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Regeneration and Control of Human Fibroblast Cell Density by Intermittently Delivered Pulsed Electric Fields

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ABSTRACT: Proliferative scarring is a human disease with neither available effective treatment nor relevant animal model. One of the hypotheses for scar formation involves deregulation of fibroblast signaling and delayed apoptosis. Here, we introduce a new chemical-free method for fibroblast density control in culture by intermittently delivered pulsed electric fields (IDPEF), which cause irreversible damage to cell membranes. Using 5–100 pulses with electric field strength of 150 V/mm, pulse duration 70 μs, and frequency of 1 Hz, we investigated the effects of PEF application on growth, death, and regeneration of normal human dermal fibroblasts in culture. We found that the fraction of fibroblasts that survive depends on the number of pulses applied and follows a Weibull distribution. We have successfully developed an IDPEF protocol that controls fibroblasts density in culture. Specifically, through application of IDPEF every 72 h for 12 days, we maintain a normal human dermal fibroblast density in the $3.1 \pm 0.2 \times 10^5$–$1.4 \pm 0.2 \times 10^5$ cell/mL range. Our results suggest that IDPEFs may prove useful as a non-chemical method for fibroblast density control in human wound healing.

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KEYWORDS: fibroblasts; irreversible electroporation; intermittently delivered pulsed electric fields; cell density control; proliferative scarring

Introduction

Wound healing is a dynamic, chronic process that is divided to four overlapping phases: hemostasis, inflammation, proliferation, and remodeling (Diegelmann and Evans, 2004; Robson, 2003; Sonnemann and Bement, 2011). During hemostasis, constriction of the damaged vessels and clot formation physically limit blood loss. During the inflammatory phase, leukocytes and then monocytes accumulate to combat infection in the wounded tissue. In this phase, multiple cytokines and growth factors are released to the wound area and contribute to the fibroblast migration, differentiation, and activity. During the proliferative phase, fibroblasts deposit new extracellular matrix and collagen and differentiate into myo-fibroblasts. In the final remodeling phase, re-organization of the closed wound environment occurs until repair is completed.

To describe this complex dynamic process, Robson et al. (2001) introduced the concept of wound healing trajectory (Fig. 1A), which demonstrates the time-dependent cumulative effects of these multiple processes that occur from injury though healing (Robson et al., 2001). According to the healing trajectory curve, normally healed tissues are characterized by complete restoration of function and structure (Lazarus et al., 1994). In contrast, chronic wounds are characterized by incomplete restoration of structure and function (Lazarus et al., 1994). In proliferative scarring, however, the healing process does not stop as it should and the tissue fails to reach a normal cell density and a balance between collagen deposition and degradation (Cuono, 1990; Robson et al., 2001).

Proliferative scarring in human wounds has adverse physical, aesthetic, functional, psychological, and social consequences (Aarabi et al., 2007; Sheridan and Tompkins, 2004). Although several systemic and genomic studies have

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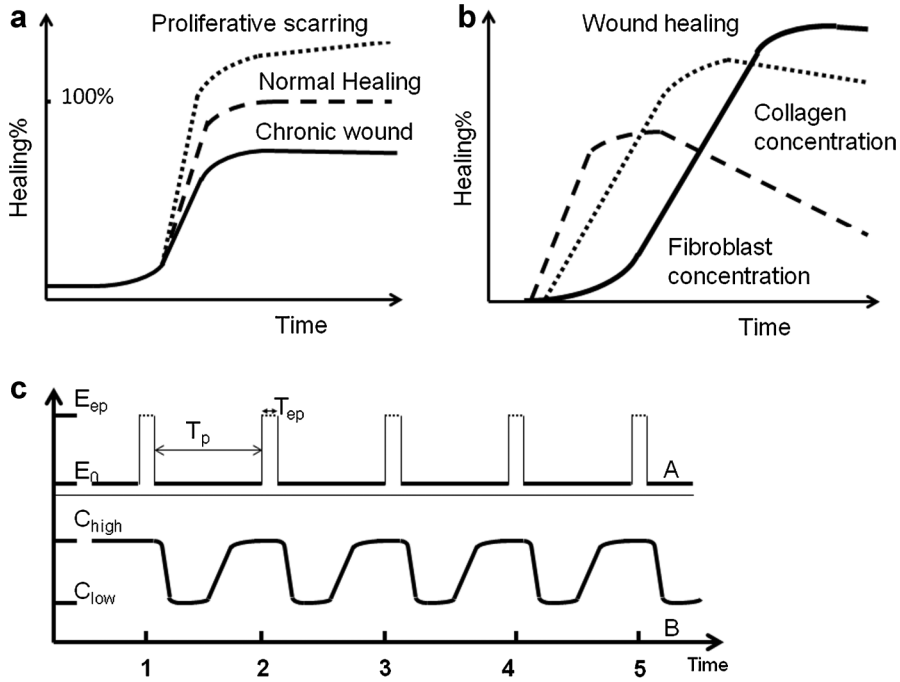

Figure 1. **a**: Wound healing trajectory. **b**: Scheme of fibroblast cell density dynamics during wound repair. **c**: Schematic presentation of IDPEF cell density control. The top line shows the timing for pulsed electric field delivery. The line on the bottom shows the respective cell concentration in the culture.

identified potential cellular and extracellular factors that mediate the formation of proliferative scar, the exact mechanism that induces proliferative scar tissue formation instead of a healthy tissue is not known. Currently available treatment strategies have limited clinical success (Garg and Longaker, 2000; Ogawa, 2010; Savage and Swann, 1985; Tan et al., 2010; Wang et al., 2008). Studies suggest that alterations in coagulation, inflammation, angiogenesis, fibroplasia, contraction, and remodeling may result in proliferative scarring (Cuono, 1990; Leask and Abraham, 2004; Polo et al., 1997; Robson, 2003; Robson et al., 1992, 2001).

The role of humeral mediators seems to play a critical role in proliferative scarring by altering fibroblast metabolism. It was shown that signaling which affects fibroblast metabolism is different in individuals who suffer from proliferative scarring from those who do not (Border and Noble, 1994; Ding et al., 2011; Garg and Longaker, 2000; O'Kane and Ferguson, 1997; Robson et al., 2001; Shah et al., 1995). The major role of fibroblasts in wound healing is to replace the fibrin-based provisional matrix established during the inflammatory phase of wound healing with collagen-rich granulation tissue. The behavior of fibroblasts in the wound is highly dynamic (Fig. 1B) and varies at each healing phase (Robson et al., 2001). Fibroblasts reach the wound during the second or third day after the injury (Iocono et al., 1998). Four days after the injury, fibroblasts are usually the major cell type in the developing granulation tissue (Peacock et al., 1993). The wound fibroblast number increases initially through migration from nearby non-injured tissue and then through cell proliferation. Fibroblast density in the wound reaches its maximum between 7 and 14 days after injury. When the anatomic function of the tissue is mostly restored, the maturing granulation tissue undergoes remodeling leading to reduction of fibroblast density by apoptosis (Desmoulliere et al., 1995; Peacock and VanWinkle, 1976; Peacock et al., 1993; Singer and Clark, 1999). Interestingly, clinical observations showed that in patients with proliferative scarring the apoptosis inhibitor—bcl-2 proto-oncogene is elevated; however, the apoptosis effector–interleukin-converting enzyme is decreased (Wasserman et al., 1998). These findings suggest that the apoptosis mechanism is altered in patients with proliferative scarring (Wasserman et al., 1998). These data led us to hypothesize that external control of fibroblast population density in the healing wound may contribute to the reduction of proliferative scarring.

Pulsed electric fields (PEFs) affect cell and tissue metabolism by regenerative stimulation at the lower amplitudes of the field strength, and permeabilization at the higher amplitudes of the field strength (Fig. 2) (Dubey et al., 2011; Neumann et al., 1982; Seil and Webster, 2007). Experimental data show that PEFs trigger multiple biochemical mechanisms in cells (Fig. 2), (Rubinsky, 2010).

A phenomenon when PEFs cause membrane permeability change is known as "electroporation" (Neumann et al.,

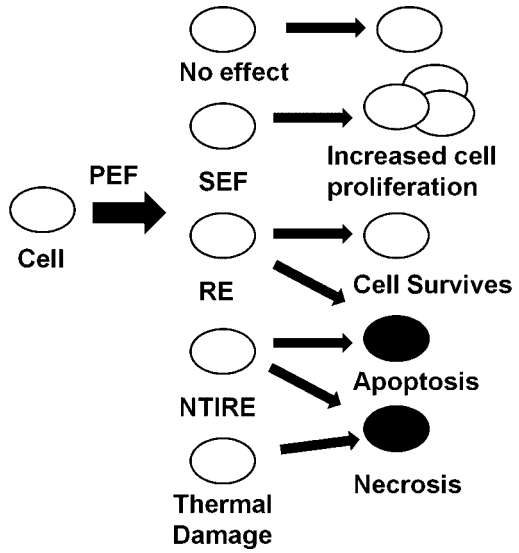

Figure 2. Schematic presentation of biochemical phenomena in cell, induced by PEF: stimulating electric fields (SEF), RE, NTIRE, and thermal damages.

1982). Reversible electroporation (RE) occurs when the cell membrane permeabilization is temporary, and cells survive. Examples of RE applications are gene delivery to cells (Neumann et al., 1982) and tissues (Titomirov et al., 1991), introduction of drugs into cells (Okino and Mohri, 1987), and cell fusion (Zimmermann, 1982). RE is the basis for a new cancer treatment therapy known as "electrochemotherapy" (Orlowski et al., 1988), where a cancer cell specific cytotoxic drug is introduced to the cell through a temporary membrane opening by PEFs. Non-thermal irreversible electroporation (NTIRE) occurs when a cell die after the exposure to PEFs (Golberg and Rubinsky, 2010). NTIRE occurs primarily when the electrical fields cause a permanent increase in membrane permeability with loss of cell homeostasis; however, the exact molecular mechanisms of electroporation are not known (Golberg and Rubinsky, 2010; Weaver and Chimadzev, 1996). Examples of NTIRE applications include cell (Sankaranarayanan et al., 2011) and tissue ablation (Rubinsky, 2010), bacteria disinfection in food (Barbosa-Canovas et al., 1998; Lelieved et al., 2007) and pharmaceuticals (Golberg et al., 2009), and plant tissue disintegration for the extraction of valuable contents (Corrales et al., 2008).

Previously, we developed an intermittently delivered PEF (IDPEF) method for long-term control of microbial loads in perishable products for food and pharmaceutical storage (Golberg, 2012; Golberg et al., 2010). In this work, we demonstrated that it is possible to control microbial cell density by IDPEF. These data suggest that it is possible to expand the use of IDPEF as a potential method to control mammalian cell density. Thus, we hypothesize that external control over the fibroblast density may improve the wound healing with less proliferative scarring.

In this work, we systematically investigated the response of normal human dermal fibroblasts culture (NDHF) to single and IDPEF exposure. We developed a protocol for NDHF cell density control by IDPEF: PEF dose and intervals that can precisely control residual fibroblast density. In light of data suggesting delayed fibroblast apoptosis may lead to proliferative scarring, our results suggest a new method for fibroblast population control that may provide a potential treatment for this common and difficult clinical problem.

Theoretical Aspects

Previous modeling of NTIRE cell ablation in cancer has demonstrated that only a fraction of cells exposed to specific PEF is killed and that surviving cells remain functional and have the ability to regenerate (Golberg and Rubinsky, 2010). Experimental work on bacteria has demonstrated that IDPEF can control microbial loads in perishable food products exposed to post processing contamination (Golberg, 2012; Golberg et al., 2010). Figure 1C shows the schematic destiny of a cell population exposed to the IDPEF. Line A in Figure 1C describes the frequency and intensity of the applied electric fields over time. Line B describes cells density response to the applied treatment over time. The different parameters of Figure 1C are described in Table I.

Materials and Methods

Cell Culture

All experiments were performed using third, fourth, and fifth passage NHDF (ATCC, PCS-201-012). NHDF were

Table I. Parameters import for planning of IDPEF cell density control NHDF cell culture.

Parameters	Units	Physical meaning
E_0	Field intensity—E (V/mm), number of pulses—N	Electric field in applied on the cells population between the treatments
E_{ep}	Pulse duration—t (s) frequency—f (Hz)	PEF applied on cell population during the treatment
T_p	s	Time interval between the treatment
T_{ep}	s	Total time of the applied PEFs
C_{high}	Cells/mL or (%)	Higher threshold cell density/confluence %
C_{low}	Cells/mL or (%)	Lower threshold for cell density/confluence %

Table II. PEF parameters used for the culture regeneration time study.

Parameters	Units	Used experimental values
E	V/mm	150
T_{ep}	μs	70
f	Hz	1
N		5,10,25,50,100

cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) (Life Technologies, Carlsbad, CA), at 37°C, in a 5% CO_2 balance air atmosphere. The cells were cultured on 6 mm nominal diameter tissue culture treated dishes (Coring Inc., New York, NY). Immediately after the PEF treatment, cells were washed three times with phosphate-buffered saline (PBS), and then fresh DMEM/FBS was added. We changed the medium 2 h after the treatment and every 24 h subsequently.

Pulsed Electric Fields Dose Response

Electric field parameters: field strength—E (V/mm), number of pulses (N) pulse duration—t (s) and frequency—f (Hz) are critical for electropermeabilization. It was recently shown that specific changes in pulse amplitude, number and duration could lead to similar electroporation outcomes (Pucihar et al., 2011). For example, longer pulses or higher number of pulses, lower amplitudes are needed for the same fraction of electroporated cells (Pucihar et al., 2011). Therefore, to investigate the recovery time of the NHDF confluent cell culture after a single PEF treatment, we tested the impact of N. The other parameters of the protocol: E (V/mm), t (μs), and f (Hz), were fixed to the parameters close to those currently used in the clinical setting (Thomson et al., 2011). For partial ablation of the NHDF culture, PEFs were delivered directly to the cell culture in a six-well plate by a BTX ECM 830 square-wave electroporator, using a PetriPulser™ electrode (Harvard Apparatus Inc., Holliston, MA).

PEFs parameters used in this culture response study appear in Table II. We investigated the NHDF culture recovery after a single PEFs treatment by live cell counting and cell culture recovery microscopic observations as described in the following sections.

Intermittently Delivered Pulsed Electric Field Protocol

To demonstrate the control of NHDF cell density by IDPEF we applied PEFs that caused ~60% cell death. The full recovery time of the survived cells was 72 h as found from the experiments described in the previous section. We applied 10 pulses with E (150 V/mm), t (70 μs) at 1 Hz every 72 h. The IDPEF protocol we used appears in Table III. We assessed culture recovery by live cell counting and microscopic observations as described in the following sections. We did not use florescent imaging in this study because of the possible leakage of the florescent markers after cell exposure to strong electric fields.

Live Cell Counting

Two hours after PEFs treatment cultured cells were detached from the plate by incubation with 1 mL of 0.25% trypsin and 0.02% EDTA for 10 min at 37°C (Life Technologies, Carlsbad, CA). The trypsin was inactivated by adding of 1 mL of DMEM/FBS media. Next, the cells were centrifuged at 800 rpm for 5 min (Allegra™6R Centrifuge, Beckman Coulter, Inc., Indianapolis, IN). The cell pellet was resuspended in PBS and an aliquot (10 mL) was removed for counting by hemocytometer (Hausser Scientific, Horsham, PA).

Cell Culture Regeneration Imaging

The treated NHDF cultures were maintained in the same six-well plates from the beginning till the end of the experiment. To monitor culture recovery, we observed the wells by phase microscopy (Zeiss Axiovert 200 M, Carl Zeiss MicroImaging, Inc., Jena, Germany). Every 24 h, 3 points in each well were captured at 2.5× magnification. Cell confluence at all three positions in the well defined the full recovery time point.

Statistical Analyses

At least three replicates were used for each experimental condition that were repeated at least three times. For statistical analyses, we used the Microsoft Office Excel 2010 external package.

Table III. IDPEF protocol for NHDF cell density control.

Parameters	Values	Comments
E_0	0	No electric field was applied between the treatments
E_{ep}	E (150 V/mm) t (70 μs) f (1 Hz) N (10)	PEF applied on cell population during the treatment.
T_p	72 (h)	Time interval between the treatment
T_{ep}	10 (s)	Total time of the delivery of PEFs
C_{high}	100%	Confluence of the 6 well plate
C_{low}	40%	Confluence of the 6 well plate

Results

Human Dermal Fibroblast Dose Response to the Pulsed Electric Fields

The survival of cells with various number of pulses delivered as described in Table II appears in Figure 3. Here, the number of cells in the six-well plate before and 2 h after the PEFs treatment is shown. We measured the number of cells surviving 2 h after treatment to wash out any injured cells that may not have detached immediately. Figure 3A shows cell survival fraction as a function of the number of pulses applied. Figure 3B–G shows the characteristic images of tissue dishes 2 h after the treatment. The outcomes from Figure 3 are summarized in Table IV.

Figure 4A shows the fraction of cells that survived first 2 h after the PEF treatment. It is important to point out that part of these cells are still electroporated for complete membrane resealing process takes hours (Weaver and Chimadzev, 1996). To describe the probability of NHDF death, we used the Weibull distribution (Equations 1–3 and Fig. 4B), commonly used to describe the probability of bacteria cell death PEFs (Peleg, 2006):

$$S = \exp\left(\frac{-N}{a}\right)^{b} \tag{1}$$

or

$$-\ln(S) = \left(\frac{N}{a}\right)^{b} \tag{2}$$

or, alternatively:

$$\ln(-\ln(S)) = b\ln N - b\ln a \tag{3}$$

where S is the survival fraction NDHF and N is the number of pulses applied to cell culture. The calculated Weibull distribution coefficients are $b = 0.72$ and $a = 38.9$. In addition, we investigated the NHDF culture recovery time to 100% confluence (days to recover-DTR) as a function of the survived cell fraction is shown on Figure 4C.

To determine the recovery profile of NHDF cell culture at different time points after a single PEFs treatment, we took images at various time points until cells 100% confluence was recovered (Fig. 5). The microscopic observations led us to the following results.

Figure 5A–C shows the recovery of the NHDF culture that was treated with 5 pulses of 150 V/mm electric field, 70 μs duration pulses delivered at 1 Hz as 2 h, 24 h, and 48 h, respectively after the treatment. It took 2 days for the culture to recover after 5 pulse-treatments.

Figure 5D–F shows the recovery of the NHDF culture that was treated with 10 pulses of 150 V/mm electric field, 70 μs duration pulses delivered at 1 Hz at 2 h, 48 h, and 72 h, respectively after the treatment. The culture fully recovered 72 h after treatment. We used this protocol in the following IDPEF studies.

Figure 5G–I shows the recovery of the NHDF culture that was treated with 25 pulses of 150 V/mm electric field, 70 μs duration pulses delivered at 1 Hz at 2 h, 96 h, and 144 h, respectively after the treatment. The NHDF cell culture recovered 6 days after the treatment.

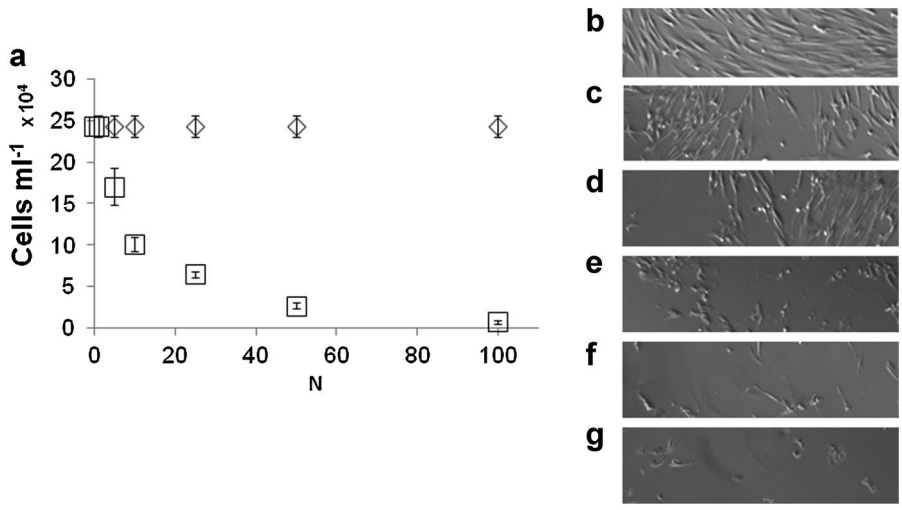

Figure 3. **a**: NHDF survival as a function of number of pulses. Here, we applied 150V/mm electric field, 70 μs duration pulses at 1 Hz. Robs represent the cell number before the application of PEFs. Squares represent the number of cells in the culture 2 h after the treatment as counted by life cell count method. Error bars show ± 1 SD. Number of cells per mL describes the total number of cells survived in a particular well that were washed by 1 mL of saline for Life cell counting method. Images of representative slice of a tissue dish 2 h after the treatment. **b**: Control (**c**) 5 pulses (**d**) 10 pulses (**e**) 25 pulses (**f**) 50 pulses (**g**) 100 pulses.

Table IV. Cell survival after different number of pulses delivered.

Number of pulses (N)	Survivals (cell/mL) $\times 10^4$
0 (control)	24.33 ± 1.28
5	17.06 ± 2.20
10	10.13 ± 0.80
25	6.46 ± 0.30
50	2.73 ± 0.41
100	0.73 ± 0.23

Figure 5J–L shows the recovery of the NHDF culture that was treated with 50 pulses of 150 V/mm electric field, 70 μs duration pulses delivered at 1 Hz at 2 h, 144 h, and 192 h, respectively after the treatment. The cell culture was fully confluent 8 days after the treatment.

Finally, Figure 5M–O shows the recovery of the NHDF culture which was treated with 100 pulses of 150 V/mm electric field, 70 μs duration pulses delivered at 1 Hz at 2, 240, and 336 h, respectively after the treatment. From the 336 h (2 weeks) observation in this experiment NHDF culture did not show any recovery after 100 pulses.

Fibroblast Proliferation and Cell Culture Recovery Under IDPEFs

To investigate the NHDF culture recovery profile under IDPEF, we applied 10 pulses of 150 V/mm, 70 μs duration each at 1 Hz every 72 h to the NHDF cultured in six-well plates. Figure 6 shows that $43 \pm 4\%$ of NHDF, which survive each PEFs treatment, recover to 100% confluent culture after 72 h, as measured by life cell count method. The line that describes cell concentration between the 100% and $43 \pm 4\%$ confluence, or $3.1 \pm 0.2 \times 10^5$ (C_{high} cell/mL) and $1.4 \pm 0.2 \times 10^5$ (C_{low} Cell/mL), was constructed using serial microscopic observations. The microscopic observations of treated cell cultures demonstrated that for the first 12 h after treatment cells show almost no proliferation. This is followed by a rapid proliferative phase that slows down approximately 48 h after the treatment. During the last 24 h, the cells grow slowly to fill any empty space. Therefore, using this specific IDPEF protocol we controlled the NHDF in the 43 ± 4–100% confluence range.

Discussion

Proliferative scar formation can occur in wounds from many etiologies and is an important unsolved clinical problem. Many non-cutaneous clinical problems, such as tendon adhesions, bile duct strictures, cirrhosis of the liver, and glomerulonephritis are also the result of proliferative scarring (Aarabi et al., 2007; Robson, 2003). Unfortunately, Phase III trials of human TGF-β3 therapy, (Occleston et al., 2008), (Juvista®, Renovo, Manchester, UK) failed to demonstrate benefit in 2011, emphasizing the complexity of interactions between extracellular and cellular components during healing. Therefore, it seems likely that targeting single mechanisms may not be effective and

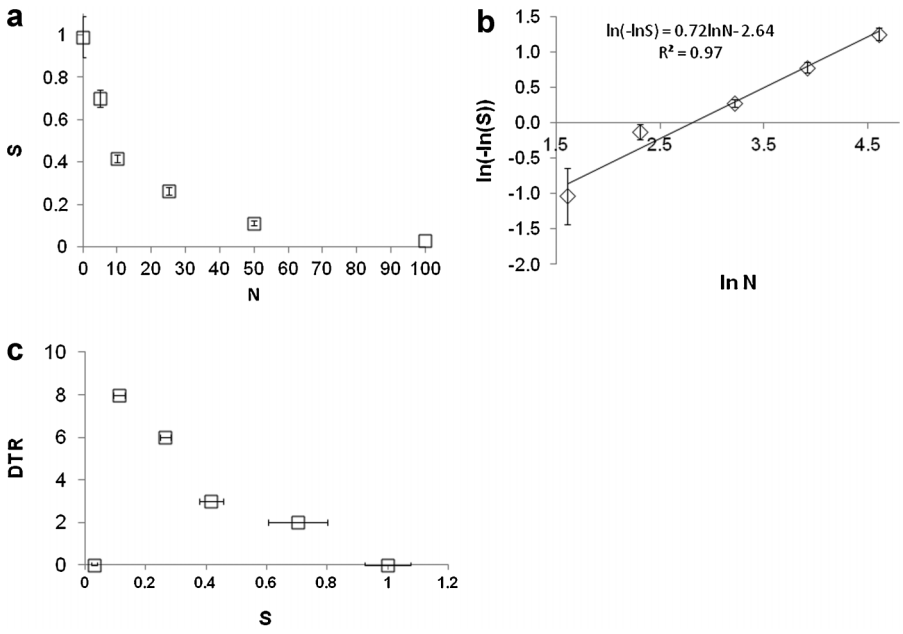

Figure 4. **a**: The impact of number of pulses on the survival rate of NHDF. **b**: Transformation of the survival function to linear form for the estimation of Weibull distribution coefficients. **c**: NHDF cell culture days to confluence recovery (DTR) as a function of the pulsed electric exposed survival fraction of cells.

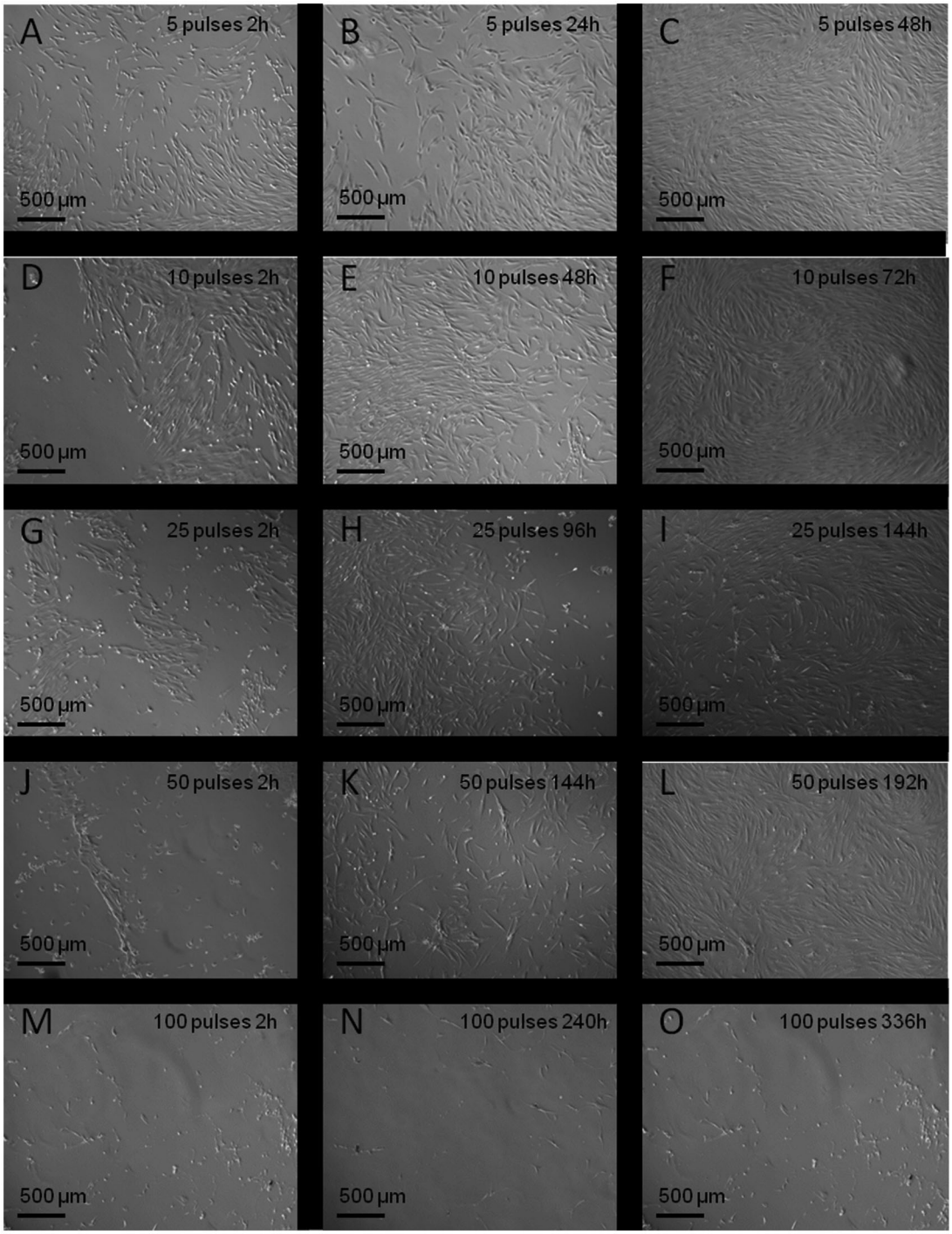

Figure 5. NHDF cell culture recovery profile at different time points after a single PEF treatment of 150 V/mm electric field, 70 µs duration pulses at 1 Hz. The number of applied pulses and the time after treatment, the image was taken appear at images A–O.

external multi-target therapies are needed. Physical therapies help a potential to affect multiple targets by externally, well-controlled interventions.

Our group previously reported on the photolysis methods for proliferative scar control (Reiken et al., 1997; Wolfort et al., 1996). Others used low amplitude electric field to regenerate tissue anatomy and to recover tissue functionality (Seil and Webster, 2007; Vrbova et al., 2008) The goal of this work is to introduce a new concept and method for controlling cell density and proliferation by IDPEF. Clinical

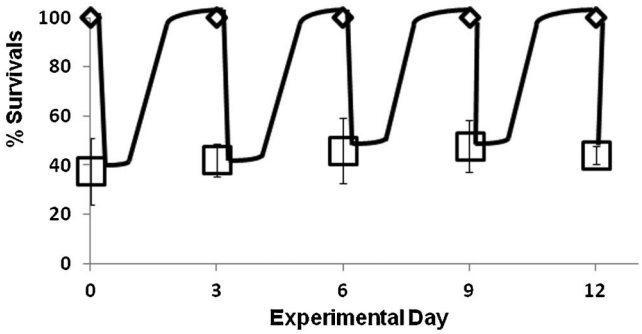

Figure 6. NHDF cell density control in culture by IDPEF. 10 pulses of 150 V/mm, 70 μs duration each at 1 Hz were delivered every 72 h to the NHDF cultured in six-well plate. The number of cell before and after each treatment was counted by the live count method. The cell survival was normalized to the number of cells on the plate before treatment. Error bars show ±1 SD.

control of cell density is usually achieved by chemical factors, which affect the cell cycle, preventing or inducing proliferation. Such agents, however, cannot be precisely targeted and affect multiple cell types. For example, Tamoxifen, a synthetic nonsteroidal anti-estrogen, has been shown to have multiple side effects. Those include altered RNA transcription, decreased cellular proliferation, delay or arrest of the cells in the G1 phase of the cell cycle, and interference with multiple growth factors such as TGF-β and insulin-like growth factor (Chau et al., 1998; Hu et al., 1998; Kuhn et al., 2002; Mikulec et al., 2001). The advantage of IDPEF as a method to control cell density is that it can precisely target the desired tissues and affect cell density locally without complex system effects (Golberg and Rubinsky, 2012).

Since no animal model for proliferative scarring exists, we made a first step towards fibroblast density control by developing IDPEF protocol in vitro. Our first goal was to characterize NHDF cell death as a function of the applied PEF protocol. Previously, we introduced a theoretical study where we used a probabilistic approach to describe mammalian cell death by PEFs (Golberg and Rubinsky, 2010). To the best of our knowledge, this is the first experimental work that describes PEFs induced cell death using probabilistic methods. We characterized the dose–response of NHDF culture in Figure 3 and Table IV and used Weibull distribution (Fig. 4 and Equations 1–3) to describe the probability of cell death as a function of number of pulses (Fig. 4). The Weibull distribution shape parameter b and scale parameter a were found to be respectively 0.72 and 38.9.

Our second goal was to investigate the NHDF culture recovery time after partial NTIRE. Previously, NTIRE was used as an efficient tissue ablation method and the parameters defined only the degree of total cell destruction in the treated area (Rubinsky, 2010). In this work, however, we suggest that cell density can be controlled by IDPEF; only a fraction of the cells are killed and those surviving are able to perform their biological function. Therefore, it is critical to characterize the recovery rate of cells under different treatment conditions. Figures 5 and 6 describe NHDF culture recovery under different PEFs treatment conditions indicating that a range of 5–50 pulses allowed the survived cells to recover in 2–8 days after the treatment. In contrast, 100 pulses inactivated 97% of cells and no cell proliferation or recovery of the survived cells was observed.

Finally, using the data collected from the NHDF cell culture response to a single PEF exposure we designed a protocol for cell density control in vitro by IDPEF. NHDF cultures were exposed to IDPEF every 72 h (3 days). In this experiment, we determined that the minimum percentage of cells needed to survive so as to recover to confluence in 72 h is $43 \pm 4\%$. We repeated the treatment five times to investigate the effects of IDPEF on the cell culture. Our results suggest that IDPEF can maintain cell density in the prescribed range, if the inactivation kinetics and recovery rates are known. One of the limitations of the current study is the 2D cell culture surface. In 3D the behavior of the system in vivo will be different, and additional in vitro 3D model is needed for future studies to understand the effect of the matrix on cell survival and migration.

For many attaching cell types, 100% confluence in vitro suggests inhibition of proliferation and growth, possibly due to contact inhibition (Puliafito et al., 2011). In vivo, however, the cell proliferation and density is controlled by multiple complex chemical, mechanical, and electrical pathways. Although the mechanisms for cell proliferation and density control are very tightly controlled, aberrations of the control mechanisms lead to diseases, such as cancer and fibrosis. We suggest that external intervention of the IDPEF type can return the balance to the system. Externally applied IDPEF in vivo may control the cell density by partial irreversible electroporation of cells, compensating the malregulated fibroblast apoptosis, which is thought to be one of the reasons for proliferative scarring (Wasserman et al., 1998). We propose to use a special electrically active bondage for the in vivo applications, which will deliver IDPEF only to the prescribed areas of the wound. The electrically active biomaterials, which deliver low amplitude PEFs for stimulation have been already reported in (Dubey et al., 2011). In addition, the methodology to achieve special control of electric field distribution in tissues was developed in (Golberg and Rubinsky, 2012). Additional in vivo studies, however, are needed to investigate the level of the partial cell ablation in tissues that still preserves the critical functions, such as infection barrier. Furthermore, additional studies are essential to understand if NTIRE can selectively ablate specific cell types and spare non-target cells in heterogeneous tissues.

Conclusions

Proliferative scarring is a condition with no known molecular mechanism. There is no effective treatment

today for this common and important pathologic condition. Current understanding of fibroblast kinetics suggests that deregulation of fibroblast signaling and delayed apoptosis are involved in pathologic scarring. Here, we introduce a novel, non-chemical method to control fibroblast cell density by IDPEF. We believe that IDPEF may contribute to the treatment of proliferative scarring in vivo by providing precise spatial control over fibroblast cell density.

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