-929 exposed for 24 h

Sample	Single measurement values of optical density					Mean value	±	S.E.M.	Stimulation in % vs. control	±	S.E.M. in %
Untreated control	89	90	120	139	98	107	±	10	0	±	9.1
Culture plate placed above Mini-Rayonex	137	171	203	184	127	164	±	14	53.4	±	8.7
Culture plate placed below Mini-Rayonex	208	198	125	130	98	152	±	22	41.6	±	14.3

Experiment # 3 - L-929 exposed for 24 h

Sample	Single measurement values of optical density					Mean value	±	S.E.M.	Stimulation in % vs. control	±	S.E.M. in %
Untreated control	160	133	148	161	165	153	±	6	0	±	3.8
Culture plate placed above Mini-Rayonex	202	197	198	189	241	205	±	9	33.9	±	4.5
Culture plate placed below Mini-Rayonex	211	248	203	171	314	229	±	24	49.5	±	10.7

Experiment # 4 - L-929 exposed for 24 h

Sample	Single measurement values of optical density					Mean value	±	S.E.M.	Stimulation in % vs. control	±	S.E.M. in %
Untreated control	244	177	235	322	244	244	±	23	0	±	9.4
Culture plate placed above Mini-Rayonex	324	338	298	294	303	311	±	8	27.4	±	2.7
Culture plate placed below Mini-Rayonex	316	246	336	369	435	340	±	31	39.3	±	9.1

Summary

Sample	Mean stimulation in % for V1-V4	±	S.E.M. in %
Culture plate placed above Mini-Rayonex	36.7	±	5.7
Culture plate placed below Mini-Rayonex	45.8	±	3.2

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Table 4: Presentation of single measurement values of all experiments obtained with HL-60 cells which have been differentiated to macrophages after an exposure time of 24 hours to the Mini-Rayonex device. The summary of the experiments presents the mean stimulation ± S.E.M. The difference between the cells below and above the Mini-Rayonex device is statistically not significant (student's *t*-test). S.E.M. = standard error of the mean.

Experiment # 1 - HL-60 adh. exposed for 24 h

Sample	Single measurement values of optical density					Mean value	±	S.E.M.	Stimulation in % vs. control	±	S.E.M. in %
Untreated control	89	90	120	139	98	107	±	10	0	±	9.1
Culture plate placed above Mini-Rayonex	114	182	155	135	198	157	±	15	46.3	±	9.7
Culture plate placed below Mini-Rayonex	178	168	159	162	150	163	±	5	52.4	±	2.9

Experiment # 2 - HL-60 adh. exposed for 24 h

Sample	Single measurement values of optical density					Mean value	±	S.E.M.	Stimulation in % vs. control	±	S.E.M. in %
Untreated control	184	133	149	136	129	146	±	10	0	±	6.9
Culture plate placed above Mini-Rayonex	203	256	207	220	229	223	±	9	52.5	±	4.2
Culture plate placed below Mini-Rayonex	196	248	212	165	208	206	±	13	40.8	±	6.5

Experiment # 3 - HL-60 adh. exposed for 24 h

Sample	Single measurement values of optical density					Mean value	±	S.E.M.	Stimulation in % vs. control	±	S.E.M. in %
Untreated control	124	103	136	137	158	132	±	9	0	±	6.8
Culture plate placed above Mini-Rayonex	177	214	207	180	128	181	±	15	37.7	±	8.4
Culture plate placed below Mini-Rayonex	170	156	168	189	178	172	±	5	30.9	±	3.2

Experiment # 4 - HL-60 adh. exposed for 24 h

Sample	Single measurement values of optical density					Mean value	±	S.E.M.	Stimulation in % vs. control	±	S.E.M. in %
Untreated control	194	178	265	287	339	253	±	30	0	±	11.8
Culture plate placed above Mini-Rayonex	255	475	356	447	253	357	±	46	41.4	±	13.0
Culture plate placed below Mini-Rayonex	330	377	280	345	406	348	±	21	37.6	±	6.2

Summary

Sample	Mean stimulation in % for V1-V4	±	S.E.M. in %
Culture plate placed above Mini-Rayonex	44.5	±	3.2
Culture plate placed below Mini-Rayonex	40.4	±	4.5

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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

CELL-BIOLOGICALLY TESTED PRODUCT
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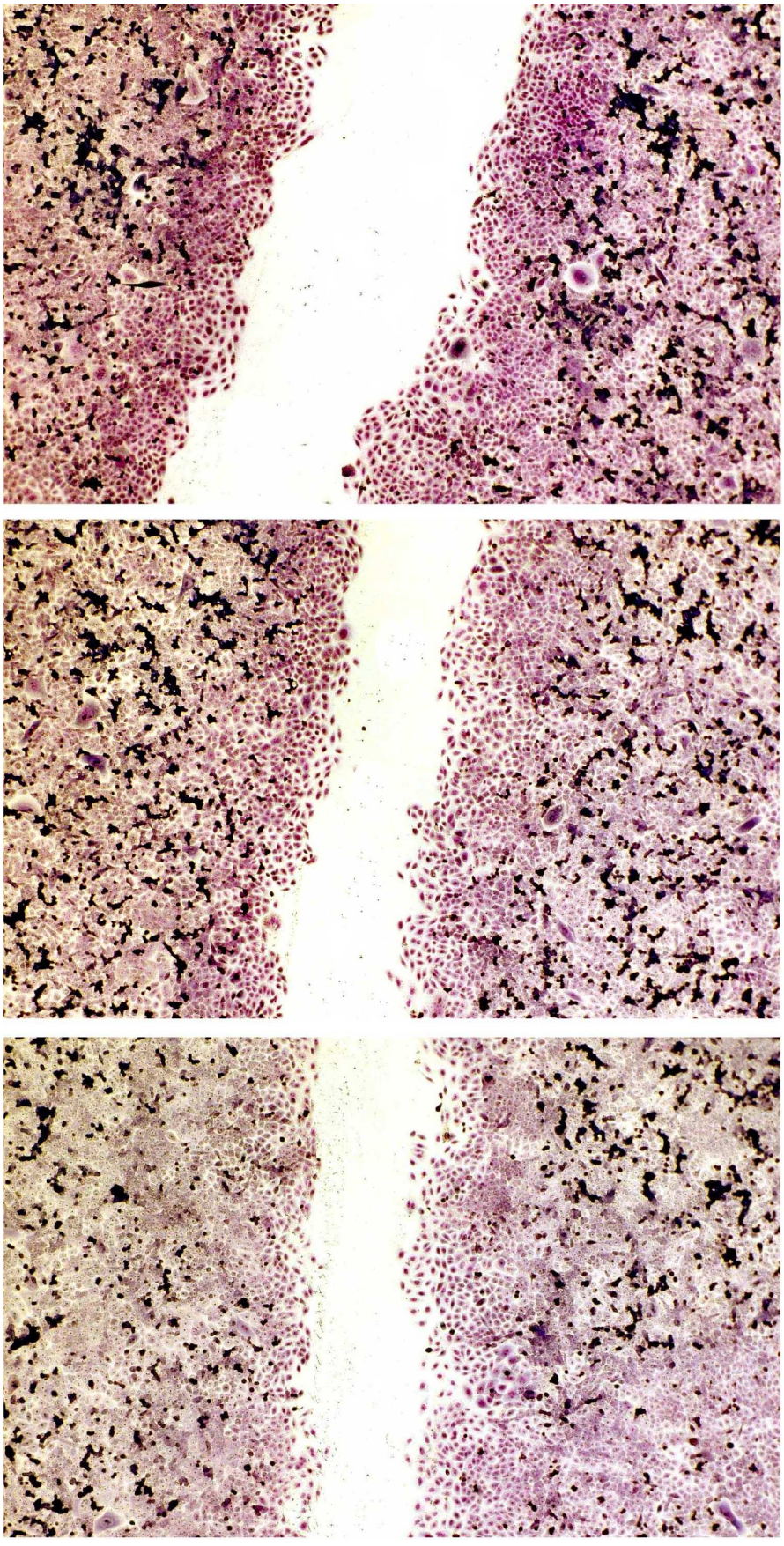

Figure 1: Micrographs of stained cell cultures using bright field after 3 days of wound healing. The cell-free area in which the cells have migrated and proliferated is good to be seen in all three micrographs. However, the wound gap is more prominent in the picture at the top. In addition, the loose 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		

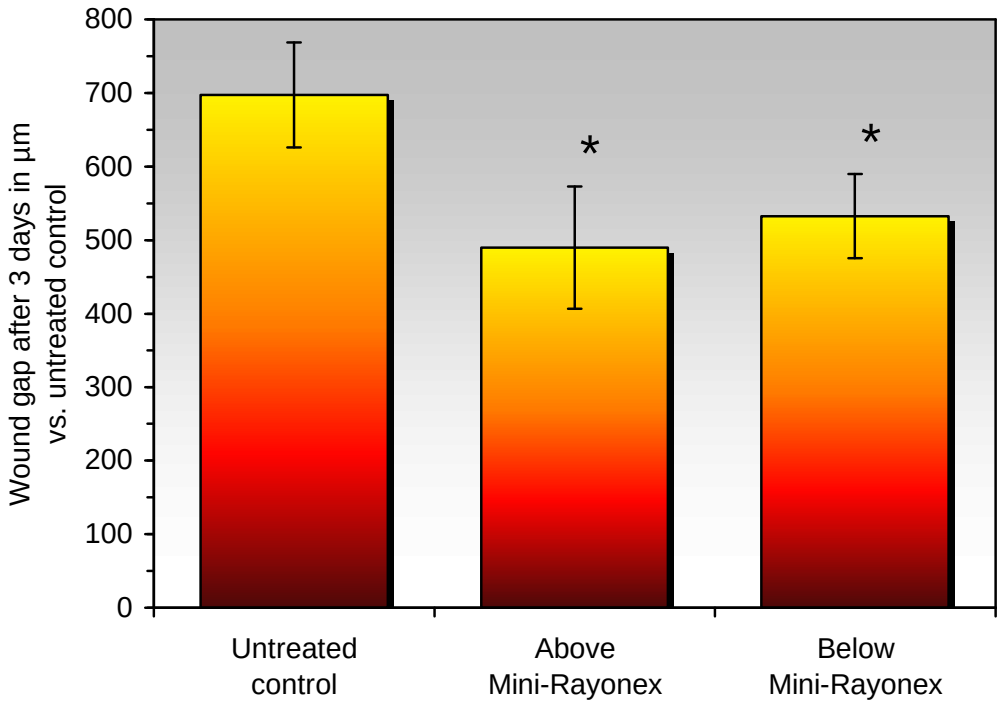

Figure 2: Presentation of measurement data in tabular and graphical form. The data show the results after 3 days of wound healing for untreated multiplates and multiplates which have been placed above and below the Mini-Rayonex device. Data represent mean value ± standard error of the mean (S.E.M.). 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).

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Institute of Cell Biological Test Systems

herewith certifies that the product named

Mini-Rayonex

manufactured and distributed by

Rayonex Biomedical GmbH from 57368 Lennestadt Germany

has been tested for its beneficial effects by using *in vitro* test systems with organ-specific cultured cells.

Results of the Test Assays

The investigations with connective tissue fibroblasts and promyelocytes which have been differentiated to macrophages have shown that cell metabolism was stimulated by 45 % after treatment with the Mini-Rayonex device for only 24 hours when compared to untreated controls. In addition, the simulated wound healing process was also stimulated by approximately 20 % demonstrating that the use of the device is beneficial for the process of wound healing.

In summary, the application of the Mini-Rayonex device can be recommended in specific situations of life such as physical burden, mental disturbances, healing processes and others.

Schongau – March 27, 2014

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