Applied Cell Biology

ISSN : 2320-1991 Online
ISSN : 2320-1983 Print
Volume 10 Issue 2
DOI: https://dx.doi.org/10.53043/2320-1991.acb90026

Research Article

Appl Cell Biol, 10(2), 2022 [57-62]

Protective Effect of Qi-Shield on the Regeneration of Cultured Connective Tissue Fibroblasts after Mobile Phone Radiation – A Study Using Continuous Monitoring by Video Micrography

Peter C. Dartsch*

Dartsch Scientific GmbH, Institute for Cell Biological Test Systems, Auf der Vosshardt 25, D-49419 Wagenfeld, Germany

***Corresponding author:** Peter C. Dartsch, Dartsch Scientific GmbH, Institute for Cell Biological Test Systems, Auf der Vosshardt 25, D-49419 Wagenfeld, Germany, E-mail: pc.dartsch@dartsch-scientific.com

ABSTRACT

Background: Mobile phones are high performance devices that consume a lot of energy and also generate heat in form of microwaves. This heat is mainly created by the high frequency electromagnetic fields that transmit voice or data in mobile communications. Moreover, mobile phones also possess non-thermal radiation which has been shown to induce oxidative stress and molecular and cellular damage.

Material and methods: In this study not only the dynamic process of cellular damage following oxidative stress from the radiation of an actively transmitting mobile phone was investigated, but also a device named Qi-Shield, which claims to protect from the adverse effects of a mobile phone & WLAN radiation at realistic conditions. The regeneration process of cultured connective tissue cells (cell line L-929) which results in the closure of an artificial cell-free area by cell proliferation and migration, was used for continuous video micrography evaluation for more than 60 hours. Three different experimental approaches were conducted: (1) Regeneration of untreated control cells; (2) Regeneration of unprotected cells after 4 hours of mobile phone radiation; (3) Regeneration of Qi-Shield protected cells after 4 hours of mobile phone radiation. For definite time points during the regeneration process the residual cell-free area was examined by a specialized software with artificial intelligence. Four independent experiments over a period of four weeks were conducted.

Results and conclusions: A quite consistent cell reaction for the three different experimental approaches was observed in all experiments. The unexposed control cells showed a nearly linear reduction of the cell-free area with a time point of closure at 50 hours. The unprotected cells after mobile phone exposure also showed a roughly linear reduction of the cell-free area, but its magnitude was significantly lower and did not result in any closure of the cell-free area. The cells protected by Qi-Shield behaved very close to the unexposed cells, although a closure of the cell-free area within 60 hours of regeneration was only observed in two of four experiments. When calculating a relation between the values at 50 hours of regeneration by setting the value for the reduction of unexposed control cells as 100 %, a reduction value of 22 % was obtained for the unprotected cells, but 82.7 % for the Qi-Shield protected cells. Therefore, Qi-Shield was able to protect cells from the adverse effects of mobile phone & WLAN radiation by more than 82 %. The results clearly demonstrate the different dynamic reactions of connective tissue cells after mobile phone radiation and the successful use of Qi-Shield to reduce adverse cellular effects.

Keywords

Mobile phone radiation
Oxidative stress
Fibroblast
L-929
Cell regeneration
Cell culture

Research Article

INTRODUCTION

Mobile phones are high performance devices that consume a lot of energy and also generate heat due to microwaves transmitted by the handset [1]. This heat is mainly created by the high frequency electromagnetic fields that transmit voice or data in mobile communications [2,3]. Moreover, mobile phone radiatiion can also cause non-thermal biological effects which have been shown to induce oxidative stress and cell damage [4-8]. The components of mobile phones also become hot during operation, due to the heating of the battery, processor, screen and radiofrequency broadcasting compnonents.

The skin is the largest organ system of the body. The integrity of a healthy skin plays a crucial role in maintaining physiological homeostasis of the human body and its protection against numerous exogenous traumata [9]. Connective tissue is one of the many basic types of animal tissue and is found between other tissues in the body. Especially dermal connective tissue connects all distinct cells and tissues of the skin to a functional organ [10]. Fibroblasts constitute the principal component of the connective tissue.

Regeneration of complex structures such as the skin after injury requires dramatic changes in cellular behaviour. Regenerating tissues initiate a program that includes diverse processes such as wound healing, cell death and dedifferentiation. Moreover, newly regenerated tissues must integrate into preexisting cellular structures [11-13].

We used a bioassay which permits the evaluation of the effect of oxidative stress resulting from the radiation of an actively transmitting mobile phone by evaluating the regeneration process of connective tissue fibroblasts. The experimental setup was adapted to a realistic situation which has been shown to be more meaningful than a controlled situation at laboratory conditions using “test” generators with invariant parameters such as intensity, frequency etc. [14].

In addition, we also studied the question whether the Qi-Shield device might be able to protect the cell regeneration process from oxidative stress due to mobile phone radiation. According to the manufacturer, Waveguard GmbH, D-02625 Bautzen, Germany, “human beings and the environment are always in direct frequency exchange. Every body needs natural waves to stay in balance. It is the unnatural frequencies, on the other hand, that disturb this harmony … We have also developed Qi technology, which protects against electrosmog with natural frequencies”.

MATERIAL AND METHODS

Qi-Shield device

Qi-Shield was kindly provided by Waveguard GmbH, D-02625 Bautzen, Germany, for the duration of the experiments.

Mobile phone

A current and commercially available mobile phone from a leading brand manufacturer with a SAR value of 0.76 W/kg was used. No distinction was made between thermal and non-thermal radiation, because both are also present in reality when making a call and have an impact on the human body and systemic health [15-17].

Cultivation of connective tissue fibroblasts

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 (Binder CB 150, Tuttlingen, Germany) 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. Cells were routinely cultivated as mass cultures and were regularly subcultured twice a week with fresh culture medium. For the four independent experiments, cells were taken within a time period of four weeks from 80-90 % confluent mass cultures.

Cell regeneration after mobile phone radiation

Cells were seeded at a density of 100,000 cells/ml into the four individual compartments of a silicone 4 well-culture insert (ibidi, Gräfelfing, Germany). The single compartments of the inserts are separated by a 500 µm thick silicone bar with an outer silicone frame of 700 µm. Due to the special adhesion area, each insert adheres firmly to the bottom of a culture dish and forms a distinct cell-free area (artificial wound), which the cells can colonize by migration and proliferation after removal of the silicone frame.

Upon reaching confluency within 48 hours after cell seeding into the inserts, the cells were exposed to the radiation of an actively transmitting mobile phone & WLAN with and without Qi-Shield. The Qi-Shield was placed in a distance of 50 cm to the incubator. The cell culture dishes were placed in separate mini-incubators without gassing. The actively transmitting mobile phone was placed for 4 hours at 37 °C below the culture dishes. To avoid any pH changes during exposure at normal air conditions in the mini-incubators, the routine culture medium was replaced by a mixture of Leibowitz L-15 and RPMI 1640 (2:1) with 5 % growth mixture, 0.5 % gentamycin and 20 mM HEPES buffer. Cells which were cultured in the same way in another mini-incubator without mobile phone radiation, served as corresponding controls. The controls were at least 20 meters away and several walls were between the exposed cells and the controls. After 4 hours of radiation, the silicone frames were carefully removed and several washings were performed

in order to eliminate any floating cells in the cell-free areas. Finally, fresh Leibowitz L-15/RPMI 1640 medium (see above) was added and the cultures were transfered to the incubator unit at the stage of the inverted microscope for continuous monitoring by video micrography for a minimum of 60 hours.

Continuous monitoring of cell regeneration by video micrography

During the regeneration process after mobile phone exposure ± Qi-Shield protection, fibroblasts were continuously monitored by an acA 1920-150uc USB 3.0 camera with the ON Semiconductor Python 2000 CMOS sensor (Basler AG, D-22926 Ahrensburg, Germany). By using the Pylon software (version 4.2.0.4240; Basler) one frame (= picture) per minute was recorded using a temperature-controlled glass incubator (ibidi, Gräfelfing, Germany) mounted on the stage of an Olympus IX 50 inverted microscope equipped with an Olympus Planachromate 10x lens. More than 4,000 single pictures for each single experiment were recorded. Thereafter, single pictures were mounted to time-lapse video clips, which were able to demonstrate the resulting effects dynamically (not shown here). In addition, at definite time points of cell regeneration, the micrographs were taken for the examination of the residual cell-free area in relation to regeneration time by a specialized software with artificial intelligence from KML Vision, Graz, Austria (IKOSA AI software). A total number of four independent experiments conducted over a period of several weeks were taken for this study.

RESULTS

As depicted in Figure 1A for unexposed control cells, the closure of the cell-free area by connective tissue fibroblasts was finished within 60 hours. In contrast, as shown in Figure 1B, mobile phone radiation caused a marked reduction in cell regenerative activity with a nearly complete inhibition of cell proliferation and migration. Moreover, numerous rounded and even detached cells with membrane damages representing dead cells were observed. The use of Qi-Shield resulted in a protection of the cells against reduction in cell regenerative activity after mobile phone radiation, i.e. connective tissue fibroblasts were still viable and proliferated and migrated only slightly slower than the control cells. As a matter of fact, there was a marginal cell-free area left after 60 hours (Figure 1C).

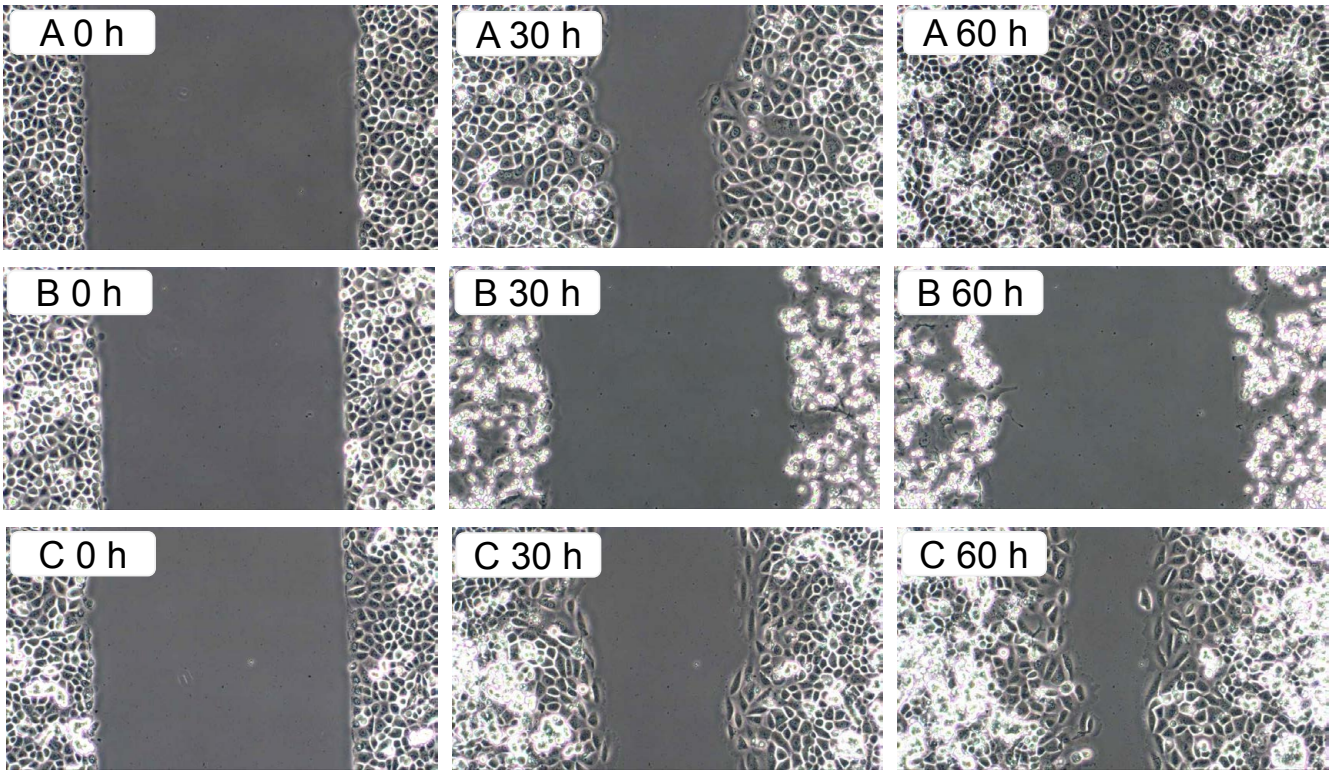

Figure 1: Original micrographs of cultured connective tissue fibroblasts closing a cell-free area within a time period of 60 hours after mobile phone & WLAN radiation. (A) Untreated control cells at the same culture conditions, but without any exposure to mobile phone radiation. (B) Unprotected cells after 4 hours of mobile phone radiation. (C) Qi-Shield protected cells after 4 hours of mobile phone radiation. Note the largely decreased colonization of the cell-free area as well as the rounding of the cells in (B). Untreated control and Qi-Shield protected cells show a very similar colonization pattern. Regeneration time points: 0 – 30 – 60 hours after mobile phone radiation.

Research Article

A quite consistent cell reaction for the three different experimental approaches was seen in all experiments (Figure 2). The unexposed control cells showed a nearly linear reduction of the cell-free area with a time point of closure at 50 hours. The unprotected cells after mobile phone exposure also showed a roughly linear reduction of the cell-free area, but its magnitude was significantly lower. The cells protected by Qi-Shield behaved very similar to the unexposed cells, although a closure of the cell-free area at 60 hours of regeneration was only observed in two of four experiments.

When summarizing all the data from the four independent experiments (Figure 3), it can be seen that the residual cell-free area of the unexposed control was reduced from 59.3 ± 4.4 % of total area to 0.2 ± 0.2 % (mean value ± standard deviation) after 50 hours. For the unprotected cells this reduction of the residual cell-free area was calculated to be from 56.4 ± 4.2 % to 43.4 ± 7.6 % (mean value ± standard deviation) after 50 hours, and for Qi-Shield protected cells the residual cell-free area was reduced from 59.4 ± 1.8 % to 10.6 ± 7.6 % (mean value ± standard deviation) after 50 hours. The reduction of cell-free area by the regeneration process was about 59 % for unexposed control, only 13 % for unprotected cells, but 48.8 % for Qi-Shield protected cells. The reduction between unexposed cells as well as Qi-Shield protected cells vs. unprotected cells was highly significant ($p \leq 0{,}01$; two-tailed Wilcoxon-Mann-Whitney test). When calculating a relation between the values at 50 hours of regeneration by setting the value for the reduction of unexposed control cells as 100 %, you get a value of 22 % for the unprotected cells and 82.7 % for the Qi-Shield protected cells. Therefore, Qi-Shield was able to protect cells from the adverse effects of mobile phone radiation & WLAN by more than 82 %.

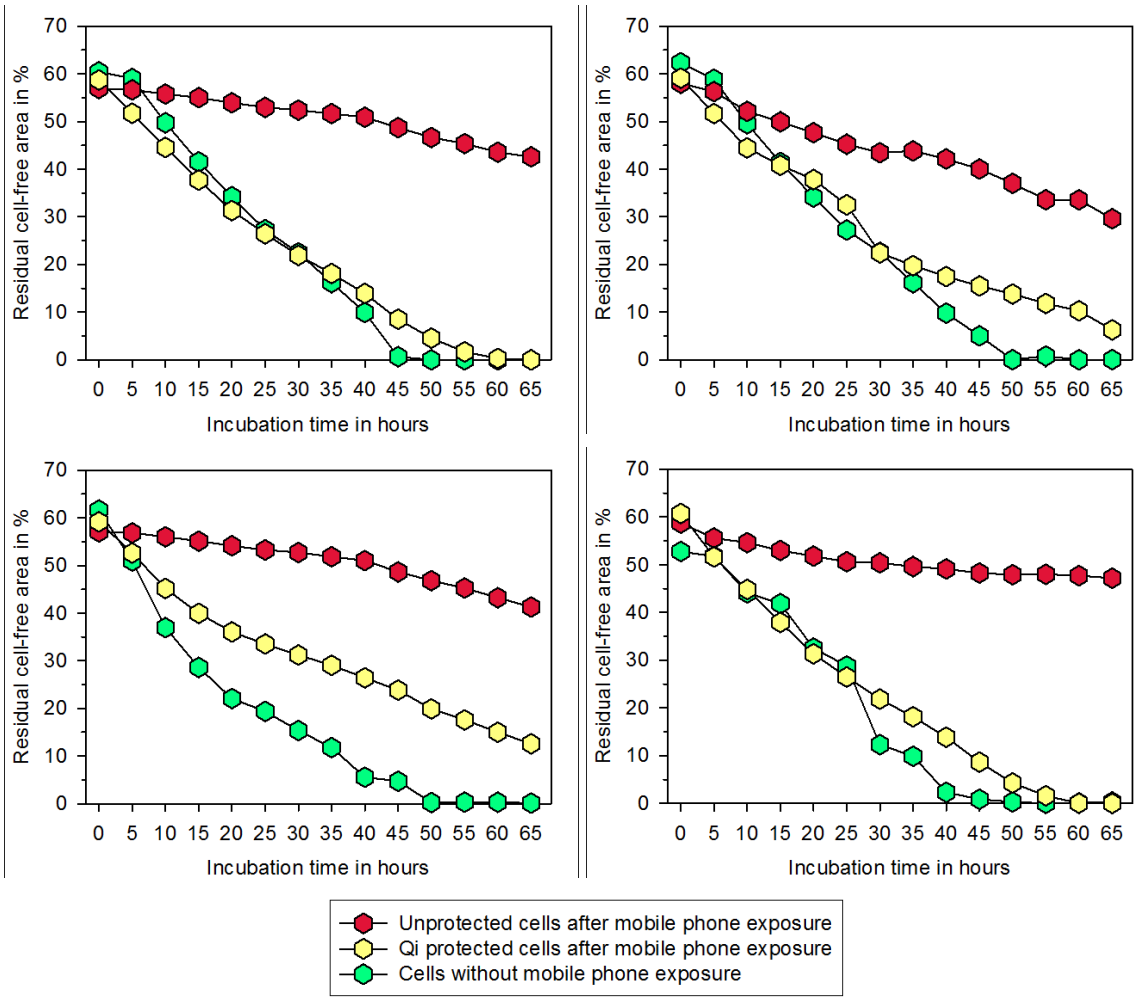

Figure 2: Presentation of the results of the four single independent experiments on cell regeneration after mobile phone & WLAN exposure with and without Qi-Shield by subsequent cultivation for 65 hours in comparison to untreated control cells. Note the consistency of the cell behaviour in all experiments which confirms the observed effects in Figure 1. The single independent experiments were conducted over a period of four weeks.

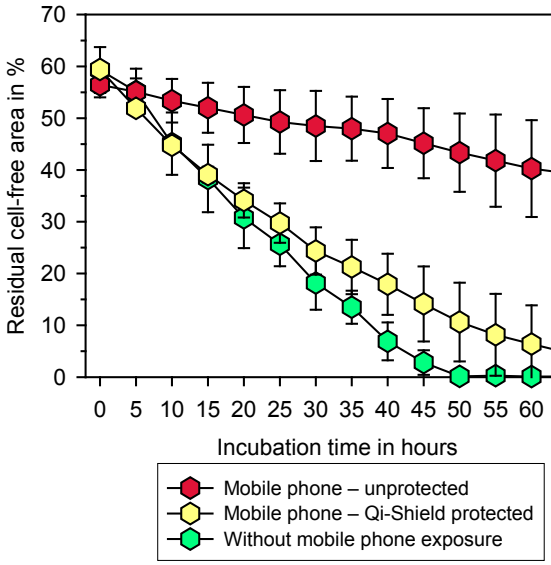

Figure 3: Summarized data of Figure 2 on cell regeneration after mobile phone & WLAN exposure with and without Qi-Shield by subsequent cultivation for 65 hours in comparison to untreated control cells. Note that the residual cell-free areas of untreated control cells and Qi-Shield protected cells differ only slightly from each other, whereas unprotected cells have a significantly higher cell-free area due to a reduced cell proliferative and migratory activity and even cell death. Data represent mean value ± standard deviation of four independent experiments.

DISCUSSION

The main problem of studies on whole multi cellular organisms such as experimental animals or volunteers is the complexity of the test systems. There are numerous unknown variables which are difficult to be established. In contrast, cultivation of eukaryotic cells can be standardized and provides the opportunity to vary different factors depending on the experimental needs. Therefore, cell culture studies which focus on one single aspect of cell behaviour such as the cell regeneration process are meaningful approaches to describe the effects of exogenous traumata such as mobile phone radiation and the resulting oxidative stress on the cells. Usually, methods by an end-point examination are used. To our knowledge, this is the first morphological study which sheds light on the dynamic cellular alterations during the regeneration process after mobile phone-induced oxidative stress.

There is another essential aspect which has been taken into account in the present study. It is the observation between the results of experimental studies that represent realistic exposures from commercially available devices (i.e. mobile phones or other telecommunication devices) and studies simulating exposures from "test" generators or "test" phones with similar parameters. While about 50 % of the studies using simulated exposures did not find any effects, studies using a realistic exposure from commercially available devices demonstrated an almost 100 % consistency in showing adverse effects [18-20].

In vivo, the cell regeneration/wound healing process can be divided into distinct phases: In-flammatory phase, granulation phase and differentiation phase [21-24]. In the present study, especially the granulation phase with an increased proliferation and migration of the cells in order to close the wound (= cell-free area) was investigated. Cultured connective tissue fibroblasts were used to examine the reduction in cell regenerative activity caused by mobile phone radiation & WLAN with and without the protection of Qi-Shield.

Although reactive oxygen species are generated as by-products of normal cellular metabolic activities which can be inactivated by endogenous enzymes such as superoxide dismutase, glutathione peroxidase and catalase, an excess of reactive oxygen species as generated by mobile phone radiation can be inactivated by these enzymes only insufficiently causing health consequences [25-27].

We were able to demonstrate that connective tissue cells are quite sensitive against oxidative stress induced by mobile phone radiation & WLAN which caused a nearly complete inhibition of cell proliferation and migration. In our study, the complete inhibition of cell activity became visible within a short period of time after radiation in the continuous video monitoring and succeded in a roughly linear way over time. This might be related to the prompt activation of intracellular signalling cascades [28] as well as to Ca^{2+} shifts [29,30].

However, the most interesting aspect of this study is the use of Qi-Shield to protect from oxidative stress resulting from mobile phone radiation. As demonstrated very consistently, Qi-Shield was able to protect the cells in all four experiments. Although there were variations from experiment to experiment, the summarized data for each time point gave a very convincing impression on the actual efficacy of the device. Except for single data points, there was no significant difference between the controls which have not been exposed to mobile phone radiation and the Qi-Shield protected cells after mobile phone radiation.

REFERENCES

1. Michaelson SM (1982) Health implications of exposure to radiofrequency/microwave energies. Br J Indust Med 39: 105-119.

2. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2013) Non-ionizing radiation Part 2: Radiofrequency electromagnetic fields. IARC Monogr Eval Carcinog Risks Hum 102: 1-460.

3. Kesari KK, Siddiqui MH, Meena R, Verma HN, Kumar S (2013) Cell phone exposure on brain and associated biological systems. Ind J Exp Biol 51: 187-200.
4. Funk RHW, Monsees TK (2006) Effects of electromagnetic fields on cells: Physiological and therapeutical approaches and molecular mechanisms of interaction. Cells Tissues Organs 182: 59-78.
5. Funk RHW, Monsees T, Özkucur N (2009) Electromagnetic effects - from cell biology to medicine. Progr Histochem Cytochem 43: 177-264.
6. Giuliani L, Soffritti M (2010) Non-thermal effects and mechanisms of interaction between electromagnetic fields and living matter. Eur J Oncol 2010: 5.
7. Yakymenko I, Sidorik E, Henschel D, Kyrylenko S (2014) Low intensity radiofrequency radiation: A new oxidant for living cells. Oxid Antioxid Med Sci 3: 1-3.
8. Yakymenko I, Tsybulin O, Sidorik E, Henschel D, Kyrylenko O, et al. (2016) Oxidative mechanisms of biological activity of low-intensity radiofrequency radiation. Electromagn Biol Med 35: 186-202.
9. Sorg H, Tilkorn DJ, Hager S, Hauser J, Mirastschijski U (2017) Skin wound healing: an update on the current knowledge and concepts. Eur Surg Res 58: 81-94.
10. Wlaschek M, Maity P, Makrantonaki E, Scharffetter-Kochanek K (2021) Connective tissue and fibroblast senescence in skin aging. J Investigat Dermatol 141: 985-992.
11. Witte M, Barbul A (1997) General principles of wound healing. Surg Clin North Am 77: 509-528.
12. King RS, Newmark PA (2012) The cell biology of regeneration. J Cell Biol 196: 553-562.
13. Carlson BM (2007) Principles of Regenerative Biology. Academic Press, Burlington, MA, USA.
14. Panagopoulos DJ (2019) Comparing DNA damage induced by mobile telephony and other types of man-made electromagnetic fields. Mut Res-Rev Mut Res 781: 53-62.
15. Gaestel M (2010) Biological monitoring of non-thermal effects of mobile phone radiation: recent approaches and challenges. Biol Rev 85: 489-500.
16. Belpomme D, Hardell L, Belyaev I, Burgio E, Carpenter DO (2018) Thermal and non-thermal health effects of low intensity non-ionizing radiation: An international perspective. Environm Poll 242: 643-658.
17. Zhao X, Dong G, Wang C (2021) The non-thermal biological effects and mechanisms of microwave exposure. Int J Radiat Res 19: 483-494.
18. Daroit NB, Visioli F, Magnusson AS, Vieira GR, Rados PV (2015) Cell phone radiation effects on cytogenetic abnormalities of oral mucosal cells. Braz Oral Res 29: 1-8.
19. Panagopoulos DJ (2017) Mobile telephony radiation effects on insect ovarian cells. The necessity for real exposures bioactivity assessment. The key role of polarization, and the "Ion Forced-Oscillation Mechanism". In: Geddes CD (ed.) Microwave Effects on DNA and Proteins, Springer, 2017.
20. Panagopoulos DJ, Johansson O, Carlo GL (2015) Real versus simulated mobile phone exposures in experimental studies. Biomed Res Int: 607053.
21. Singer AJ, Clark RA (1999) Cutaneous wound healing. N Engl J Med 341: 738-746.
22. Broughton II G, Janis JE, Attinger CE (2006) The basic science of wound healing. Plastic Reconstruct Surg 117: 12-34.
23. Wallace HA, Basehore BM, Zito PM (2019) Wound Healing Phases. In: StatPearls. Stat Pearls Publishing, Treasure Island (FL).
24. Cañedo-Dorantes L, Cañedo-Ayala M (2019) Skin acute wound healing: A comprehensive review. Int J Inflammation 2019: 3706315.
25. Balci M, Devrim E, Durak I (2007) Effects of mobile phones on oxidant/antioxidant balance in cornea and lens of rats. Curr Eye Res 32: 21-25.
26. Meral I, Mert H, Mert N, Deger Y, Yoruk I, et al. (2007) Effects of 900-MHz electromagnetic field emitted from cellular phone on brain oxidative stress and some vitamin levels of guinea pigs. Brain Res 1169: 120-124.
27. Oktem F, Ozguner F, Mollaoglu H, Koyu A, Uz E (2005). Oxidative damage in the kidney induced by 900-MHz-emitted mobile phone: protection by melatonin. Arch Med Res 36: 350-355.
28. Friedman J, Kraus S, Hauptman Y, Schiff Y, Seger R (2007) Mechanism of short-term ERK activation by electromagnetic fields at mobile phone frequencies. Biochem J 405: 559-568.
29. Nazıroğlu M, ÇIğ B, Doğan S, Uğuz AC, Dilek S, et al. (2012) 2.45-Gz wireless devices induce oxidative stress and proliferation through cytosolic Ca^{2+} influx in human leukemia cancer cells. Int J Radiat Biol 88: 449-456.
30. Wood A, Karipidis K (2021) Radiofrequency fields and calcium movements into and out of cells. Radiat Res 195: 101-113.

Citation: Dartsch PC (2022) Protective Effect of Qi-Shield on the Regeneration of Cultured Connective Tissue Fibroblasts after Mobile Phone Radiation – A Study Using Continuous Monitoring by Video Micrography. Appl Cell Biol, 10(2), 2022 [57-62]