

Wound Healing Activity of Consciousness Energy Healing Treatment on HFF-1 Cells and DMEM Using Scratch Assay

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Abstract

The wound healing activity using scratch assay is considered as a convenient *in vitro* tool for the assessment of wound healing. The present study deals with the optimization of Biofield Energy Treatment (Consciousness Energy Healing Treatment-The Trivedi Effect[®]) in the HFF-1 cell line (Human Foreskin Fibroblast) and DMEM (Dulbecco's Modified Eagle Medium) using scratch assay against positive control, recombinant Human Epidermal Growth Factor (Hu EGF, 30 ng/mL). This method was used for the determination of cell proliferation and migration of fibroblast quantitatively in the scratched wounded area. The scratched area was monitored after 24 hours of wound closure in the Biofield Energy Treated HFF-1 cells and the Biofield Energy Treated DMEM groups, and the representative photomicrographs were taken in each wells using WimScratch Image analysis software. The results showed that the Biofield Energy Treated DMEM was significantly higher percentage of fibroblast migration i.e. 51.8%, while the migration was altered in the Biofield Energy Treated HFF-1 cell line compared to the baseline control group. In addition to, the percentage of scratch area was significantly decreased by 2.7% in the Biofield Energy Treated DMEM group, while it was increased by 12% in the Biofield Treated cells group compared to the baseline control group. Overall, the experimental results concluded that The Trivedi Effect[®] has the significant capacity and wide implications in wound healing activity *via* cell culture media, DMEM as compared with the HFF-1 cell line directly. Biofield Energy Healing would be a complementary and alternative medicine that can be used against burn injury cases, acute wound, skin regeneration, eczema, diaper rash, chickenpox, measles, warts, acne, hives, wrinkles, ringworm, Rosacea, psoriasis, seborrheic dermatitis, skin cancer, *etc.*

Keywords: Consciousness Energy Healing Treatment; Scratch assay; Wound healing; The Trivedi Effect[®]; HFF-1 cells; Fibroblasts

Introduction

“Wound” results from an opening or breaking of the skin results in a physical injuries and “wound healing”, which a complex multifactorial process is including integrated cell responses to injury. These processes maintain the integrity and function of the damaged tissues. Healing involves the inflammation followed by remodeling and formation of the damaged tissues^[1]. New tissues formation begins with the keratinocytes migration in injured epidermis and hair follicles, further leads to cells proliferation at wound edge and results in the formation of new tissue known as re-epithelialization phase. Keratinocytes re-differentiation occurs in order to restore the barrier function. In addition to, fibroblast plays a major role to repair the injured dermis and results in the synthesis of new extracellular matrix (ECM)^[2,3]. The use of alternative and complementary medicines since ancient times, to accelerate the wound healing process^[4,5]. However, their use is not based on scientific approach and it's just merely based on the irradiation due to less knowledge on mode of action of alternative medicines. Biofield Energy Healing-based therapies are considered as a Complementary and Alternative Medicine (CAM) against many therapeutic

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tic aspects. Pre-clinical and clinical data have been reported in favor of Biofield Energy Healing against cancer research that suggest better results of Energy Medicine as compared with other CAM approaches^[6]. It was reported that during the year 2007, approximately 40 % of the U.S. population have used some form of CAM for their health benefits^[7]. National Center for Complementary and Alternative Medicine (NCCAM), now categorized the Biofield Energy Therapies in subcategory of Energy Therapies as complementary medicine domain. Biofield Energy Therapies have been reported with therapeutic potentials in case of an improved personal well-being in cancer patient^[8], better functional ability in arthritis patient^[9], reduced pain and anxiety^[10], and in wound healing^[11,12]. There are a growing number of wound healing studies related with Biofield Healing Modalities. Mr. Mahendra Kumar Trivedi's Biofield Energy Treatment (The Trivedi Effect[®]) has been scientifically studied and reported in various fields. The significance of The Trivedi Effect[®] has been reported in cancer research^[13,14], microbiology with changed antimicrobial sensitivity pattern^[15-18], improved the overall productivity of crops in agriculture and livestock^[19-22], significant results in different nutraceutical and pharmaceutical compounds^[23-28], and altered structural, physical, and thermal properties of several metals and ceramics^[29-31]. Different number of wound healing evaluation methods are available, but *in vitro* assays are always preferred not only due to ethical and financial constraint, but also they can be carried out in small-scale bioassays, analyze the cell migration and cell interactions. Among different *in vitro* assay, scratch assay has been proven as an inexpensive and valuable tool to study the cell migration. Scratched surface can disrupt the cell to cell contacts, thus results in increase proliferation and migration of various cells like keratinocytes and fibroblasts. The progression of various cellular events can be monitor by imaging at various time points using time-lapse microscopy^[32].

The present study evaluated the effect of Biofield Energy Treatment (The Trivedi Effect[®]) on (Human foreskin fibroblast-1) HFF-1 cell line and Dulbecco's Modified Eagle Medium (DMEM) using scratch assay for quantitative determination of fibroblast migration and proliferation.

Materials and Methods

Chemicals and Reagents: Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), and Recombinant Human Epidermal Growth Factor (Hu EGF) were purchased from Gibco, Genex Life Sciences Pvt. Ltd., India. Ethylenediaminetetraacetic Acid (EDTA), trypsin, and NaHCO₃ were purchased from Sigma, USA. Antibiotics solution, Penicillin-Streptomycin was procured from HiMedia Pvt. Ltd., USA. All the other chemicals used in this experiment were analytical grade procured locally from India.

Cell Culture Maintenance (HFF-1, ATCC[®] SCRC-1041[™]):

HFF-1 (human foreskin fibroblast) cells were procured from American Type Culture Collection (ATCC), SCRC-1041[™], USA, originated from normal human skin fibroblast cells. HFF-1 cell line was maintained in the growth medium DMEM supplemented with 15 % FBS, with added antibiotics penicillin (100 U / mL) and streptomycin (100µg / mL). The growth condition of cell lines were maintained at 37°C, 5 %CO₂, and 95 % humid-

ity. The cells were sub-cultured by trypsinization followed by splitting the cell suspension into fresh flasks and supplementing with fresh cell growth medium. Hu EGF (100 µg / mL) in PBS stock (positive control) was diluted in DMEM to achieve the working concentration corresponding of 30 ng / mL in cell plate.

Consciousness Energy Healing Strategy: An aliquot of HFF-1 cells in a T-25 cell culture flask and an aliquot of DMEM culture medium were received Biofield Energy Treatment (Consciousness Energy Healing Treatment-The Trivedi Effect[®]) by a renowned Biofield Energy Healer, Mahendra Kumar Trivedi, who participated in this study under laboratory conditions for ~3 minutes from a distance of ~25 cm. The energy transmission was done without touching the cells and media. Following Biofield Energy Treatment, the medium and the cell line were used for estimation of *in vitro* wound healing potential using scratch assay. Following treatment, the above Biofield Energy Treated and untreated T-25 flask was incubated till one week in a CO₂ incubator at 37°C, 5 %CO₂, and 95 % humidity. The Biofield Energy Treated and untreated DMEM were stored at 4°C till cell culture.

Experimental Design: Group I served as cells in the untreated medium (200 µL of phenol-free DMEM supplemented with 10 % CD-FBS). Group II defined as positive control (Hu-EGF in DMEM), i.e. cells in DMEM with Hu-EGF (30 ng / mL). Media was changed with 900 µL of DMEM followed by addition of 100 µL of 300 ng / mL of Hu EGF. Group III was referred as HFF-1 cells in the Biofield Energy (known as The Trivedi Effect[®]) Treated DMEM. Group IV was denoted as Biofield Energy Treated HFF-1 cells in the untreated DMEM.

***In vitro* Wound Healing Assay:** The HFF-1 cell lines were counted using a hemocytometer and plated in 12-well plates at the densities 0.08 X 10⁶ /well / mL of cell growth medium. The cells were incubated overnight under growth conditions and allowed for cell recovery and exponential growth. After overnight incubation, the Biofield Energy Treated and untreated cells were subjected to the serum starvation in the treated and untreated DMEM for 24 hours. Mechanical scratch representing wounds were created in the near confluent monolayer of cells by gently scraping using sterile 200 µL micropipette tip. The scratched area was then monitored after 24 hours for closure of wound area. The photomicrographs was done at the selected time point's for quantitative assessment of migrated cells and its area of wound closure using digital camera, which was connected to the inverted microscope. Further, fibroblast cells migration distance in each wells were monitored using Wim Scratch Image analysis software. All the observations were calculated and compared with baseline values^[33].

Results and Discussion

***In vitro* Wound Healing Assay:** *In vitro* wound healing activity by scratch assay was performed in order to measure the cell migration rate in HFF-1 cell line. The Biofield Energy Treated DMEM in HFF-1 cells were monitored and represented images are presented in Figure 1.

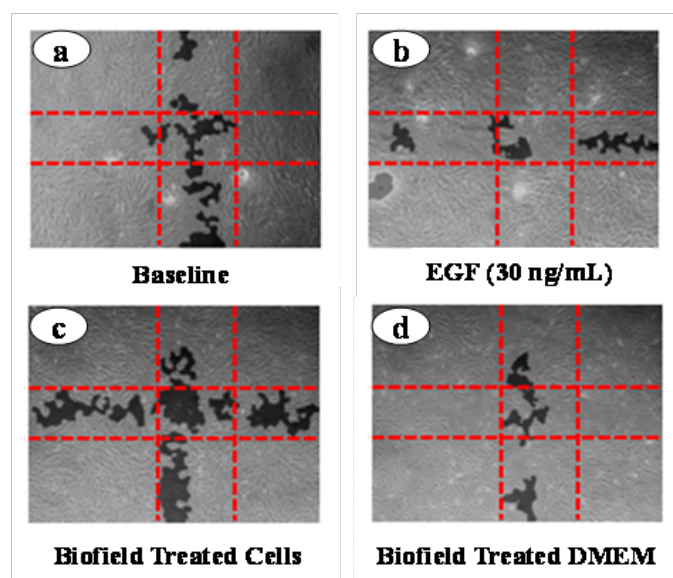


Figure 1: Effect of the Biofield Energy Treatment on HFF-1 cell line and DMEM in scratch assay for the assessment of wound healing activity. All the pictures were taken after 24 hours of the induced scratch at 50X magnification. Representative pictures were of (a) untreated baseline control group; (b) Epidermal growth factor (EGF) at 30 ng/mL; (c) Biofield Energy Treated HFF-1 cells; and (d) Biofield Energy Treated DMEM

The representative images showed that the rate of fibroblast migration in the Biofield Energy Treated DMEM group was higher as compared with the Biofield Energy Treated HFF-1 cells group, when compared with the positive control (EGF) group. The percentage of cell migration and the scratch area measured after 24 hours in all the tested groups were identified and summarized in Table 1.

Table 1: Effect of the Biofield Energy Treatment on HFF-1 cell line and DMEM on cell migration in scratch assay

Treatment Group	% Scratched Area	% Migration
Baseline control	5.6	0
EGF (30 ng/mL)	2	64.3
Biofield Treated DMEM	2.7	51.8
Biofield Treated HFF-1 cells	12	-114.3 (No migration)

Thus, the Biofield Energy Treated DMEM media showed a significant migration of fibroblast cells compared with the Biofield Energy Treated HFF-1 cells. The obtained result suggests that the Biofield Energy Treated DMEM significantly improved the fibroblast migration, as monitored in 24 hours period, which implicates its wound repair activity. Confluent monolayer was scratched and cells migrations were recorded at different time-points. Representative photomicrographs showed a significant migration of cells at time-point 24 hours was monitored. From the experimental observations, it was found that the percent migration in EGF group was increased from baseline values to 64.30 %, while it was 51.80 % in the Biofield Energy Treated DMEM group, and no migration was reported in the Biofield Energy Treated cells. Similarly, the scratch area in the untreated baseline group was 5.6 %, while it was significantly decreased to

2% in EGF (30 ng / mL). However, the Biofield Energy Treated DMEM also showed a significant decreased scratched area upto 2.7 %, while the Biofield Energy Treated cells showed no migration with increase scratched area by 12 % (Table 1).

Scratch assays for assessing the wound healing potential were first used as the best *in vitro* wound healing models for epithelial or mesenchymal cells^[34]. It is well-developed method for monitoring the cell migration, to study the cell-matrix and cell-cell interactions events occurred during wound healing and migration^[32]. Wound repair process required cell migration and proliferation, which are the important process that includes collagen deposition in tissue injury. This assay includes the HFF-1 cells seeded into a multiwell assay plate that were allowed to attach, spread, and ultimately formed a confluent monolayer. Further, scratch was made using a pin tool or needle to remove cells from a discrete area of the confluent monolayer in order to create a cell-free zone at the edges of the wound can migrate^[32].

To promote wound healing, several traditional, complementary and alternative medicine have been used by the wound care professionals with several challenges^[36]. Biofield Energy Treatment, as an alternate treatment approach has been reported clinically with significant outcomes in burn injury cases, acute wound, etc. Healing as an alternative therapy in helping wounds to heal has gained popularity over the past two decades^[37,38]. Biofield Energy might be beneficial to promote the growth of the cells and its migration rate. It was reported that exposure of the cells to low pulsed electric fields could enhances the adsorption and uptake of macromolecules and associated with processes of development, regeneration and wound healing^[39]. Song *et al.*, 2002 using animal model reported that electric cues could regulate the orientation and frequency of cell division with an improved rate of wound healing^[40]. The wound healing scratch assay generally covers the second phase of the wound healing and it was characterized by the proliferation and migration of either keratinocytes or fibroblasts^[2]. It can be suggested that human Biofield, a low electromagnetic field might have the capacity to alter the cell differentiation as well as improves the migration of either keratinocytes or fibroblasts. On the basis of the results, Mahendra Kumar Trivedi's Consciousness Energy Healing Treatment (The Trivedi Effect®) showed a significant improvement in wound healing rate in HFF-1 cell lines through Biofield Energy Treated DMEM. Overall, the Biofield Energy Treated DMEM would be a new aspect in order to maintain the cells integrity and life.

Conclusions

Based on the study outcomes, the Consciousness Energy Healing Treatment (The Trivedi Effect®) showed significant results in *in vitro* scratch assay for 24 hours study period. The results of cell migration showed 51.8 % increased rate in Biofield Energy Treated DMEM group and 64.3 % in the positive control (EGF), while cell migration was altered in the Biofield Treated cells. Similarly, the percentage scratch area was reduced by 2.7 % in the Biofield Energy Treated DMEM group, while it was increased by 12 % in the Biofield Energy Treated cells. This showed that Mr. Trivedi's Biofield Energy Healing capacity has the potential to improve the wound healing rate in HFF-1 cells using DMEM. It can also be suggest that the cell migration

rate and decreased scratch area in the Biofield Energy Treated DMEM would be due to an improved migration of either keratinocytes or fibroblasts that would improve the wound healing process.

Overall, the Biofield Energy Treated test formulation can be used as a Complementary and Alternative Medicine (CAM) with a safe therapeutic index for various wound healing related disorders such as eczema, warts, acne, psoriasis, seborrheic dermatitis, skin cancer, rashes from bacterial or fungal infections, rashes from allergic reactions, raised bumps that are red or white, cracked skin, discolored patches of skin, fleshy bumps, warts, or other skin growths, changes in mole color or size, a loss of skin pigment, scaly or rough skin, peeling skin, ulcers, open sores or lesions, dry, excessive flushing. Overall, Biofield Energy Treatment as an alternate treatment approach that can likely be used and contribute to the wound healing in the prevention of temporary and permanent skin disorders, anti-aging, an improved overall health, and quality of life.

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