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Biomedical GmbH

Dartsch study
for MINI-Rayonex

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– 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

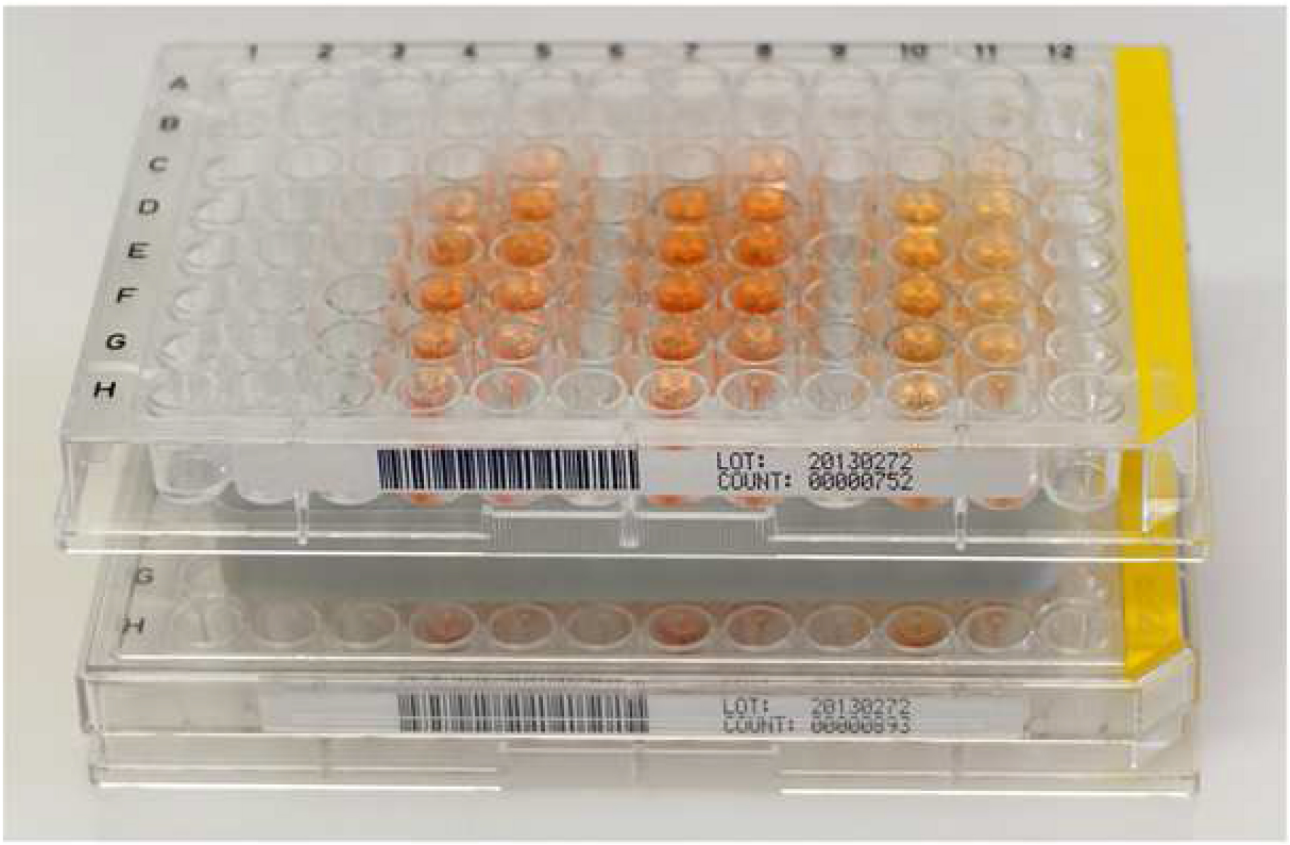

Figure 1: Alignment of 96-well plates below and above a Mini-Rayonex device with its lettering pointing upside and to the front. The multiplates were placed exactly in this way into the external incubator at 37.2 ± 0.2 °C. The lower part of the figure represents the wells with seeded and exposed cells 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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-实验报告与专业信息–

迷你瑞欧内克斯

器官特异性细胞培养的细胞新陈代谢活化*体外*研究

背景

根据位于德国 D-57368 伦纳斯塔特市 (Lennestadt) 的瑞欧内克斯生物医学有限公司的说法，对于物理压力和问题，最常被侦测到的（失衡）共振频率点落在 12.5 的基本频率值。在保罗施密特生物能反馈概念里，此频率值即代表能量，这正是生物体面对任何干扰时所需要的频率。迷你瑞欧内克斯内部配置了具有通用正谐振的偶极子天线系统，并调校至 12.5 的基频值。使用保罗施密特生物能反馈系统，您可以在迷你瑞欧内克斯 2~3 米的半径范围内检测到它。

使用技巧：迷你瑞欧内克斯装置上的文字应朝上或朝向身体外侧。当迷你瑞欧内克斯放于固定的位置时，您可以根据装置上的标示将它朝东西向摆放，则效果更佳。每周一至二次将装置用流动的冷水（自来水）冲洗二十秒，将可发挥最大效用。

本*体外*研究背后的问题

到目前为止，全世界已有许多用户感受到迷你瑞欧内克斯的正面共振。本*体外*研究是检验不同的特异性器官细胞培养是否也能够响应装置的正面共振。此效应应使用客观且科学界普遍接受的实验方法来确定。

实验设置

本实验采取了两种不同的细胞株：（1）老鼠结缔组织纤维母细胞（L-929 细胞株，ACC 173，P128 继代），根据 EN ISO 10993-5 国际标准规定，它们通常是为了试验医疗设备的生物兼容性而采用的，及（2）分化为负责身体组织中第一道非特异性防御的巨噬细胞的贴壁生长细胞（HL-60 细胞株，ACC 3，P3 继代）。两种细胞株皆采购自位于 D-38124 布劳恩施魏格市的莱布尼兹研究所－德国微生物及细胞培养物采集有限公司（ Leibniz-Institut DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH，D-38124 Braunschweig）。

这些细胞在 37 °C、5% 二氧化碳及 95% 空气的潮湿大气的恒温箱内大量培养。培养基为 RPMI 1640 辅以 5% 胎牛血清、100 单位/毫升青霉素和 100 微克/毫升链霉素。所有的细胞培养试剂皆来自位于 D-35091 科尔贝市的 GE 医疗保健生命科学公司（GE Healthcare Life Sciences，Cölbe）。

在实验中，细胞从 80~90%的融合大量培养物中抽取，以各自的细胞密度分别植入五个一排的培养孔中（96 孔盘，200 微升培养基／孔）。在单次实验中，细胞密度从 5,000~20,000 个细胞／孔不等。植入的细胞置于恒温箱中培养 48 小时以便进行新陈代谢的附加、散布及正常化。然后，吸出培养基，替换成一种 pH 稳定曝光介质（180 微升／孔），其中一部分为 RPMI 1640，一部分为含钙、镁、5 mM 葡萄糖、5%胎牛血清、100 单位/毫升青霉素、100 微克/毫升链霉素和 15 mM HEPES 缓冲液的磷酸盐缓冲生理食盐水。

之后将此多孔盘移至一特殊设计，可维持温度在 37.2 ± 0.2 °C 的外部恒温箱中。恒温箱分别放置在至少距离 4 米的不同房间内，以避免未处理的控制组被迷你瑞欧内克斯的共振所影响。控制组的多孔盘直接放置在外部恒温箱底部，而另一个外部恒温箱中则分别在装置的上下方各放一个多孔盘，使培养孔暴露在迷你瑞欧内克斯的共振中（图一）。使用前已将迷你瑞欧内克斯以自来水冲洗，且以东西向摆置，装置上的文字朝上朝前。

持续暴露在迷你瑞欧内克斯装置的共振中 2 小时（重复三次实验） 和 24 小时后（重复四次实验），从外部恒温箱中取出多孔盘，每个孔加入 20 微升的二甲氧唑黄（XTT），然后将多孔盘放回恒温箱内同一位置再培养一小时。之后，使用双波长酵素免疫分析测读仪（ELISA reader， BioTEK Elx 808）以 450 - 690 纳米的差异测量检测每个孔的光密度。XTT 为 c;3,3'-[1-(苯氨酰基)-3,4-四氮唑]-二(4-甲氧基-6-硝基)苯磺酸钠的溶液，呈淡黄色。活细胞内的粒腺体去氢酶会切开 XTT 上的四唑环，产生溶于水的三苯基甲酯橙色结晶。获得的橙色溶液密度与细胞活性及新陈代谢活性度直接相关。这些结果以绝对性的测量数值与百分比数值表示，以便和未处理控制组相比较。

实验结果与结论

如表 1 和表 2 的细节所示，使用迷你瑞欧内克斯装置的时间只有 2 小时，此装置的共振对两种细胞类型的细胞新陈代谢都产生了出色的刺激效应。置于迷你瑞欧内克斯装置上下方的细胞之间的差值，在统计学上并不显着（学生的 *t*-测试）。和未处理控制组相比，促进比率为 32~38%。这意味着该装置的应用使细胞的新陈代谢活性增加了大约三分之一。迷你瑞欧内克斯的此种刺激效应，在使用 24 小时后可进一步达到 45%的百分比（表 3 和表 4）。同样的，置于迷你瑞欧内克斯上下方的细胞之间并无明显差异（学生的 *t*-测试）。

综上所述，目前使用两种不同细胞类型的*体外*研究已证实迷你瑞欧内克斯装置的正面效果，正如世界各地众多用户所描述的那样。高达 45%的细胞新陈代谢促进率是非常令人印象深刻的，而且只需连续使用一天即可达成。因此在特定的生活状况下，例如身体负担、精神障碍、愈合过程.....等，皆可推荐使用迷你瑞欧内克斯装置。

研究者应为呈现出的实验和结果的正确性负责。

尚高镇 – 19.03.2014

CELL-BIOLOGICALLY TESTED PRODUCT
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2014
PRODUKT ZELLBIOLOGISCH GEPRÜFT

生化博士彼得•C.•达曲教授
(Peter C. Dartsch)

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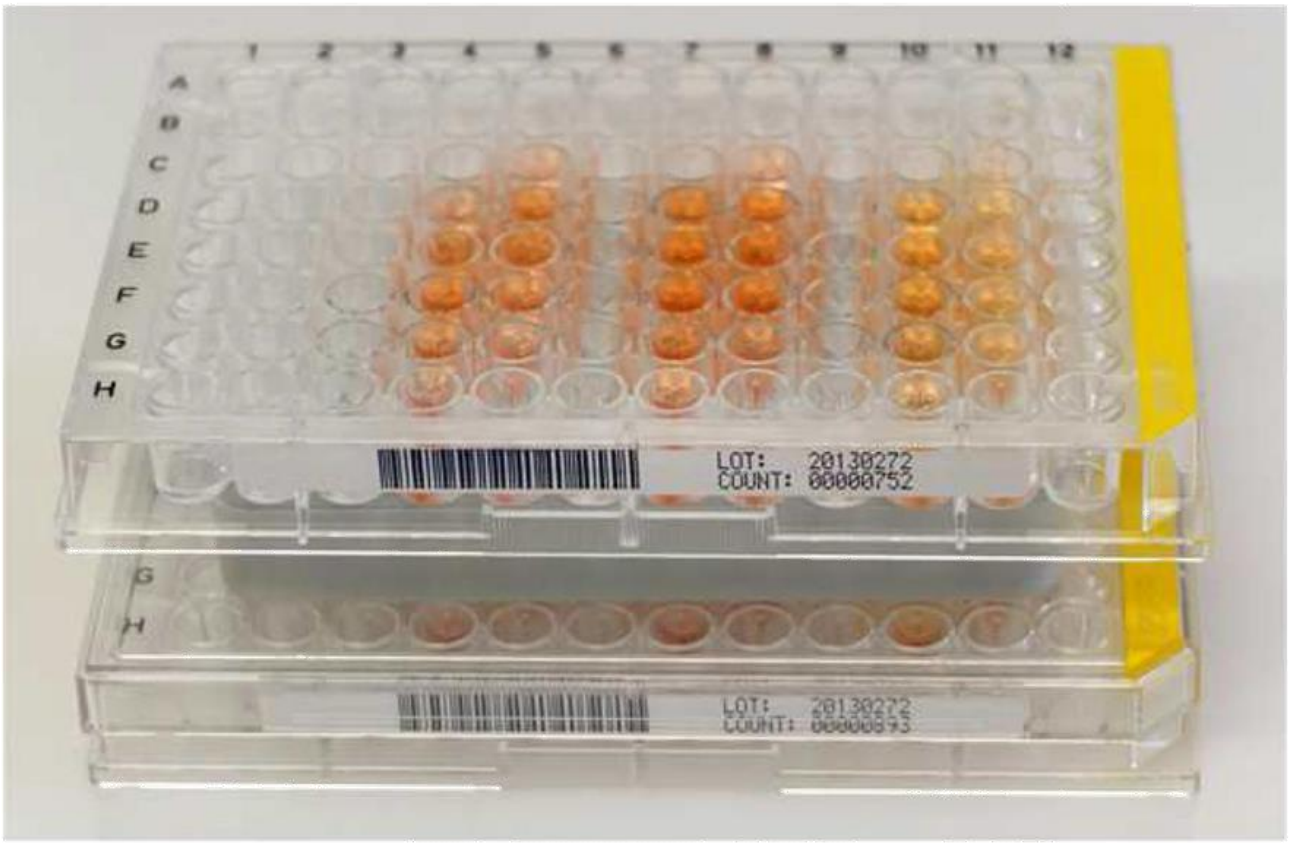

图 1：分别置于迷你瑞欧内克斯装置上下方的 96 孔盘的放置方式，装置上的文字朝上朝前。 多孔盘维持同样摆置放入一维持在 37.2± 0.2 °C 的外部恒温箱中。图中下方显示植入且暴露的细胞培养孔中呈现因粒线体去氢酶活力与活动而裂解的四唑染（呈橙色孔）。

表 1：在接触迷你瑞欧内克斯装置 2 小时后，用结缔组织纤维母细胞（细胞株 L-929）获得的所有实验的单次测量值的介绍。实验结果以促进平均值± S.E.M. 显示。迷你瑞欧内克斯装置下方和上方的细胞之间的差值在统计学上并不显着（学生的 *t*-测试）。S.E.M. = 平均值标准误差。

实验＃1 - L-929 暴露 2 小时

样本	光密度单次测量值					平均值	±	S.E.M.	对比控制组之促进%	±	S.E.M.(%)
未处理控制组	142	124	173	121	109	134	±	11	0	±	8.3
迷你瑞欧内克斯上方盘	184	161	181	190	175	178	±	5	33.2	±	2.8
迷你瑞欧内克斯下方盘	195	228	168	135	187	183	±	15	36.5	±	8.4

实验# 2 - L-929 暴露 2 小时

样本	光密度单次测量值					平均值	±	S.E.M.	对比控制组之促进%	±	S.E.M.(%)
未处理控制组	110	123	119	118	107	115	±	3	0	±	2.6
迷你瑞欧内克斯上方盘	206	180	153	128	119	157	±	16	36.2	±	10.3
迷你瑞欧内克斯下方盘	136	163	159	182	174	163	±	8	41.1	±	4.8

实验# 3 - L-929 暴露 2 小时

样本	光密度单次测量值					平均值	±	S.E.M.	对比控制组之促进%	±	S.E.M.(%)
未处理控制组	167	128	179	164	143	156	±	9	0	±	5.8
迷你瑞欧内克斯上方盘	208	198	209	187	206	202	±	4	29.1	±	2.0
迷你瑞欧内克斯下方盘	188	201	254	158	174	195	±	16	24.8	±	8.4

结论

样本	V1-V3 促进%平均值	±	S.E.M.(%)
迷你瑞欧内克斯上方盘	32.8	±	2.1
迷你瑞欧内克斯下方盘	34.1	±	4.8

表 2：在接触迷你瑞欧内克斯装置 2 小时后，用分化为巨噬细胞的 HL-60 细胞获得的所有实验的单次测量值的介绍。实验结果以促进平均值± S.E.M. 显示。迷你瑞欧内克斯装置下方和上方的细胞之间的差值在统计学上并不显着（学生的 *t*-测试）。S.E.M. = 平均值标准误差。

实验 # 1 – HL-60 adh. 暴露 2 小時

样本	光密度单次测量值					平均值	±	S.E.M.	对比控制组之促进%	±	S.E.M.(%)
未处理控制组	96	123	178	145	131	135	±	13	0	±	10.0
迷你瑞欧内克斯上方盘	246	198	148	187	152	186	±	18	38.3	±	9.6
迷你瑞欧内克斯下方盘	166	206	211	165	175	185	±	10	37.1	±	5.4

实验# 2 – HL-60 adh. 暴露 2 小時

样本	光密度单次测量值					平均值	±	S.E.M.	对比控制组之促进%	±	S.E.M.(%)
未处理控制组	110	123	119	118	107	115	±	3	0	±	2.6
迷你瑞欧内克斯上方盘	180	166	205	132	145	166	±	13	43.5	±	7.8
迷你瑞欧内克斯下方盘	176	144	161	200	177	172	±	9	48.7	±	5.4

实验# 3 – HL-60 adh. 暴露 2 小時

样本	光密度单次测量值					平均值	±	S.E.M.	对比控制组之促进%	±	S.E.M.(%)
未处理控制组	136	147	159	133	154	146	±	5	0	±	3.4
迷你瑞欧内克斯上方盘	181	177	189	203	206	191	±	6	31.1	±	3.0
迷你瑞欧内克斯下方盘	166	213	223	169	175	189	±	12	29.8	±	6.3

结论

样本	V1-V3 促进%平均值	± S.E.M.(%)
迷你瑞欧内克斯上方盘	37.7	± 3.6
迷你瑞欧内克斯下方盘	38.5	± 5.5

表 3：在接触迷你瑞欧内克斯装置 24 小时后，用结缔组织纤维母细胞（细胞株 L-929）获得的所有实验的单次测量值的介绍。实验结果以促进平均值± S.E.M. 显示。迷你瑞欧内克斯装置下方和上方的细胞之间的差值在统计学上并不显着（学生的 *t*-测试）。S.E.M. = 平均值标准误差。

实验 # 1 - L-929 暴露 24 小時

样本	光密度单次测量值					平均值	±	S.E.M.	对比控制组之促进%	±	S.E.M.(%)
未处理控制组	92	155	173	98	112	126	±	16	0	±	12.8
迷你瑞欧内克斯上方盘	219	136	153	192	132	166	±	17	32.1	±	10.2
迷你瑞欧内克斯下方盘	143	219	275	182	144	193	±	25	52.9	±	12.9

实验# 2 - L-929 暴露 24 小時

样本	光密度单次测量值					平均值	±	S.E.M.	对比控制组之促进%	±	S.E.M.(%)
未处理控制组	89	90	120	139	98	107	±	10	0	±	9.1
迷你瑞欧内克斯上方盘	137	171	203	184	127	164	±	14	53.4	±	8.7
迷你瑞欧内克斯下方盘	208	198	125	130	98	152	±	22	41.6	±	14.3

实验# 3 - L-929 暴露 24 小時

样本	光密度单次测量值					平均值	±	S.E.M.	对比控制组之促进%	±	S.E.M.(%)
未处理控制组	160	133	148	161	165	153	±	6	0	±	3.8
迷你瑞欧内克斯上方盘	202	197	198	189	241	205	±	9	33.9	±	4.5
迷你瑞欧内克斯下方盘	211	248	203	171	314	229	±	24	49.5	±	10.7

实验# 4 - L-929 暴露 24 小時

样本	光密度单次测量值					平均值	±	S.E.M.	对比控制组之促进%	±	S.E.M.(%)
未处理控制组	244	177	235	322	244	244	±	23	0	±	9.4
迷你瑞欧内克斯上方盘	324	338	298	294	303	311	±	8	27.4	±	2.7
迷你瑞欧内克斯下方盘	316	246	336	369	435	340	±	31	39.3	±	9.1

结论

样本	V1-V4 促进%平均值	±	S.E.M.(%)
迷你瑞欧内克斯上方盘	36.7	±	5.7
迷你瑞欧内克斯下方盘	45.8	±	3.2

表 4：在接触迷你瑞欧内克斯装置 24 小时后，用分化为巨噬细胞的 HL-60 细胞获得的所有实验的单次测量值的介绍。实验结果以促进平均值± S.E.M. 显示。迷你瑞欧内克斯装置下方和上方的细胞之间的差值在统计学上并不显着（学生的 *t*-测试）。S.E.M. = 平均值标准误差。

实验 # 1 – HL-60 adh. 暴露 24 小时

样本	光密度单次测量值					平均值	±	S.E.M.	对比控制组之促进%	±	S.E.M.(%)
未处理控制组	89	90	120	139	98	107	±	10	0	±	9.1
迷你瑞欧内克斯上方盘	114	182	155	135	198	157	±	15	46.3	±	9.7
迷你瑞欧内克斯下方盘	178	168	159	162	150	163	±	5	52.4	±	2.9

实验# 2 – HL-60 adh. 暴露 24 小时

样本	光密度单次测量值					平均值	±	S.E.M.	对比控制组之促进%	±	S.E.M.(%)
未处理控制组	184	133	149	136	129	146	±	10	0	±	6.9
迷你瑞欧内克斯上方盘	203	256	207	220	229	223	±	9	52.5	±	4.2
迷你瑞欧内克斯下方盘	196	248	212	165	208	206	±	13	40.8	±	6.5

实验# 3 – HL-60 adh. 暴露 24 小时

样本	光密度单次测量值					平均值	±	S.E.M.	对比控制组之促进%	±	S.E.M.(%)
未处理控制组	124	103	136	137	158	132	±	9	0	±	6.8
迷你瑞欧内克斯上方盘	177	214	207	180	128	181	±	15	37.7	±	8.4
迷你瑞欧内克斯下方盘	170	156	168	189	178	172	±	5	30.9	±	3.2

实验# 4 – HL-60 adh. 暴露 24 小时

样本	光密度单次测量值					平均值	±	S.E.M.	对比控制组之促进%	±	S.E.M.(%)
未处理控制组	194	178	265	287	339	253	±	30	0	±	11.8
迷你瑞欧内克斯上方盘	255	475	356	447	253	357	±	46	41.4	±	13.0
迷你瑞欧内克斯下方盘	330	377	280	345	406	348	±	21	37.6	±	6.2

结论

样本	V1-V4 促进%平均值	±	S.E.M.(%)
迷你瑞欧内克斯上方盘	44.5	±	3.2
迷你瑞欧内克斯下方盘	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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2014
PRODUKT ZELLBIOLOGISCH GEPRÜFT

Prof. Dr. Peter C. Dartsch
Diplom-Biochemist

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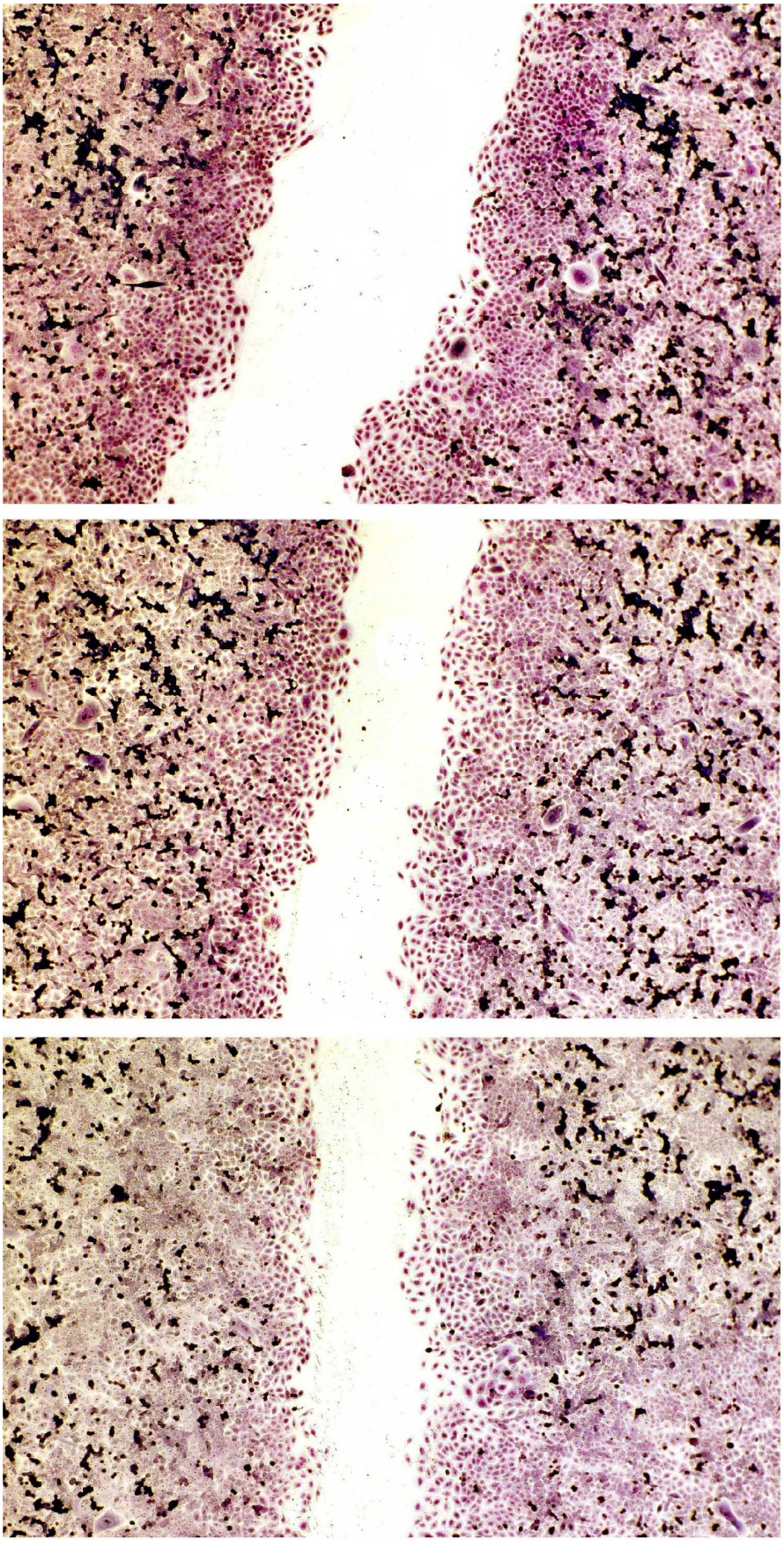

Figure 1: Micrographs of stained cell cultures using bright field after 3 days of wound healing. The cell-free area in which the cells have migrated and proliferated is good to be seen in all three micrographs. However, the wound gap is more prominent in the picture at the top. In addition, the loose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

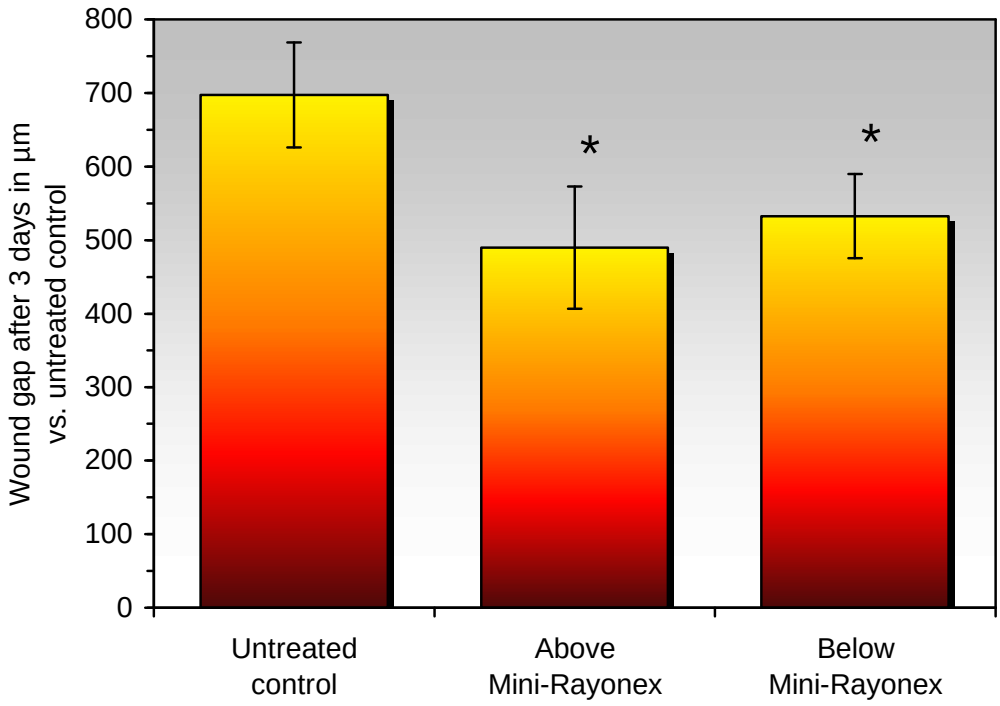

Figure 2: Presentation of measurement data in tabular and graphical form. The data show the results after 3 days of wound healing for untreated multiplates and multiplates which have been placed above and below the Mini-Rayonex device. Data represent mean value ± standard error of the mean (S.E.M.). 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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09.04.2014

– 实验报告与专业信息 –

迷你瑞欧内克斯

培养结缔组织纤维母细胞对于伤口愈合过程之促进的*体外*研究

研究的背景与问题

伤口愈合的阶段分为止血、发炎、增生或造粒以及重塑或成熟。这里使用的测试系统是刺激造粒阶段，其特征是主要结缔组织纤维母细胞的转移和增生，以闭合伤口间隙。
到目前为止，全世界已有许多用户感受到迷你瑞欧内克斯的正面共振。本*体外*研究是检验使用迷你瑞欧内克斯装置是否也有利于刺激结缔组织成纤维母细胞的转移和增生，从而更快地缩小伤口间隙。
这种刮伤愈合试验已被研究人员广泛调整和修改，以研究各种实验条件对细胞转移和增生的影响。其基本原则是细胞单层中的伤口间隙（=无细胞空间）随后朝间隙的中心闭合。

实验设计与数据分析

本研究采用老鼠结缔组织纤维母细胞（L-929 细胞株，ACC 173， P132 继代），根据 EN ISO 10993-5 国际标准规定，它们通常是为了试验医疗设备的生物兼容性而采用的。细胞株采购自位于 D-38124 布劳恩施魏格市的莱布尼兹研究所－德国微生物及细胞培养物采集有限公司（Leibniz-Institut DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH，D-38124 Braunschweig）。这些细胞在 37 °C、5% 二氧化碳及 95% 空气的潮湿大气的恒温箱内

大量培养。培养基为 RPMI 1640 辅以 5% 胎牛血清、100 单位/毫升青霉素和 100 微克/毫升链霉素。所有的细胞培养试剂皆来自位于 D-35091 科尔贝市的 GE 医疗保健生命科学公司（GE Healthcare Life Sciences， Cölbe）。

在实验中，细胞从 80~90%的融合大量培养物中抽取，并以 50,000 个细胞／孔（2 毫升培养基／孔）的密度分别植入 12 孔盘中。这些细胞在恒温箱中培养三天直到呈现融合状态。然后使用 5,000 微升吸管的尖端，轻轻刮取孔内中心的细胞单层。当刮取孔内表面时，吸管尖端的长轴始终垂直于孔内底部。由此产生的伤口间隙（=无细胞空间）的距离为 1,400 微米。先从一个方向刮取一条直线，另一条刮取直线则与第一条垂直，在每个孔中形成一个十字型。刮取完成后，使用含钙和镁的磷酸盐缓冲生理食盐水冲洗孔洞，以清除分离的细胞。之后再以新鲜的 pH 稳定培养基（2 毫升／孔）补充孔洞，其中一部分为 RPMI 1640，一部分为含钙、镁、5 mM 葡萄糖、5%胎牛血清、100 单位／毫升青霉素、100 微克／毫升链霉素和 15 mM HEPES 缓冲液的磷酸盐缓冲生理食盐水。

为了避免培养孔在三天的培养期间干掉，这些多孔盘以胶带密封，然后移至一特殊设计，可维持温度稳定在 37.2± 0.2 °C 的外部恒温箱中。恒温箱分别放置在至少距离 4 米的不同房间内，以避免未处理的控制组受到迷你瑞欧内克斯生物共振的影响。控制组的多孔盘直接放置在外部恒温箱底部，而另一个外部恒温箱中则分别在装置的上下方各放一个多孔盘，使培养孔暴露在迷你瑞欧内克斯的生物共振中。使用前已将迷你瑞欧内克斯以自来水冲洗，并且在恒温箱中以东西向摆置，装置上的文字朝上朝前。

经过连续三天暴露在迷你瑞欧内克斯装置的生物共振之下，或是无任何生物共振接触（未处理控制组）后，将细胞依照吉姆萨染色法（Romanowsky-Giemsa）进行固定与染色，结果产生了蓝紫色的细胞质与红色的细胞核。

每个孔内取两个不同的位置，以显微照相测量其间隙距离。评估以表格和图形形式进行。以 P 值 ≤0.01（学生的 *t*-测试）来计算暴露样本与未处理控制组之间的统计学意义。

结果与结论

如图 1 所示，结缔组织纤维母细胞在 3 天的培养期间在无细胞空间中转移和增生。在未处理控制组中，伤口间隙从 1,400 微米愈合至约 700 微米（图 2）。迷你瑞欧内克斯装置的生物共振刺激了放置于其上方及下方的培养物中的伤口愈合过程。因此，伤口间隙显着分别缩小至 490 微米或 549 微米（图 2）。当计算促进率百分比时，相较于未处理控制组，迷你瑞欧

内克斯的促进效果大约为 20%。对比未处理控制组，此促进率是具有统计学意义的（$p \leq 0.01$; 学生的 *t*-测试）。装置上方和下方的多孔盘之间并无显着差距。

综上所述，本结缔组织纤维母细胞的*体外*研究证实了迷你瑞欧内克斯装置的正面效果，正如世界各地众多用户所描述的那样。大约 20% 的伤口愈合促进率是非常令人印象深刻的，因为这个过程包括促进细胞转移和增生。因此，可以推荐使用迷你瑞欧内克斯装置作为诱导和刺激伤口愈合过程的应用。

研究者应为呈现出的实验和结果的正确性负责。

尚高镇 – 09.04.2014

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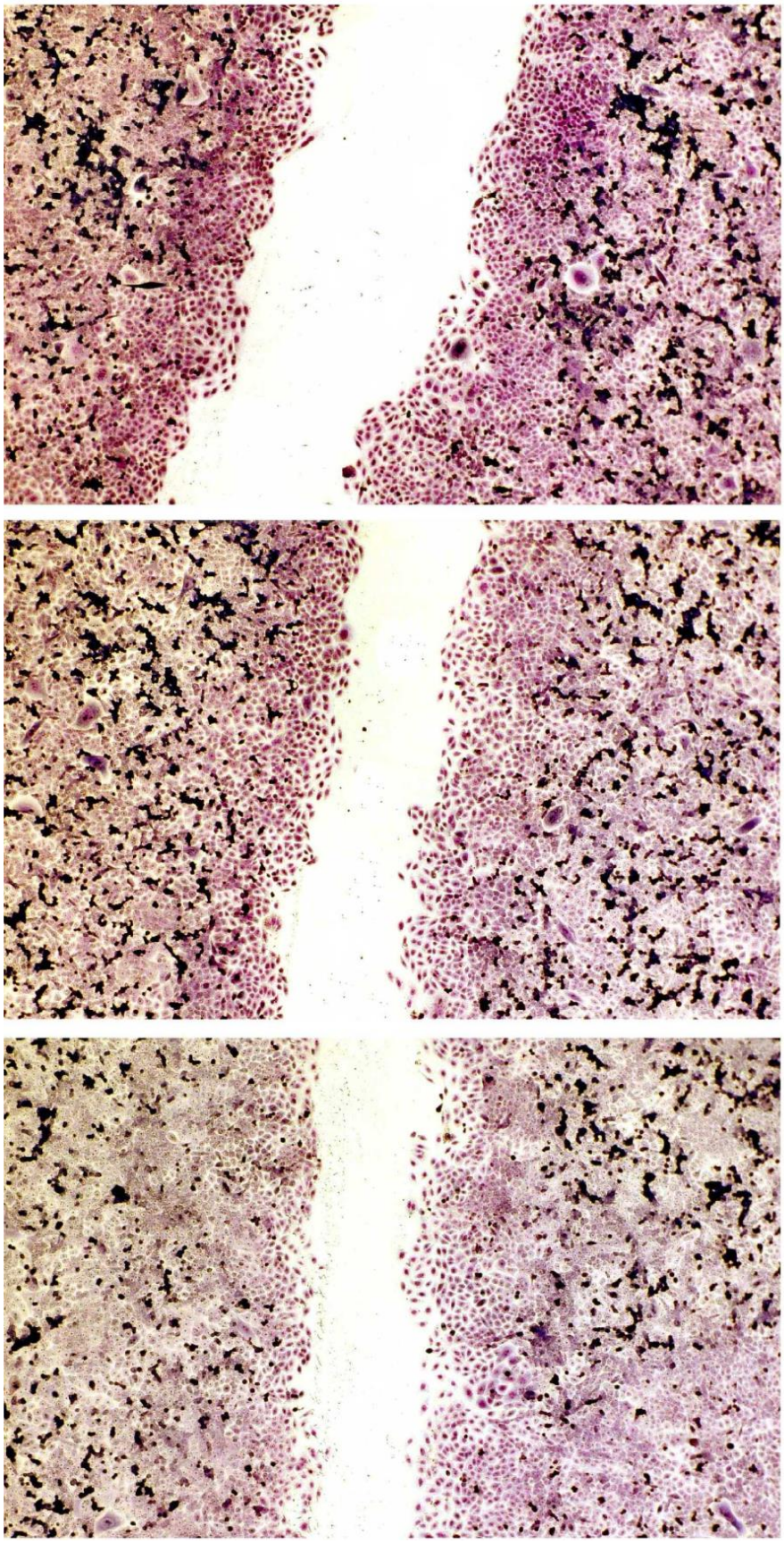

图 1：伤口经过三天愈合后，使用明亮场的染色细胞培养物的显微图。三张显微图内皆可清楚看到，无细胞区域旁的细胞皆有转移与增生，但是上图中的伤口间隙较为突出。此外，更能清楚分辨出由于细胞转移导致伤口边缘处排列松散，而离伤口间隙较远的地方则是排列紧密被细胞外基质覆盖的细胞层。
上图：未处理控制组的伤口愈合
中图：迷你瑞欧内克斯上方盘的伤口愈合
下图：迷你瑞欧内克斯下方盘的伤口愈合

样本	三天后的伤口间隙 单次测量值 (微米)				平均值 (n = 3)	S.E.M. (n = 3)
未处理控制组	591	682	819	573		
	591	727	764	682	697	71
	545	672	736	986		
迷你瑞欧内克斯上方多孔盘	709	682	467	610		
	473	564	362	228	490	83
	482	364	364	571		
迷你瑞欧内克斯下方多孔盘	410	500	672	546		
	400	510	474	624	532	57
	476	541	506	729		

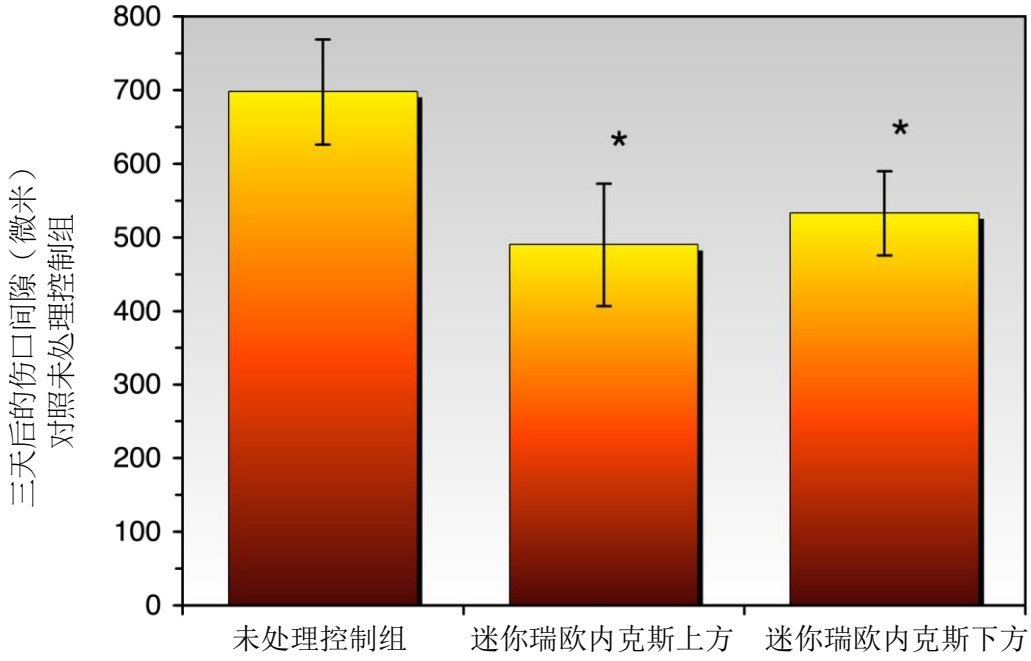

图 2：以表格和图形形式表示测量数据。数据显示伤口经过 3 天愈合后，未处理控制组与迷你瑞欧内克斯装置上下方多孔盘个别的结果。数据为平均值 ± 平均值标准误差（ S.E.M.）。具有统计学意义的促进伤口愈合过程（= 较小的伤口间隙距离）以箭头标记（$p \leq 0.01$; 学生的 *t*-测试）。

DARTSCH SCIENTIFIC GMBH

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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