Application of a Static Magnetic Field as a Complementary Aid to Healing in an in Vitro Wound Model

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Application of a static magnetic field as a complementary aid to healing in an *in vitro* wound model

Objective: Static magnetic field (SMF) has long been used as a therapeutic means, though its effects on the activity of cells and the mechanism(s) involved remain unknown. The purpose of this study is to determine the effect of a moderate-intensity SMF on the activity, growth and migration of mouse embryonic fibroblast (NIH 3T3), aiming to mimic wound healing and to study it in real time.
Method: A cell-free area (a scratch with a 200–500µm width) was formed in NIH 3T3 cultured cells and used as a wound model. The effects of a SMF (10, 50, 80 and 100mT) on the survival rate (MTT assay), integrity of cell membranes (lactate dehydrogenase (LDH) assay), the morphology of the cell (circularity, number and length of filopodia), cell orientation, and migration (speed, direction, rate) were studied as a function of the incubation time in a time-lapse manner.
Results: The exposure of cells to SMF at all intensities had no cytotoxic effect, as revealed by the MTT assay. The integrity of the membranes of the SMF-treated cells studied by the LDH assay test showed no effects. The structure of the membrane at the leading edge of the cells changed and showed several filopodia extended parallel to the field direction. The exposure to the SMF elongated the cells and decreased their circularity at SMF 10mT. The migration of the cells from one edge of the gap towards the other was affected by the applied SMF. The maximum and minimum effects were monitored at 80mT and 10mT, respectively. Analysis of cell migration revealed an average directness of 0.73, 0.66, 0.78, 0.78 and 0.69 under SMF 10, 50, 80, 100mT and control, respectively.
Conclusion: The morphological and functional changes of the cells in the presence of SMF revealed particular effects on the membrane and cytoskeleton. Cells were affected by physicochemical changes caused by the applied SMF, though the extent of the incurred effects was not a linear function of the field intensity. This low cost, non-invasive approach can be used as a magneto-manipulative means to tailor a practical, independent or complementary means of manipulating the activities of cells and tissues for clinical purposes.
Declaration of interest: The authors have no conflicts of interest.

biophysics • migration • static magnetic field • survival • wound repair model

Clinical application of static magnetic field (SMF) has been used by physicians and scientists, yet the mechanism(s) involved are still to be identified.[1] Many studies have demonstrated the safe use of SMF for diagnostic and imaging purposes, for example in the magnetic resonance imaging (MRI) used by MRI machines.[2] The effects of exogenous magnetic fields on biological processes have also been considered for various clinical diagnostic and treatment purposes, including bone unification, pain relief, soft tissue oedema and wound healing.[1]

The biological effects of SMF differ in various cells, for example, long-term (64 hours) *in vitro* exposure of human tumour cells to SMF 7 Tesla (T) decreased their proliferation,[3] though no change was observed in human foetal lung fibroblast (HFLFs) cells when they were exposed to a SMF at up to 10T.[4] The consecutive five-day exposure of both synchronised and non-synchronised HFLF cells to a SMF at 0.2, 1.0 and 1.5T for one hour a day did not show any changes in their cell cycle.[5] However, exposure of ovarian carcinoma cells, (HTB77 IP3), melanoma (HTB 63) and lymphoma (CCL 86) to SMF 7T decreased their viability and proliferation.[3]

Occurrence of cell apoptosis in SMF-treated cells has been attributed to an incurred change in the intracellular calcium concentration, $[Ca^{2+}]_i$, and associated with the stimulation of apoptosis in S49 cell lymphoma. However, the increased concentration of $[Ca^{2+}]_i$ does not always correlate with the apoptosis in different cell types,[6] yet it lowered the extent of stress-induced apoptosis in human lymphocytes and U937 cells.[7]

Exposure to SMF changes the alignment of exposed cells; for example, erythrocytes position their disk planes parallel to the direction of the SMF,[8] while sickled erythrocytes position themselves perpendicular to the SMF direction when exposed to a SMF at 0.35T.[9] A high-intensity magnetic field of 14T affected the arrangement of smooth muscle cell assemblies by aligning their colonies with the direction of the magnetic flux.[10] At the molecular level, exposure of

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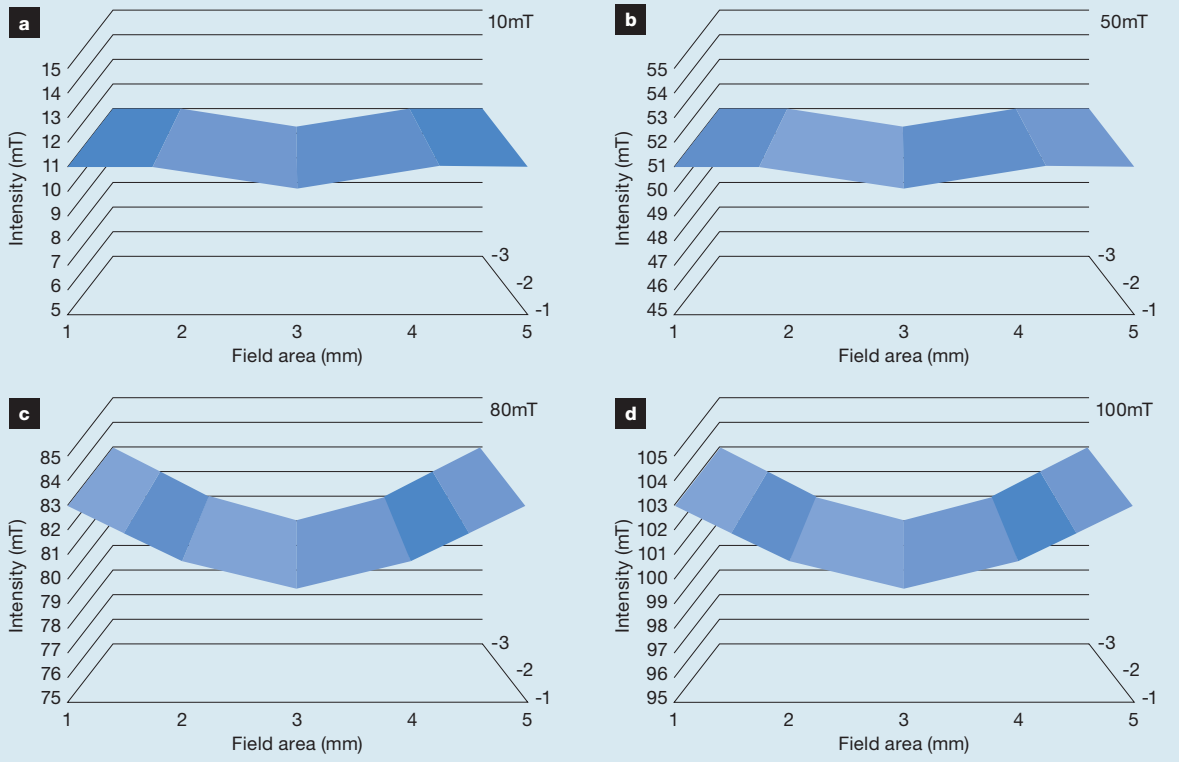
Fig 1. The profile of the static magnetic field (SMF) applied to the cell culture area. The magnetic field was measured at every millimetre in the vicinity of the culture wells in the presence of SMF 10mT (**a**), 50mT (**b**), 80mT (**c**) and 100mT (**d**). The scratch (200–500µm) was formed in the mid area (at the line crossing 3mm on X axis) where a homogeneous field with minimum deviation was applied



collagen macromolecules to SMF 1T positioned them perpendicular to the field direction,[11] and a SMF at 4.0 and 4.7T made human foreskin fibroblast cells grow according to the alignment of the collagen molecules.[12] The exposure of cells to SMF induces certain forces on the intracellular molecules, orients them in a certain direction and facilitates cell elongation, motility and migration.[13]

Wound healing is a complex process that involves various interactions between fibroblasts, endothelial cells and the extracellular matrix (ECM) constituent. The process can be categorised into five overlapping phases, including; homeostasis, inflammation,[14] migration and proliferation,[15] protein synthesis,[16] and wound contraction and remodelling,[17] which involve cellular, immunological, dynamics, metabolic and tissue alteration aspects.[14–17] Wound healing in patients with diabetes, and the high risk of developing a diabetic ulcer that can lead to lower limb amputations,[18] is a major challenge. However, certain physicochemical conditions can impair the wound healing process as well as cell signaling and motility[19] that could be considered for wound treatment in patients with diabetes. and approaches are being directed towards non-chemical, noninvasive treatments.[20]

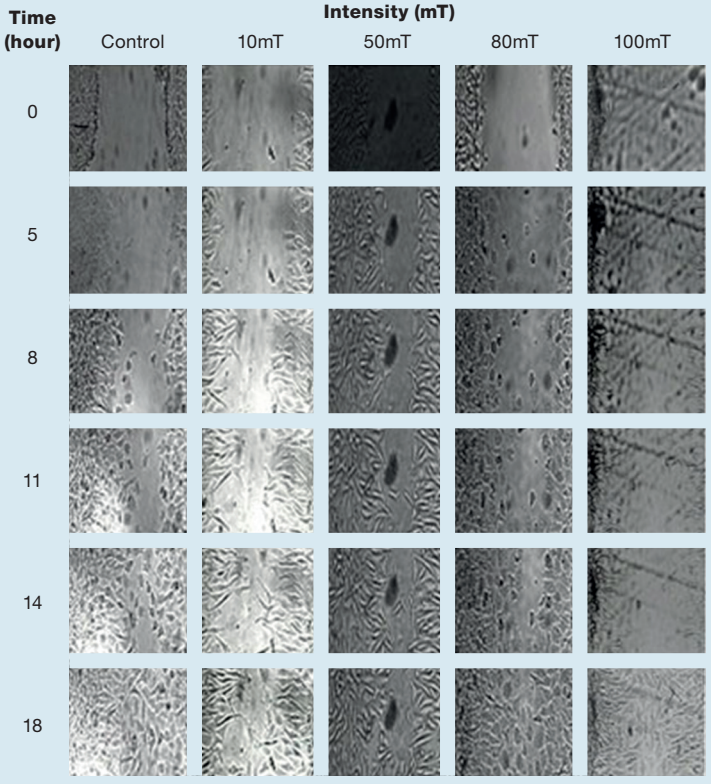
An essential part of the wound healing process *in vivo* is the proliferation process, which is initiated by the release of different metabolites and signal peptides including platelet-drived growth factor (PDGF), transforming growth factor-beta (TGF-b), epidermal growth factor (EGF), and fibronectin.[21] The responded fibroblast cells then migrate to the wound area, synthesise fibrin, fibronectin glycosaminoglycan, hyaluronic acid and other ECM constituents.[15 14]

Different approaches have been used *in vitro* to study wound healing at the tissue, cellular and molecular levels. Microfluidic chamber, cell exclusion zone, scratch wound model, and Boyden chamber and transfer assays through membranes have been widely applied to study cell migration.[22] The main aims of these approaches are real-time monitoring and recording of the morphology, activity and dynamics of cells in a time-lapse manner in the presence and absence of different influencing factors.[22]

Application of SMF is progressively considered as a noninvasive and effective therapy for different purposes, including acceleration of bone formation,[23] wound healing[24] pain relief, and control of soft tissue oedema.[1] Experimental animal studies have indicated that SMF is capable of improving tissue healing in both non-diabetic[25,26] and diabetic cases with no side-effects.[27]

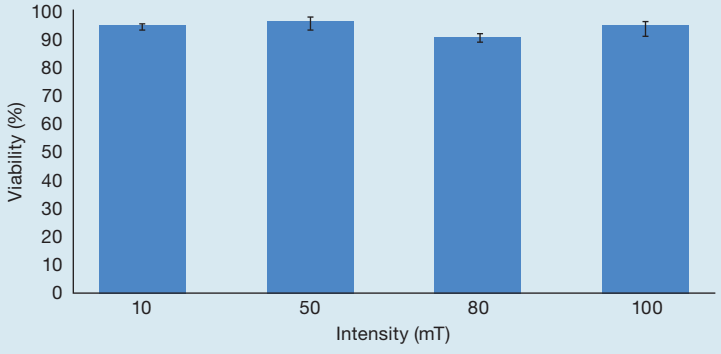
Accordingly, application of SMF can be considered as an alternative therapy to improving different aspects

Fig 2. Activity, morphology and motility of NIH 3T3 cells in the absence and presence of static magnetic field (SMF) at different intensities and time points. The cells showed a wider spectrum of morphological changes and motility rate, but similar viability and division in the presence of SMF at 10, 50, 80 and 100 mT intensities. The images were taken at five minute intervals by means of a time-lapse experiment and their representative appearance, recorded at 5, 8, 11, 14 and 18 hour intervals, are shown



involved in wound healing.[28] Exposure of cells to the SMF causes significant changes in the shape of cell and plasma membrane.[29] Chionna et al. reported a modified spherical shape of the suspended cells to a disordered elongated form, and some variations in the shape of attached cells that led to their suspension.[4] Furthermore, SMF causes certain modifications in the microvilli, blebs, structure of the membrane and reduction in the smoothness of the cell surface in a time-dependent manner.[30] The primary effects of SMFs have been attributed to the bioelectric properties of the plasma membrane,[31] as well as the reorientation of diamagnetic molecular domains of the membrane constituent molecules.[32] The manifested changes in the morphology and structure of the plasma membrane reveal significant deviation in the living status of the cells, cytoskeleton arrangement, phagocytosis capability, receptor distribution and ion flux.[33]

Fig 3. Effect of static magnetic field (SMF) on the viability of NIH 3T3 cells. Exposure of NIH 3T3 cells to SMF at 10, 50, 80 and 100mT for 30 hours caused no significant cytotoxic effect on the viability of the treated cells. Data are presented as means ± standard deviation, n=3–5



Aim

In this study, SMF with moderate-intensity was applied to cells to promote their migration and orientation, and to define an *in vitro* model of wound healing driven by a noninvasive, extrinsic-inducing force. The experiments aimed to reveal the effect of SMF as a non-chemical biophysical stimulant in mimicking the attracting effect of the chemotactic factors routinely released by the cells located at the either sides of wound during wound healing process. Accordingly, the study was conducted in a single cell line system with no supporting cells involved in the release of chemotactic factors, signal peptides and metabolites. Quantitative analysis of cell viability, morphology, membrane integrity, division, migration and confluency was conducted in a time-lapse manner to reveal real-time effects of the applied field on cell activities.

Materials and methods

Cell culture

Mouse embryonic fibroblast cells (NIH 3T3) were provided by the Pasteur Institute (Iran, Tehran). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with foetal calf serum (10% v/v), streptomycin (10,000µg/ml, 2% v/v) and penicillin (10,000U/ml, 2% v/v) (37°C, 5% CO_2). Cells were harvested after three passages and cultured for 30 hours to reach 90% confluency. Cell migration assays were performed in a scratch-model formed in the confluent cell cultures obtained from the passages three and seven.

In vitro wound model

A wound model was created by means of a single line scratch formed in a monolayer of confluent NIH 3T3 cells, using a tailored plastic needle (~300–400µm in diameter). Accordingly, a linear cell-free gap was formed and used to study the migration of cells and their morphological changes, survival rate, and division during the course of the occupation of the cell-free gap. The cells arrayed on each edge of the gap, divided and represented different morphologies during their migration across it. Different status of cells, including survival, morphology, dynamics, motility, area size, orientation and migration were monitored, recorded in a time-lapse manner, and analysed against time in the

presence and absence of SMF at different intensities using Juli Inverted Microscope (Fargene Pouyesh, Iran, Juli, NanoEnTek Inc., Korea). The incubation was carried out for 24–30 hours, the confluency of the cells reached after 14–18 hours, and analysis of different aspects of cell activities was conducted along the course of the experiment.

Considering the patterns of cell distribution across the scratch, gap length (Lg), gap width (Wg), and the gap area (Ag) was recorded at five-minute intervals, and measured and plotted as a function of time.[34] Accordingly, considering the initial gap area (Ag), the time required to reach a 50% confluency, T1/2, was derived based on Equation 1:

$$T1/2=Ag/2\times|slope|$$

The rate of cell migration (Vm) was obtained using Equation 2. The gap area was plotted versus time during cell migration and, using Microsoft Excel software, the results were fitted by Linear Trendline tool, where Vm=m.x+b ('m' and 'b' representing the slope and y-intercept of the line, respectively). Equation 2:

$$Vm=|slope|/2\times Lg$$

The activities and morphologies of the cells, direction and rate of migration, and the progress of healing in the gap area were recorded and analysed using ImageJ and Gradientech softwares. Accordingly, the area, orientation and the directness of cell migration in the gap area were studied quantitatively. The rate of wound closure for the control and SMF-treated cells was calculated by means of the exponential plot based on Equation 3 and use of Sigma Plot software:

$$f=ae(-b\times t)$$

where, 'f' represents the area of the wound, 'a' represents the initial gap area, 'b' the index of the wound closure, and 't' the time at which the corresponding data was obtained.

Fig 4. Effects of static magnetic field (SMF) on the integrity of the NIH 3T3 cell membranes. Exposure of cells to SMF at 10, 50, 80 and 100mT for 30 hours caused no damages to the integrity of cell membranes with respect to the control (cells not exposed to SMF) and thus, the release of lactate dehydrogenase (LDH) through the cell membranes of the treated cells remained constant. Data are shown as mean ± standard deviation, n=3–5

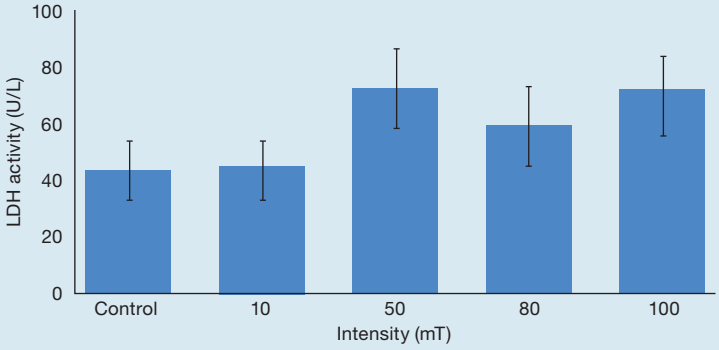
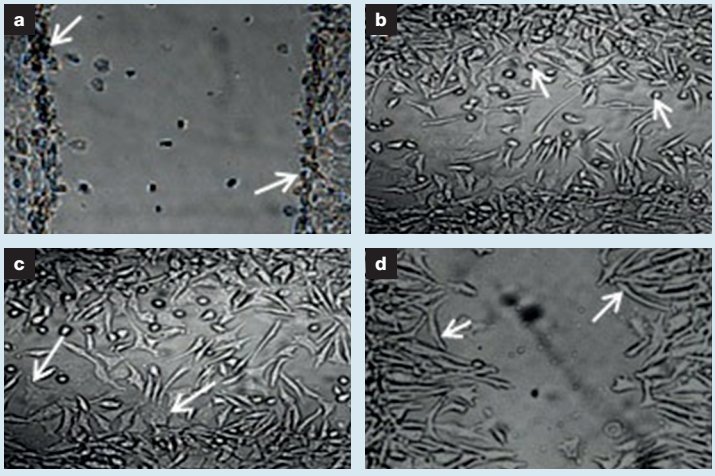


Fig 5. Different morphologies of NIH 3T3 migrating cells during the repair process. While the majority of cells in the absence of static magnetic field (SMF) possessed small circular (SC) (**a**, **b**) and spread out (SO) (**c**) morphologies in the absence of SMF, the dominant ones in the presence of SMF represented elongated (EL) morphology (**d**). Magnification x40



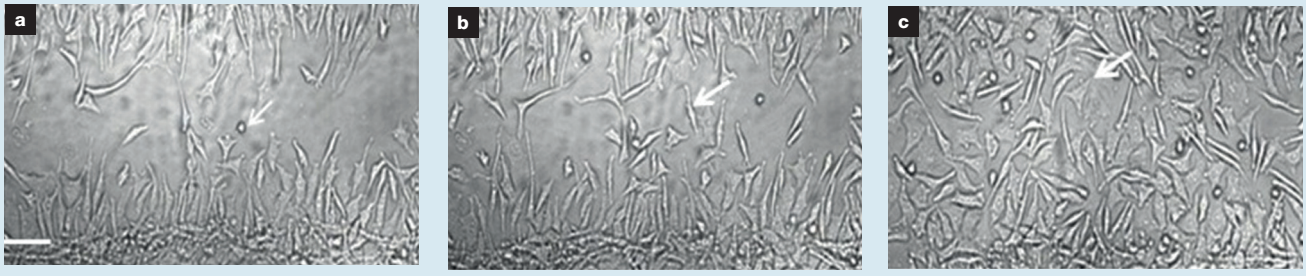
Application of SMF

SMFs with intensities of 10, 50, 80 and 100mT were applied by means of NdBrF neodymium permanent magnets. The SMF was applied in a chamber with specific spaces for the cell culture well and magnets. The intensity and homogeneity of the applied SMFs at different SMF-exposed locations and cell culture area in the well were assessed by a Gauss Meter (HIRST, GM08, UK) with a sensitivity of 1µT–1T. The cell culture well was positioned between the north and south poles of magnets so that cells grown at the bottom of the well, in particular those in the vicinity of the gap area, experience a homogeneous field (Fig 1).

Analysis of cell viability

The viability of cells was performed by MTT assay where yellow tetrazolium salt is used as a reagent to assess the survival and growth of cells. The MTT method is based on the restoration of the colour of dimethylthiazol diphenyl tetrazolium bromide to an insoluble formazan, violet blue, that is fulfilled by the activity of mitochondrial reductase enzyme in living cells.[35] The living cells normally absorb salt and form insoluble formazan purple crystals. The relevance between the number of viable cells and absorption for each cell is linear and provides a precise measure of changes in the rate of cell proliferation. The cells were incubated in the presence of MTT reagent (dimethylthiazol diphenyl tetrazolium bromide, 0.5mg/l, Sigma, US) for 3–5 hours at 37°C, and their absorption was measured at 570nm using HT Microplate reader Spectrophotometer (Bioteck, Power Wave XS2, US).

Fig 6. Transitional reversible morphologies shown by NIH 3T3 cells. Cells switched between different small circular (SC) (**a**), elongated (EL) (**b**) and spread out (SO) (**c**) morphologies (shown by arrows) during the course of experiment in the presence and absence of static magnetic field (SMF). Bar=100µm, x40 magnification



Effects of SMF on the area size of the NIH 3T3 cells

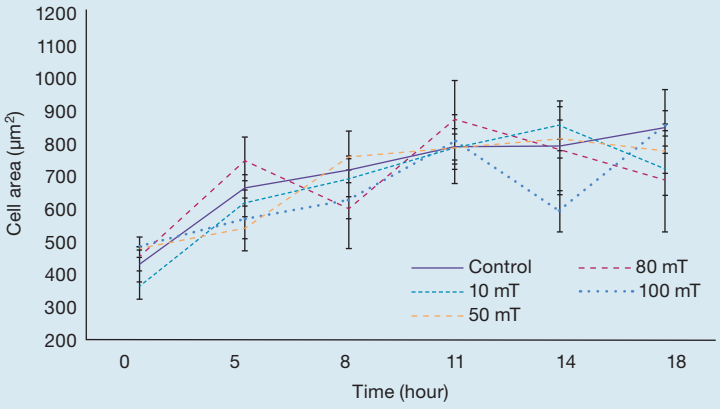
The changes in the area of cells (Ac) were monitored, recorded and analysed in the presence and absence of the SMF over time. ImageJ software was used to identify each cell as an object and then its area was measured in μm^2 during the course of the experiment. In some cases, the software was trained by the operator to correctly choose the edge of the cells and avoid inclusion of the artifacts (non-clear cell edges, due to the low intensity or overlaps, that had to be further evaluated and approved in order to have individual cells identified, thus, misjudgment of the software is avoided and the validity of the analysis increased). The average area of the cell at different stages of wound healing was analysed by ImageJ and Microsoft Excel and reported against time.

Effects of SMF on the circularity of the NIH 3T3 cells

The morphology of cells was recorded over the course of the experiment, analysed by ImageJ, and their roundness (circularity) was considered as one of the indices of cell shape. The circularity index was calculated by means of the ratio between the cell area and the squared perimeter of the cell.[36] The larger the index, the higher the circularity of the cell and, consequently, the lower the elongation. Accordingly, considering the perimeter of cells (Pc), the circularity value (Cv) increases as the cell elongated, as shown in Equation 4:

$$Cv=Pc2/4\times Ac\times\pi$$

Fig 7. The area of NIH 3T3 cells after 18-hour exposure to static magnetic field (SMF) at different intensities. The image analysis of SMF-treated cells (at 10, 50, 80 and 100 mT) was performed 5, 8, 11, 14 and 18 hours after wound creation and was compared with their initial status at zero hours and also to that of control. The changes in the cell area were not as stable as in the control group and fluctuated at different SMF intensities during the course of healing. The reported values represent the mean ± standard deviation



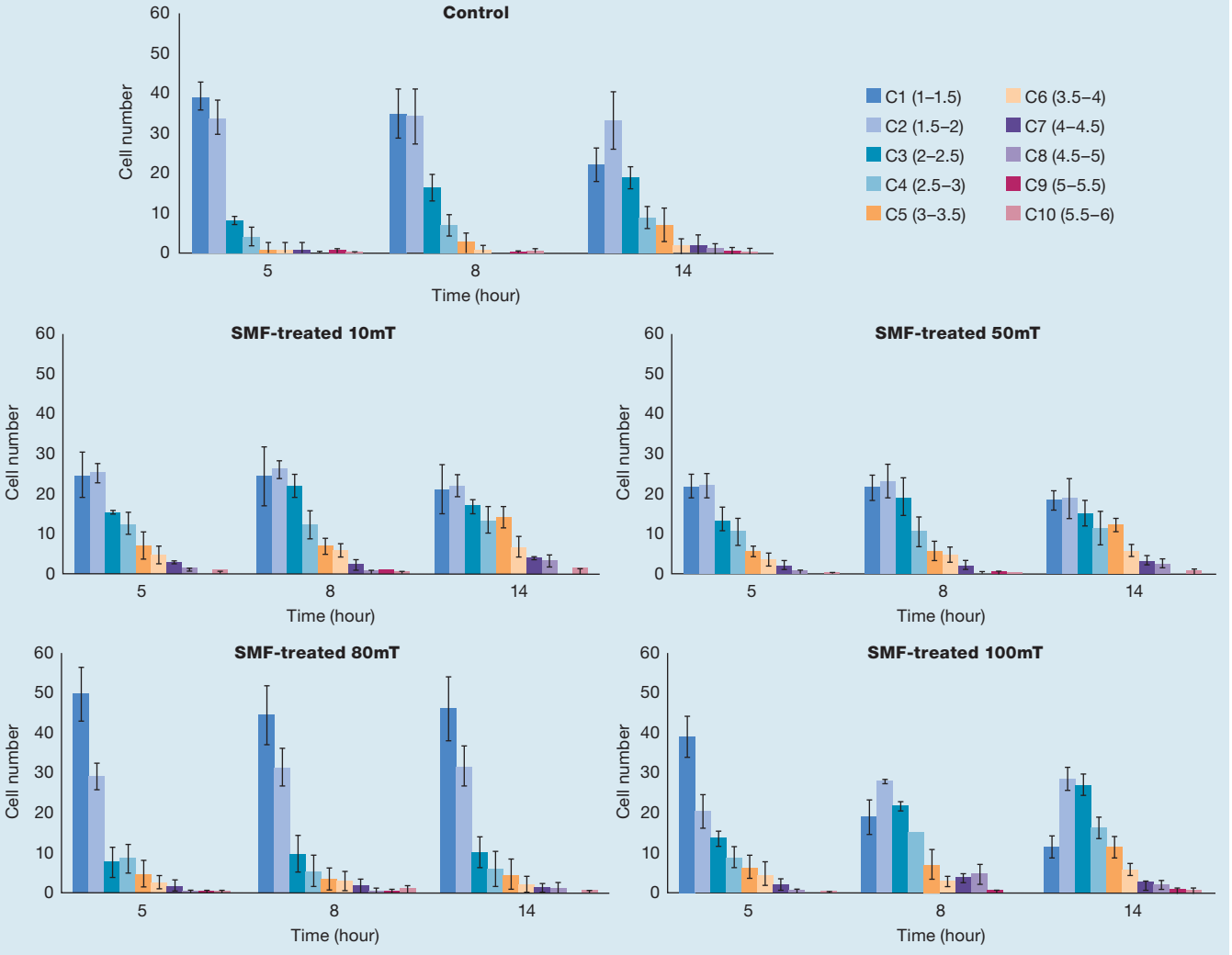
Effects of SMF on the integrity of the NIH 3T3 cell membrane

The effect of SMF on the membrane permeability in the NIH 3T3 cells was assayed by means of the release of lactate dehydrogenase (LDH) from control and SMF-treated cells using the LDH assay (Pars Azmun Company, Iran). LDH makes L-lactate and nicotinamide adenine dinucleotide (NAD) to be transmuted to pyruvate and NADH (the reduced form of NAD). The assay defines the extent of the integrity of the cell membranes based on the release of LDH which converts L-lactate and NAD to pyruvate and NADH subsequently. The cytotoxicity of SMF was conducted in 50µl of supernatant supplemented with 50µl of reconstituted substrate mix that incubated at room temperature for 30 minutes in the dark. The absorbance of the SMF-treated and non-treated cells were measured at 340nm and RT by HT Microplate Spectrophotometer (Bioteck, Power Wave XS2, US) in kinetics mode. The same number of cells were exposed to a SMF at 10, 50, 80 and 100mT. SMF was used in each well for LDH assay and the results were compared to their corresponding control group incubated in the same way but in the absence of SMF.

Theoretical and statistical analysis

Statistical analysis of the results was carried out using one-way ANOVA (SPSS 15). A p-value of <0.05 was considered statistically significant. Each experiment was repeated at least three times and the results were reported as mean ± standard deviation (SD). Sigma plot was used for fitting the trends and formulation of the correlations.

Fig 8. Effects of static magnetic field (SMF) on the circularity index of NIH 3T3 cells at different intensities. The average circularity value of cells changed during the migration and exposure to SMF at 10, 50, 80 and 100mT. The results showed that elongation of cells, C2 (1.5–2) and C3 (2–2.5), increased as a result of the exposure to SMF at 10, 50 and 100mT at the early stages of healing (<5 hours), and decreased at the late stage (~14 hours) when the number of spread out (SO) cells, C1 (1–1.5 circularity), increased. Exposure of cells to SMF at 80mT did not significantly effect the morphology of the cells during 14 hours' incubation time. Data are presented as means ± standard deviation, Bin=10 (each bar holds the information gathered from a maximum of 10 recorded data), n=3–5



Results

Activities of NIH 3T3 cells at different SMF intensity

The activities of the cells migrating across a scratch with a certain width size and area were studied to mimic the healing process in real time. Once the gap was formed, the cells located on the edge of each side took small circular (SC) morphology (Fig 2). These cells subsequently changed their morphology into spread out (SO) and/or elongated (EL) types at various frequencies in the presence and absence of SMF during the course of the experiment.

Further to the morphology of the cells, their dynamics, motility, formation of filopodia, direction, and the speed of migration as well as the orientation of the cells with respect to the direction of SMF was changed during the course of incubation.

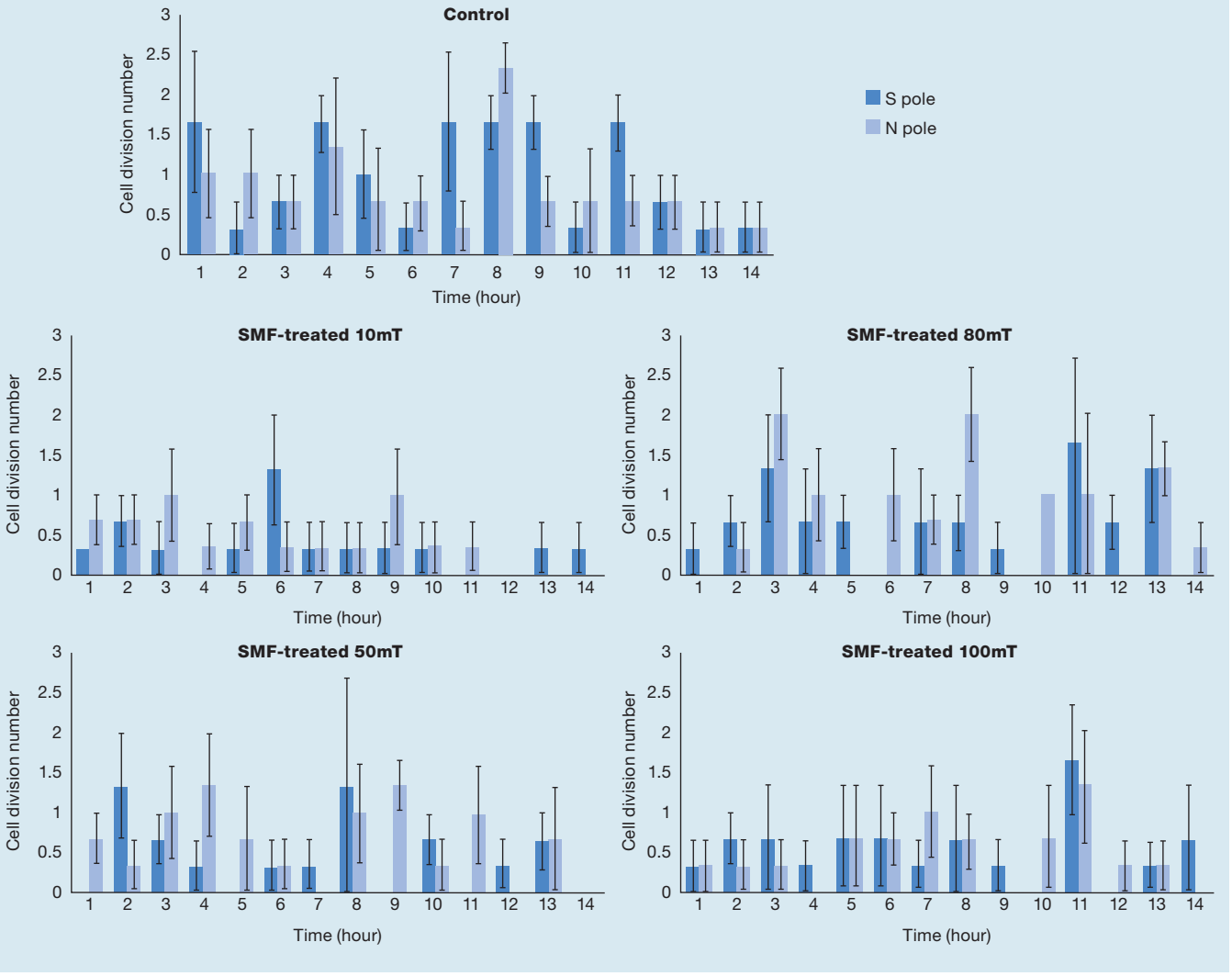
Effect of SMF on the viability of NIH 3T3 cells

The viability of 12,000 cells cultured in each well (40,000 cells/cm^2) was calculated based on the activities of their mitochondria. The results of MTT assay for at least three independent experiments revealed no significant changes in the viability of the cells exposed to a SMF at 10, 50, 80 and 100mT for 30 hours (Fig 3).

Effect of SMF on the integrity of the NIH 3T3 cell membranes

In order to probe the effect of different intensities of SMF on the integrity of cell membranes, the release of LDH through the membranes of SMF-treated cells was evaluated. The activity of LDH in the NIH 3T3 cell culture medium demonstrated no significant changes

Fig 9. The frequency of cell division in NIH 3T3 cells in the presence and absence of static magnetic field (SMF). The number of cell divisions was evaluated in the control as well as cells exposed to SMF at 10, 50, 80 and 100mT for 18 hours. Though there was no significant difference in the number of cell divisions occurring at certain times during the course of the experiment, it is obvious that the total number of cell divisions declined in the presence of SMF, in particular at SMF 10mT. Representative values shown have been extracted from all data, at one-hour intervals. Data are presented as means ± standard deviation, n=3–5



when the cells were exposed to 10–100mT for 30 hours, showing no SMF-induced damage in their cell membranes (Fig 4).

Effect of SMF on the morphology of NIH 3T3 cells

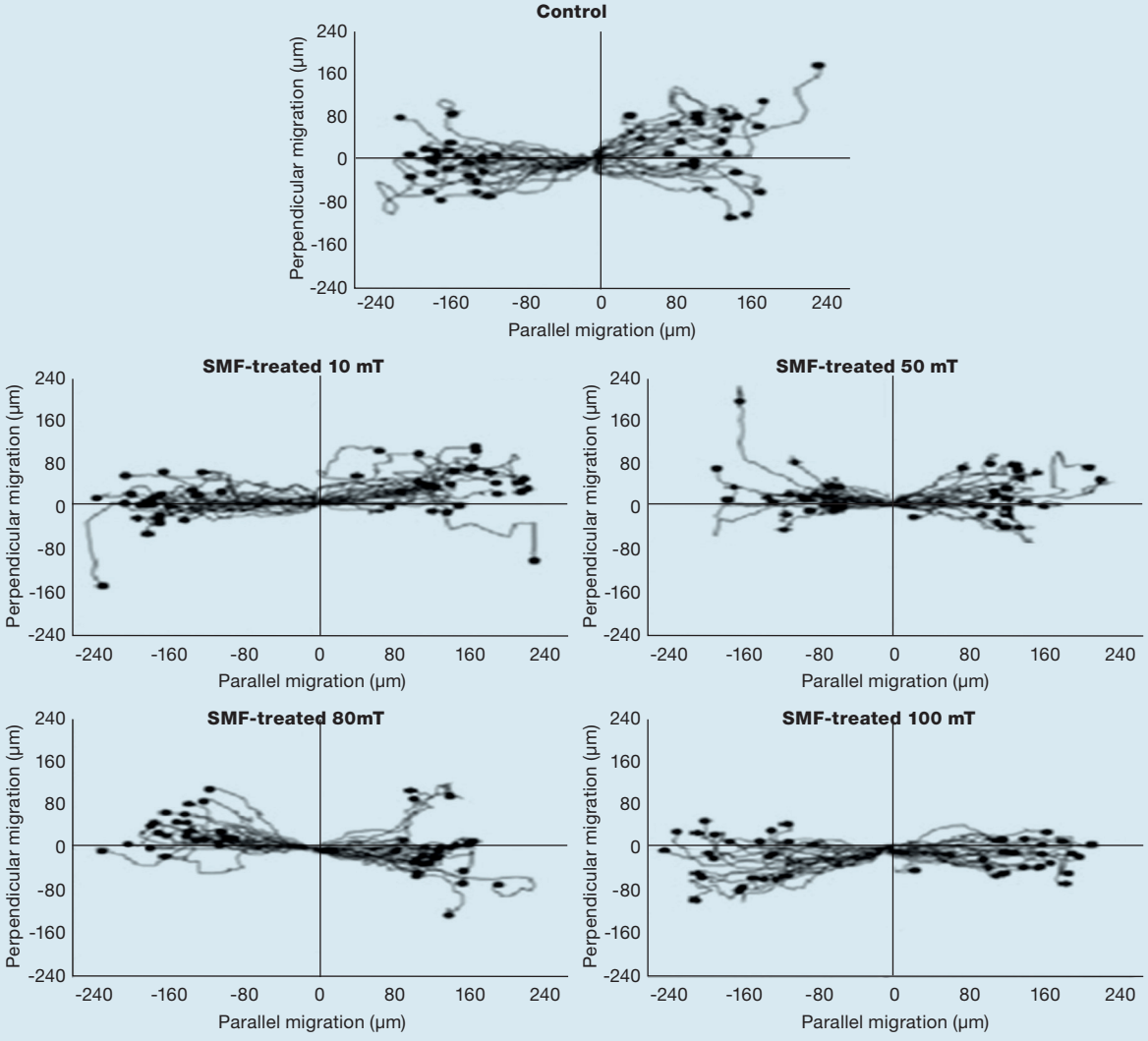
The morphology of the cells located at the gap-edge and gap area were studied and assessed during the course of exposure to SMF. The round-like SC cells were formed on the edge of the gap as soon as the scratch on the plane of confluent cell culture was created. The EL cells were mainly formed in the presence of SMF with a length-to-width ratio of 3:10. Each cell switched morphology (either to SO, EL or SC) but at different frequencies depending on the intensity of the applied SMF (Fig 5). SO cells were dominant at the end of the repair process.

The cells showed three distinct morphologies as a result of exposure to SMF, incubation time and consequent changes in the physicochemical condition of the medium (Fig 6). However, the cells took the SC morphology to initiate cell division, and SO/EL configuration for migration purposes.

Effect of SMF on the number and distribution of protrusions in NIH 3T3 cells

The EL cells showed almost no protrusions on their lateral sides while several protrusions (filopodia and lamellipodia) continuously formed and retracted on their front leading edge, that were mainly arranged towards the SMF direction. The rate of podia dynamics and continuous formation and retraction in EL cells was

Fig 10. Pattern of NIH 3T3 cell migration in the plain of cell culture in the presence and absence of static magnetic field (SMF). The analysis of cell tracking images demonstrated increased persistence of the cells exposed to SMF (at 10, 50, 80 and 100mT) after 18 hours. Though the representative SMF-treated cells seem to be affected by the north or south poles of the field, statistical analysis of all the data revealed no significant directness tendencies toward them. In the groups treated at 50 and 80mT, some cells migrated faster than others across the gap, reached the other side and then moved parallel to the gap edge, as shown at 10mT and 50 mT. Data was analysed by the Gradientech programme



lower than that of SO cells, which formed and retracted at different parts of their surface during the course of repair (Fig 2). In other words, the SO cells made a great number of evenly distributed filopodias across their surface in the absence and presence of SMF (Fig 2), while the EL cells formed filopodias mainly at the front edge of their cell body when exposed to a SMF at 10, 50 and 100mT (Fig 2, 6).

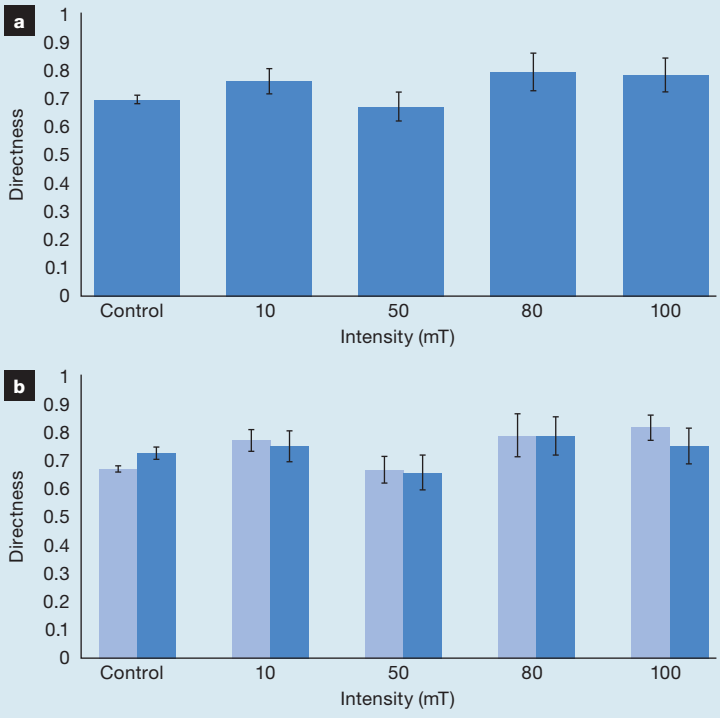
Effect of SMF on the area size of the NIH 3T3 cells

The area of NIH 3T3 cells changed following their exposure to a SMF at 10, 50, 80 and 100mT (Fig 7). Following the creation of the wound, the cell cycle in cells located at the gap edge changed, causing less proliferation, forming a SC shape (with a radius of 3–5µm) and migrating into the gap area. The average area size of the cells increased after about five hours and reached a plateau, representing when the cells from either side of the gap reached each other. The average area of the cells was reduced after 16–18 hours' incubation, when the gap was filled and cell culture became confluent.

The circularity of NIH 3T3 cells in the presence and absence of SMF

The circularity of the cells was significantly changed at a SMF at 10, 50 and 100mT. The treated cells were categorised into 10 groups (C1–C10) based on their circularity values

Fig 11. The persistence (directness) of NIH 3T3 cells exposed to the north (N) and south (S) poles of SMF. The cells were exposed to SMF (at 10, 50, 80 and 100mT) for at least 18 hours and their directness was measured, based on their location and orientation recorded at five-minute intervals. Analysis of data by one-way analysis of variance (ANOVA) followed by Tukey's post-test revealed significant differences between cell directness at 50mT and 80mT ($p \leq 0.05$) (**a**). Separate analysis of cell directness towards the N and S poles showed no tendencies towards one in particular (**b**). The black columns represent cells migrating toward the S pole and gray ones towards the N pole. Data are presented as means ± standard deviation, n=3–5



at different intensities (Fig 8). SMF-treated cells were elongated during migration and their circularity values varied, creating groups with different characteristics whose circularity index increased in the C2 (1.5–2) and C3 (2–2.5) groups, but decreased in the C1 (1–1.5) group. A remarkable increase in the circularity index was observed in the cells exposed to 10, 50 and 100mT SMF but remained about the same at 80mT (Fig 8).

Cell division in the control and SMF-treated NIH 3T3 cells

The number of cell divisions per hour was investigated at short timescales, however, no significant differences were identified when the average behaviour of different groups of control and SMF-treated cells (at 10, 50, 80 and 100mT) were compared statistically (Fig 9). In other words, considering the area formed under the curve of mean values, it is obvious that the total number of cell divisions has decreased in the presence of SMF (i.e., at SMF 10mT) however, the analysis of data in all groups showed no significant differences. Maximum number of cell division occurred at about 6–12 hours in the control cells and in the SMT-treated cells exposed to 50, 80 and 100mT, while the minimum number of divisions was identified in cells exposed to a SMT at 10mT. The number of cell divisions significantly decreased at the end of the experiment, when the cells reached confluent status.

Cell migration in the control and SMF-treated NIH 3T3 cells

The cell migration across the gap was evaluated *in vitro* by means of time-lapse recording for 18–30 hours (Fig 2). Following the creation of a gap of 300–400µm in the control experiments, the leader cells derived from cells located at the edge of each side migrated towards other side. The number of daughter and migrating cells was less in the presence of SMF because most forefront cells kept their initial point of contact and elongated towards the other side and in the direction of the field vectors.

Migration pattern of NIH 3T3 cells in the presence and absence of SMF

The directness of the cell migration was assessed according to the Euclidean distance (µm) over time (minutes) (Fig 10). Cells were distributed in the plain of culture in the presence and absence of SMF. Considering the centre coordinates as the starting point, cells in the control group moved and spent their time equally in each of the four quarters (the initial position of the cell on the bottom of the plate was the origin of the polar coordinates and then worked out its migration as vectors formed at different directions taking place in the four quarters) and were mainly directed by chemotactic factors. However, cells exposed to a SMF at 10mT were under the influence of both chemotactic factors and SMF effects. Consequently, most of the cells migrated mainly towards quarters 1 and 2 at 50mT, while they moved towards quarter 2 and 4 at 80mT, and 3 and 4 at 100mT (Fig 10).

Accumulated distance (µm) over time (minutes) revealed the average directness value of the migrating cells and was used to determine the tendency of cells to move towards a certain direction according to the technique reported by Spadinger et al.[45] The analysis of the migration of more than 100 cells tracked by Gradientech software revealed an average directness of 0.76, 0.66, 0.79, 0.78 and 0.69 for cells exposed to a SMF at 10, 50, 80, 100mT and control group, respectively (Fig 11).

According to the results, the increased speed of migration caused by the exposure to SMF 80mT and 100mT was accompanied by an increase in the cell directionality (persistence). The cell persistence toward both north and south poles was determined in cells exposed to SMF at different intensities of 10, 50, 80 and 100mT, but no significant tendency toward north and south poles was identified (Fig 11b).

Wound closure and migration speed of NIH 3T3 cells in the presence and absence of SMF

The complete occupation of the gap area by the NIH 3T3

cells was carried out at different rates in the presence of SMF at different intensities (Fig 12a). Assuming the wound closure have taken place in a linear manner, based on the Equation 3 and the data presented in Fig 12a, the rate of gap closure for the control and cells exposed to 10, 50, 80 and 100 mT for 18 hours, with initial gap area of 350000, 300000, 260000, 240000 and 17,5000µm^2 and final open area of 2415, 1735, 2114, 2671 and 2252 µm^2, was 2608.7, 3112.4, 2213.8, 1617.4 and 1398.8µm^2/hour, respectively. The speed of cells migrating from the opposing edges of the gap was evaluated during the course of the repair. Accordingly, the time took to occupy half of the gap area (50% wound closure) was calculated (Fig 12b). The exposure of the cells to a SMF at 10mT lowered their migration speed to less than that of the control, while those treated with a SMF at 80mT migrated faster (Fig 12c).

Discussion

A moderate SMF has been considered as an effective means to manipulate different cell and tissue activities. Different systems, including the circulatory system,[24] cardiovascular, nervous and musculoskeletal systems[37] have been studied. There have been almost no reports on the lethal effect of SMF on targeted cells, in other words, their growth and viability remained uneffected.[27] Therefore, application of SMF has been considered as an appropriate complementary therapeutic means to be used along with a dressing-based wound healing approach.[1]

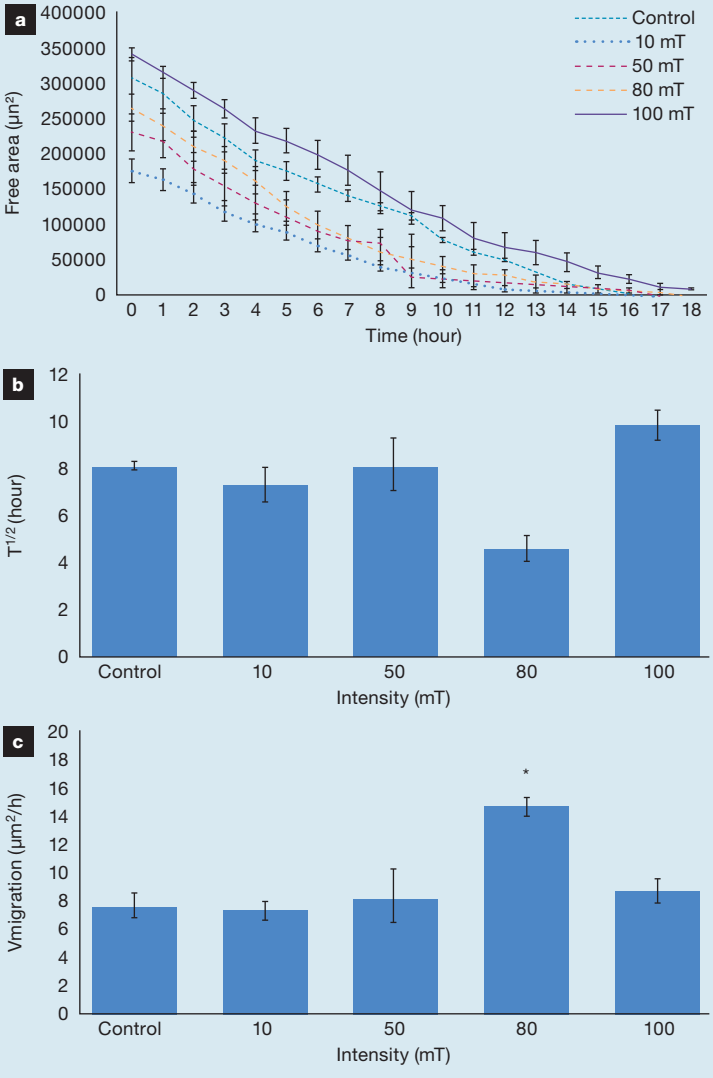
The presence of electric currents in the skin is known as one of the most effective factors involved in the promotion of cell migration towards the vacant area of the wound.[38] Endogenous electric currents of about 1µA are formed at the wound area of the human skin.[37] The finding is consistent with the intrinsic map of potential differences set across skin wounds known as 'skin battery'.[39] Measurement of the potential differences through electrical equipment involves mounting electrodes on each side of the wound/injury, and several significant features such as electrode size, polarity and spacing should be considered when calculating how to deliver a sufficiently stimulating current in order to obtain reliable and repeatable results. However, stimulation and monitoring of the same conditions by means of SMF is more practical, with less artefacts and uncertainty involved. The correlation between the electrical current and its consequent indirect effect has paved the way for the application of an exogenous magnetic field to improve wound healing.

The effects of SMF on cell viability obtained in this study is consistent with results of the studies on HFLF cells.[5] However, the cell viability was shown to be reduced in human macrophages exposed to a SMF at 0–670mT in another study.[40]

The results of the LDH assay in this study showed that SMF caused no distortion and negative effects on the integrity of cell membranes in the exposed cells, representing the diamagnetic anisotropy of biological systems.[33] This is consistent with the study on MG-63 osteoblasts that demonstrated no effects on cell membrane integrity when they were exposed to a SMF at 320mT for one hour a day.[40]

According to the time-lapse-based morphological analysis used in this study, the exposure of fibroblast to a SMF at 10, 50 and 100mT caused no significant damage to their membrane and, thus, no consequent cell death incurred. The extent of cell elongation significantly increased but was not a linear function of SMF intensity,

Fig 12. Effect of static magnetic field (SMF) on the wound closure and migration speed of NIH 3T3 cells. The time required to fill the gap area (wound closure time) was recorded in the NIH 3T3 cells exposed to SMF at 10, 50, 80 and 100mT for 18 hours and revealed their migration rate (**a**). The time required for cells to fill half the gap area in the presence and absence of SMF at different intensities is shown as T1/2 (**b**). The speed of migration (migration rate) was significantly higher at 80mT (**c**). The data derived from the recorded activities of NIH 3T3 cells were analysed by one-way analysis of variance (ANOVA) followed by Tukey's post-test for various SMF intensities and presented as means ± standard deviation, n=3-5 and considered significant when p≤0.05



so that the maximum and minimum elongation was identified in the cells exposed to a SMF at 10mT and 80mT, respectively. Elongation of SMF-treated cells has also been shown in U937 cells after their exposure to a SMF at 6mT for 24 hours.[33] By contrast, exposure to a SMF at 8, 30, and 300mT changed the morphology of glioblastoma cells into a round shaped one. The authors mention the rearrangement and dramatic modification of the cytoskeleton and membrane constituents such as receptors, adhesion molecules, tubulin networks and so on under exposure to SMF. Consequently, the resulting morphological changes may be due to the new arrangement of the actin molecules caused by the SMF.[30]

In this study, the captured images showed formation of protrusions in different areas of SO and EL cells as a result of exposure to SMF 10, 50 and 100mT. The low level of cell elongation and short microvili appeared in the cells exposed to SMF at 80mT revealed the significance of the field strength on the sensitivity of the cells and consequent polymerisation of the cytoskeleton at a certain SMF intensity, which is consistent with the results of other studies.[30] The plasma membrane consists of special proteins involved in signal transduction and trafficking that are also localised in the microvilli, filopodia and lamellipodia area and govern cell volume and fluctuations.[41] Consequently, the effect of SMF on the membrane and formation of the membrane-active sites and protrusions, reveals sophisticated actions and manipulative means triggered by the applied SMF. The exposure of NIH 3T3 cells to SMF altered the migration speed in the exposed cell. Cell motility towards the field direction changed in the presence of SMF, with a minimum at 10mT and maximum at 80mT (Fig 12). Cell movement results from a set of concerted actions taking place in the proteins that are involved in the adhesion of the cell to the surface.[42] Consequently, changes in the migration rate and speed, caused by the applied SMF, is the consequence of the global changes occurring in the conformation, location and binding affinity of the cell to the substrate.

According to the results of this study, persistence (directness) has been mainly augmented in the presence of SMF, and each individual movement of the cells was affected by the field vectors and led to an acceleration of the migration process. This is consistent with the results of another study where SMF-treated (at 8T) fibroblasts moved directly and parallel to the magnetic fields. The diamagnetic constituents of cells including membranes, microtubules and actin molecules possess anisotropy property which cause orientation toward external SMF.[10]

Cell elongation was augmented during cell migration in the course of this study. Cell migration rate increased at SMF 80mT, thus, cell elongation attenuated migration but in a non-dose-dependent manner. This was further proved by the results that revealed that there was no linear function between the intensity of the applied SMF and the indices of cell circularity, migration speed and division. However, faster and more sensitive means of monitoring cell activities at molecular and atomic levels may show more detail. Cell movement is influenced by the exchange and transfer of ions through the plasma membrane that is conducted by the voltage-sensitive ion channels. Any changes in membrane potential results in the direct/indirect deviation of different ion concentrations, including potassium (K^+), sodium (Na^+), Cl^-, Ca^{2+}, subsequently triggering a certain cascade of events. Accordingly, the exposure of cells to SMF, that effect the distribution of ions and corresponding ion channels, can initiate both local and global changes in the sensitivity and activity of the cells causing the formation of new protrusions in the membrane. This is consistent with the influence of moderate SMF demonstrated in a number of biological systems.[6,29]

The results gained in this study demonstrate that the polarisation and elongation of SMF-treated cells did not lead to an increased rate of migration. The cell polarity is often modulated by various intrinsic and extrinsic electrical and chemical means that lead to the reorganisation of cytoplasm components, organelles, cytoskeleton and constituent proteins of the membrane. Furthermore, the actin filaments dictate the structure and dynamics of the microvilli and act as a target site for SMF. Accordingly, the involved actin filaments, adhesion molecules and integrins that are responsible for the morphology of cells at different conditions, respond to the applied SMF. This is in agreement with the effects of SMF on the actin filaments and its possible consequent effects on the movement of condensed counter-ion clouds along the filament axis where the displacement of charges produces ion transfer across the filaments and facilitates the cation conductance,[43] electron hopping along the filament and extended polymerisation. Consequently, SMF can be used as a means to alter the mechanical forces in the cells and considered as physical therapy at the macroscopic level. Mechanical forces can modulate cell activities.[44] Cells sense their niche through mechanotransduction and adjust their activities by the mechanical forces.[45] SMF can be considered as a way to effect on the calcium channels and cytoskeleton proteins,[30,31] and the intracellular traffic and consequent changes in the cell activities.

In our study, the reduction in cell circularity as a result of exposure to SMF and migration rates suggest that elongated cells tend to move slower. On the other hand, persistence has been augmented in cells exposed to 80mT. Thus, the cells located at the edges of gap on either sides, were affected by the applied SMF which accelerated their migration process. The results showed that the index of cell circularity, migration speed and increasing cell division do not change as a function of the increased intensity in a linear manner overtime. In other words, the actual micro- or nano-environment of the cells that we cannot monitor precisely at atomic and molecular levels, may play a major role in the ultimate macroscopic activities of the cell.

It has been shown that the attenuation of cdc42 signalling abolishes electrotaxis and enhances contact guidance, whereas inhibiting rho signalling enhances electrotaxis and rho stimulation enhances contact guidance.[46] Accordingly, we propose that the changes in the migration pattern of the NIH 3T3 exposed to SMF at 80mT in this study, may have also involved both electrotaxis and contact guidance. The adhesion of NIH 3T3 cells was partially improved by SMF and led to slower movement and decreasing cell migration. The consequence of increased adhesion that was monitored by means of decline in the migration of SMF-treated cells (at 10mT), might point to opposite mechanism(s) where the higher number of pseudopodia identified in PEMF (pulsed electro magnetic field)-treated osteoblasts, i.e. increased number of cationic microfilaments and proteins responsible for cell attachment,[47] play the major roles.

The 'bioelectric signals' are known as one of the main means of communication between various cells, though chemical gradients are also considered. Most of the cells produce bioelectric signals by means of ion channels and pumps. They also receive these signals from neighbouring cells as a result of changes in their medium voltage gradient.[48] The voltage gradient intercedes long-range communication between cells and is able to modulate the expression of ion pumps and channels. Consequently, the ion fluxes throughout cell membrane are modulated remotely, leading to different modification, including changes in the cell shape, proliferation, apoptosis and cell migration.[49] We have not applied electric field (EF) in this study, however, the consequent effect of SMF on different aspects of cell life, shows similar effects through the extracellular electric signals it can cause.

Many studies have examined the interaction between magnetic fields and biological systems[50] and proposed diverse models that involve deviation in the charge transfer caused by the applied SMF. For example, Teodori et al. revealed that SMF causes increased influx of capacitative calcium in human glioblastoma cells[51] that was also proved by the augmentation of calcium fluxes in other cellular systems.[52,53] Exposure to magnetic fields has been shown to affect the concentration of intracellular Ca^{2+},[54] reactive oxygen species, lipid peroxidation,[55] DNA breakage[56] as well as signal transduction pathways.[57]

Limitations

Although the time-lapse approach revealed the morphological responses of the cells to the applied SMF in real-time, there were some limitations, including the low resolution of the images taken as well as the lack of fluorescent labelling of individual molecules whose expression might have been down- or up-regulated by the SMF. Accordingly, we are to pursue the study by means of confocal microscopy to obtain the effects of SMF on the intracellular traffic, polymerisation and membrane structure. Further studies are also planned to examine the reversibility of SMF bioeffects.

Conclusion

SMFs act as an efficient means of controlling cell behaviour (migration) and its constituent molecules (i.e. cytoskeleton proteins, ion pump/channel and so on) that is manifested by the orientation, morphology and migration of the cells. Considering many diseases with a defective function in their biological components, the manipulation of their structure/function by means of a remote SMF might assist in repairing their activity. Having achieved this, the approach could be used to overcome different biological malfunctions, including wound healing in diabetes, lack of cell activity and growth in spinal cord injuries. SMF can be considered as an inexpensive, noninvasive, portable, convenient, fast and remote means of treatment with minimal side effects. The results of our ongoing research together with complementary studies of other groups will define certain regimes of SMF, in terms of intensity, direction, duration and so on, to pave the path for the development of an efficient, complementary means of clinical treatment. JWC

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References

1 Markov, MS. Magnetic field therapy: a review. Electromagn Biol Med 2007; 26:1–23. https://doi.org/10.1080/15368370600925342
2 Ghodbane S, Lahbib A, Sakly M. Abdelmelek H. Bioeffects of static magnetic fields: oxidative stress, genotoxic effects, and cancer studies. Biommed Res Int 2013; 2013:602987. https://doi.org/10.1155/2013/602987
3 Raylman RR, Clavo AC, Wahl RL. Exposure to strong static magnetic field slows the growth of human cancer cells in vitro. Bioelectromagnetics 1996; 17(5):358–363. https://doi.org/10.1002/(SICI)1521-186X(1996)17:5<358::AID-BEM2>3.0.CO;2-2
4 Wiskirchen J, Groenewaeller EF, Kehlbach R et al. Long-term effects of repetitive exposure to a static magnetic field (1.5 T) on proliferation of human fetal lung fibroblasts. Magn Reson Med 1999; 41(3):464–468. https://doi.org/10.1002/(SICI)1522-2594(199903)41:3<464::AID-MRM6>3.0.CO;2-R
5 Wiskirchen J, Grönewäller EF, Heinzelmann F et al. Human fetal lung fibroblasts: in vitro study of repetitive magnetic field exposure at 0.2, 1.0, and 1.5 T. Radiology 2000; 215(3):858–862. https://doi.org/10.1148/radiology.215.3.r00jn11858
6 Chionna A, Dwikat M, Panzarini E et al. Cell shape and plasma membrane alterations after static magnetic fields exposure. Eur J Histochem 2009; 47(4):299–308. https://doi.org/10.4081/840
7 Fanelli C, Coppola S, Barone R et al. Magnetic fields increase cell survival by inhibiting apoptosis via modulation of Ca 2+ influx. FASEB J 1999; 13(1):95–102. https://doi.org/10.1096/fasebj.13.1.95
8 Higashi T, Yamagishi A, Takeuchi T et al. Orientation of erythrocytes in a strong static magnetic field. Blood 1993; 82(4):1328–1334
9 Murayama M. Orientation of sickled erythrocytes in a magnetic field. Nature 1965; 206(4982):420–422. https://doi.org/10.1038/206420a0
10 Iwasaka M, Ueno S. Magnetic-field parallel motion of living cells. J Appl Phys 2005; 97(10):10Q907. https://doi.org/10.1063/1.1851876
11 Torbet J, Ronzière MC. Magnetic alignment of collagen during self-assembly. Biochem J 1984; 219(3):1057–1059. https://doi.org/10.1042/bj2191057
12 Guido S, Tranquillo RT. A methodology for the systematic and quantitative study of cell contact guidance in oriented collagen gels. Correlation of fibroblast orientation and gel birefringence. J Cell Sci 1993; 105(Pt 2):317–331
13 Mousavidoust S, Mobasheri H, Riazi GH. Effects of static magnetic fields on the structure, polymerization, and bioelectric of tubulin assemblies. J Biomol Struct Dyn 2016; 1102:1–14
14 Monaco JL, Lawrence WT. Acute wound healing. Clin Plast Surg 2003; 30(1):1–12. https://doi.org/10.1016/S0094-1298(02)00070-6
15 Cines DB, Pollak ES, Buck CA et al. Endothelial cells in physiology and in the pathophysiology of vascular disorders. Blood 1998; 91(10):3527–3561

16 White JG, Gerrard JM. Ultrastructural features of abnormal blood platelets. A review. Am J Pathol 1976; 83(3):589–632
17 Hynes RO. Fibronectins. Sci Am 1986; 254(6):42–51. https://doi.org/10.1038/scientificamerican0686-42
18 Alexiadou K, Doupis J. Management of diabetic foot ulcers. Diabetes Therapy 2012; 3(1):4. https://doi.org/10.1007/s13300-012-0004-9
19 Nobes CD, Hall A. Rho GTPases control polarity, protrusion, and adhesion during cell movement. J Cell Biol 1999; 144(6):1235–1244
20 Belik DV, Pedonova ZN, Bukovsky MP. Magnetotherapy biotechnical system for rapid wound healing. 2014 12th International Conference on Actual Problems of Electronic Instrument Engineering, APEIE 2014 - Proceedings. 2015; 509–512. http://dx.doi.org/10.1109/APEIE.2014.7040735]
21 Grazul-Bilska AT, Johnson ML, Bilski JJ et al Wound healing: The role of growth factors. Drugs Today (Barc) 2003; 39(10):787–800
22 Hulkower KI, Herber RL. Cell migration and invasion assays as tools for drug discovery. Pharmaceutics 2011; 3(4):107–124. https://doi.org/10.3390/pharmaceutics3010107
23 Aaron RK, Ciombor DM, Simon BJ. Treatment of nonunions with electric and electromagnetic fields. Clin Orthop Relat Res 2004; 419(419):21–29. https://doi.org/10.1097/00003086-200402000-00005
24 Ohkubo C, Okano H, Ushiyama A, Masuda H. EMF effects on microcirculatory system. Environmentalist 2007; 27(4):395–402. https://doi.org/10.1007/s10669-007-9082-z
25 Man D, Man B, Plosker H. The influence of permanent magnetic field therapy on wound healing in suction lipectomy patients: a double-blind study. Plast Reconstr Surg 1999; 104(7):2261–2266. https://doi.org/10.1097/00006534-199912000-00051
26 Henry SL, Concannon MJ, Yee GJ. The effect of magnetic fields on wound healing: experimental study and review of the literature. Eplasty 2008; 8:e40
27 Jing D, Shen G, Cai J et al. Effects of 180 mT static magnetic fields on diabetic wound healing in rats. Bioelectromagnetics 2010; 31(8):640–648. https://doi.org/10.1002/bem.20592
28 Yu S, Shang P. A review of bioeffects of static magnetic field on rodent models. Prog Biophys Mol Biol 2014; 114(1):14–24. https://doi.org/10.1016/j.pbiomolbio.2013.11.002
29 Lisi A, Pozzi D, Pasquali E et al. Three dimensional (3D) analysis of the morphological changes induced by 50 Hz magnetic field exposure on human lymphoblastoid cells (Raji). Bioelectromagnetics 2000; 21(1):46–51. https://doi.org/10.1002/(SICI)1521-186X(200001)21:1<46::AID-BEM7>3.0.CO;2-Z
30 Teodori L, Albertini MC, Uguccioni F et al. Static magnetic fields affect cell size, shape, orientation, and membrane surface of human glioblastoma cells, as demonstrated by electron, optic, and atomic force microscopy. Cytometry A 2006; 69A(2):75–85. https://doi.org/10.1002/cyto.a.20208
31 Rosen AD. Mechanism of action of moderate-intensity static magnetic fields on biological systems. Cell Biochem Biophys 2003; 39(2):163–174. https://doi.org/10.1385/CBB:39:2:163
32 Rosen AD. Membrane response to static magnetic fields: effect of exposure duration. Biochim Biophys Acta BBA Biomembr 1993; 1148(2):317–320. https://doi.org/10.1016/0005-2736(93)90145-P
33 Dini L, Abbro L. Bioeffects of moderate-intensity static magnetic fields on cell cultures. Micron 2005; 36(3):195–217. https://doi.org/10.1016/j.micron.2004.12.009
34 43 Jonkman JE, Cathcart JA, Xu F et al. An introduction to the wound healing assay using live-cell microscopy. Cell Adhes Migr 2014; 8(5):440–451. https://doi.org/10.4161/cam.36224
35 Fotakis G, Timbrell JA. In vitro cytotoxicity assays: comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. Toxicol Lett 2006; 160(2):171–177. https://doi.org/10.1016/j.toxlet.2005.07.001
36 Spadinger I, Agnew D, Palcic B. 3T3 cell motility and morphology before, during, and after exposure to extremely-low-frequency magnetic fields. Bioelectromagnetics 1995;16(3):178–187. https://doi.org/10.1002/bem.2250160306
37 Saunders R. Static magnetic fields: animal studies. Prog Biophys Mol Biol 2005; 87(2-3):225–239. https://doi.org/10.1016/j.pbiomolbio.2004.09.001
38 Tyler, SEB, Bioelectricity in Wound Healing and Regenerative Processes in Animals, Humans, and Plants. Front Physiol 2017; 8: 627. https://doi.org/10.3389/fphys.2017.00627
39 Barker AT, Jaffe LF, Vanable JW Jr. The glabrous epidermis of cavies contains a powerful battery. Am J Physiol 1982; 242(3):R358–R366
40 Mariani F, Cappelli G, Eremenko T, Volpe P. Influence of static magnetic fields on cell viability necrosis and apoptosis. Boll Soc Ital Biol Sper 2001; 77(4–6):71–84
41 Lange K. Microvillar Ca++ signaling: a new view of an old problem. J Cell Physiol 1999; 180(1):19–34. https://doi.org/10.1002/(SICI)1097-4652(199907)180:1<19::AID-JCP3>3.0.CO;2-K
42 Tayeb M, Skalski M, Cha M et al. Inhibition of SNARE-mediated membrane traffic impairs cell migration. Exp Cell Res 2005; 305(1):63–73. https://doi.org/10.1016/j.yexcr.2004.12.004
43 Teodori L, Albertini MC, Uguccioni F et al. Static magnetic fields affect cell size, shape, orientation, and membrane surface of human glioblastoma cells, as demonstrated by electron, optic, and atomic force microscopy. Cytometry A 2006; 69(2):75–85. https://doi.org/10.1002/cyto.a.20208
44 Ross TD, Coon BG, Yun S et al. Integrins in mechanotransduction. Curr Opin Cell Biol 2013; 25(5):613–618. https://doi.org/10.1016/j.ceb.2013.05.006
45 Alonso LJ, Goldmann HW. Cellular mechanotransduction. AIMS Biophysics. 2016; 3(1):50–62. https://doi.org/10.3934/biophy.2016.1.50
46 Rajnicek AM, Foubister LE, McCaig CD. Prioritising guidance cues: Directional migration induced by substratum contours and electrical. Developmental Biology 2007; 312(1):448–460. https://doi.org/10.1016/j.ydbio.2007.09.051
47 Wang E, Zhao M, Forrester JV, MCCaig CD. Re-orientation and faster, directed migration of lens epithelial cells in a physiological electric field. Exp Eye Res 2000; 71(1):91–98. https://doi.org/10.1006/exer.2000.0858
48 Adams DS, Levin M. Endogenous voltage gradients as mediators of cell-cell communication: strategies for investigating bioelectrical signals during pattern formation. Cell Tissue Res 2013; 352(1):95–122. https://doi.org/10.1007/s00441-012-1329-4
49 Robinson KR, Messerli MA. Left/right, up/down: the role of endogenous electrical fields as directional signals in development, repair and invasion. BioEssays 2003; 25(8):759–766
50 Foster KR. Mechanisms of interaction of extremely low frequency electric fields and biological systems. Radiat Prot Dosimetry 2003;106(4):301–310. https://doi.org/10.1093/oxfordjournals.rpd.a006364
51 Teodori L, Göhde W, Valente MG et al. Static magnetic fields affect calcium fluxes and inhibit stress-induced apoptosis in human glioblastoma cells. Cytometry 2002; 49(4):143–149. https://doi.org/10.1002/cyto.10172
52 Rink TJ, Merritt JE. Calcium signalling. Curr Opin Cell Biol 1990; 2(2):198–205. https://doi.org/10.1016/0955-0674(90)90007-2
53 Aldinucci C, Palmi M, Sgaragli G et al. The effect of pulsed electromagnetic fields on the physiologic behaviour of a human astrocytoma cell line. Biochim Biophys Acta BBAMol Cell Res 2000; 1499(1-2):101–108. https://doi.org/10.1016/S0167-4889(00)00111-7
54 Lyle DB, Fuchs TA, Casamento JP et al. Intracellular calcium signaling by jurkat T-lymphocytes exposed to a 60 hz magnetic field. Bioelectromagnetics 1997; 18(6):439–445. https://doi.org/10.1002/(SICI)1521-186X(1997)18:6<439::AID-BEM6>3.0.CO;2-3
55 Ishisaka R, Kanno T, Inai Y, Nakahara H. Effects of a magnetic fields on the various functions of subcellular organelles and cells. Pathophysiology 2000; 7(2):149-152
56 Fiorani M, Cantoni O, Sestili P et al. Electric and/or magnetic field effects on DNA structure and function in cultured human cells. Mutat Res 1992;282(1):25–29
57 Zhao M. Electrical fields in wound healing–an overriding signal that directs cell migration. Semin Cell Dev Biol 2009; 20(6):674–682. https://doi.org/10.1016/j.semcdb.2008.12.009