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Antiaging Potential of Biofield Energy Healing Treatment in HFF-1 Cell Line Using Collagen level and Cellular Proliferation

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Abstract

Skin health and aging are co-related with a complex biological process that are influenced by a combination of factors such as intrinsic (or endogenous) and extrinsic (or exogenous). Many skin based therapies including physical and chemical methods are in practice to rejuvenate the skin, but they might be related with some significant side-effects such as scarring. The aim of the present study was to evaluate the effect 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) for evaluation of skin health parameters such as proliferation rate and collagen level. The cell growth rate was evaluated at 24, 48, and 72 hours and the results observed with a significant growth in the Biofield Treated DMEM group compared with the Biofield Treated HFF-1 cells. The HFF-1 cellular proliferation rate was significantly ($p \leq 0.001$) increased by 85% in the Biofield Treated DMEM, while positive controls, ascorbic acid (10µM) and FBS (15% fetal bovine serum) reported with increased cell proliferation by 21% and 44%, respectively. Similarly, the collagen level was significantly ($p \leq 0.01$) increased in the Biofield Treated DMEM by 44.2%, while the Biofield Energy Treated cells did not showed significant change in collagen and proliferation rate as compared with control. Hence, the results concluded significant improved collagen and proliferation in the Biofield Energy Treated media (DMEM) for improving skin health. It can be concluded that The Trivedi Effect® might be a complementary and alternative approach with respect to the skin health, anti-aging in DMEM as compared with the HFF-1 cell line.

Keywords: Anti-aging; HFF-1 cell line; Consciousness Energy Healing Treatment; Biofield Energy Healing; The Trivedi Effect®; Fibroblast cells; Collagen.

Introduction

Skin is the largest organ in the body and plays an important functional and psychological mechanism. Loss of skin function, color, and texture might be responsible for many conditions such as aging, chronic metabolic diseases, trauma, etc. Aging and loss of skin function result in infection, atrophy, laxity, chronic wounds, and rhytids [1]. Internal and external aging factors are very complex biological process that results in skin aging. Intrinsic factors include genetics factors, hormone, cellular metabolism, and metabolic processes, while external factors include light exposure, chemicals, pollution, ionizing radiation, toxins, etc. [2]. Both of these factors are responsible for structural and physiological alterations in skin layers. Most of the skin manifestations are influenced by decrease in the collagen level, reduced skin elasticity, atrophy, and gradual bone resorption that lead to wrinkled and dry skin. Epidermal atrophy results in thinning and increased fragility of skin and was found that most of the individuals around 60 years of age. The epidermis thinning, and destruction of dermal collagen fibers can induce the fine wrinkles with exaggeration of the facial expression lines, pallor, and laxity [3]. A report suggests that more than 2 crores skin rejuvenation procedures cases were performed in the United States in 2013 [4].

In order to eliminate these skin blemishes of the face, many different treatment approaches have been practiced world-wide. Skin barriers that are healthy and functioning are considered as vital skin protector and helps against dehydration, irritants, microorganism protection, allergens, ROS (reactive oxygen species) and from UV radiation. In addition, various photo aging products, antioxidants, pharmacological agents that have anti-aging properties (i.e. Vitamin B3, C, and E), many invasive procedures like gene therapy, chemical peels, and several devices such as laser energy, injectable, and many more were used for skin health and rejuvenation [5,6]. However, the studies outcomes suggest that these procedures does not ensure a natural skin look and very optional, as it was found that most of the techniques are applied without any clear understanding of the skin anatomy and physiology of skin aging. The Complementary and Alternative Medicine (CAM) systems are widely used against various therapeutic aspects along with skin treatment or wound repair therapies [7]. Biofield Energy Healing is one of the CAM reported with significant outcomes in clinical and pre-clinical studies. In addition, National Center for Complementary and Alternative Medicine (NCCAM), defined biofield therapies in subcategory of

Energy Therapies. Biofield Energy Therapies have been reported with significant outcomes in case of arthritis patient [8], cancer patient [9], pain and anxiety cases [10], wound healing [11], and many other clinical studies with recommendations [12]. Human Biofield is a cumulative outcome of measurable magnetic and electric field, exerted by the human body [13]. Mr. Mahendra Trivedi's unique Biofield Energy (The Trivedi Effect®) has been scientifically studied and reported with significant outcomes in living organisms and nonliving materials in a different manner. The results of The Trivedi Effect® have been reported in the field of microbiology [14-16], agriculture [17-19], livestock [20], pharmaceutical sciences [21-24], and materials sciences [25-28]. With the increased number of growing acceptances of Biofield Energy Healing as a conventional medicine, present study was designed to evaluate the effect of Biofield Energy Treatment (The Trivedi Effect®) on HFF-1 cell line and DMEM for skin health and aging potential with respect to cellular proliferation assay and effect on collagen level.

Materials and methods

Reagents

Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco, Genex Life Sciences Pvt. Ltd., India. Ethylenediaminetetraacetic acid (EDTA), trypsin, L-ascorbic acid and $NaHCO_3$ were purchased from Sigma, USA. Antibiotics solution (Penicillin-Streptomycin) was procured from HiMedia Pvt. Ltd., USA. Dimethyl sulphoxide (DMSO) was obtained from Thermo Fisher Scientific, USA. All the other chemicals used in this experiment were analytical grade procured from local vendors.

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 37ºC, 5% CO_2, and 95% humidity. The cells were sub cultured by trypsinization followed by splitting the cell suspension into fresh flasks and supplementing with fresh cell growth medium. L-ascorbic acid and FBS (positive control) was diluted in DMEM to achieve the working concentration corresponding in cell plate.

Biofield 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 (The Trivedi Effect®) under standard laboratory conditions. This Biofield Energy Healing Treatment was provided by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi, who participated in this study and performed the Biofield Energy Treatment for ~3 minutes from a distance of ~25 cm. The energy transmission was done without touching the cells and DMEM. This Biofield Energy Treatment was administered through the Healer's unique Energy Transmission process to the HFF-1 cells and DMEM under laboratory conditions. After that, the Biofield Energy Treated and untreated samples were kept in similar sealed conditions for experimental study. Following Biofield Energy Treatment, the medium and the cell line were used for estimation of *in vitro* wound healing potential using scratch assay. The Biofield Energy Treated and untreated T-25 flask were incubated till one week in a CO_2 incubator at 37°C, 5%CO_2, and 95% humidity. Besides, the treated and untreated DMEM were stored at 4°C till cell culture.

Experimental Design

Group I served as untreated cells in untreated medium (200µL of phenol-free DMEM supplemented with 10% CD-FBS) at two different time-point (i.e. t=0 and t=72 hours). Group II served as positive control (L-ascorbic acid), i.e. cells in DMEM with ascorbic acid (10 and 50µM) in cellular proliferation and for collagen estimation, while 15% FBS was used as another positive control in proliferation assay. Group III was referred as the untreated HFF-1 cells in the Biofield Energy Treated DMEM. Group IV was served as the Biofield Energy Treated HFF-1 cells in the untreated DMEM.

Estimation of Cell Growth in the Biofield Treated DMEM

HFF-1 cells were trypsin zed at the density corresponding to $5X10^3$ cells/well/180µL of growth medium followed by incubation. Further, the cell were subjected to the treatment with the Biofield Energy Treated and untreated DMEM. After treatment, the plates were kept in standard condition, which were visualized under phase contrast microscope for monitoring the cell growth at three time points, 24, 48, and 72 hours.

Assessment on Cellular Proliferation Assay

The Biofield Energy Treated HFF-1 cells were trypsin zed, counted and plated at density of $5X10^3$ cells/well/180µL of growth medium followed by overnight incubation for cell recovery and exponential growth. Further, the cells were subjected to serum starvation so as to synchronize cell growth. These cells were treated as per experimental procedure with positive controls (ascorbic acid and FBS) and test items (Biofield Energy Treated cells and DMEM) followed by the incubation for 72 hours in a CO_2 incubation at 37 °C, 5% CO_2, and 95% humidity. 20µL of 5mg/mL of MTT 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide solution was added followed by additional incubation for 3 hours at 37°C. The supernatant was aspirated and 150µL of DMSO was added to each well to dissolve the formazan crystals followed by measurement of absorbance at 540 nm using Synergy HT microplate reader.

Estimation of Collagen Synthesis

The Biofield Energy Treated HFF-1 cells as well as untreated HFF-1 cells were trypsinized, counted and plated in wells of 48-well plates at a density corresponding to $10X10^3$ cells/well/0.5mL of cell growth medium followed by overnight incubation. Further, the cells were subjected to serum starvation in order to synchronize the cell growth. These cells were treated as per experimental procedure with the positive control (ascorbic acid)

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and test item (Biofield Treated cells and DMEM) followed by the incubation for 72 hours in a CO_2 incubation at 37°C, 5% CO_2, and 95% humidity. After incubation, the plates were taken out and the amount of collagen accumulated in HFF-1 cells corresponding to each treatment group was measured by direct Sirius red dye binding assay [29,30].

Statistical Analysis

Each experiment was carried out in three independent assays and were expressed as mean values ± Standard Deviation (SD). For statistical comparison, values were subjected to one way analysis of variance (ANOVA) with Bonferroni post-test using Graphpad prism software version 4.01. Statistical significance was considered at $p<0.05$.

Results and Discussion

Cell Growth Rate

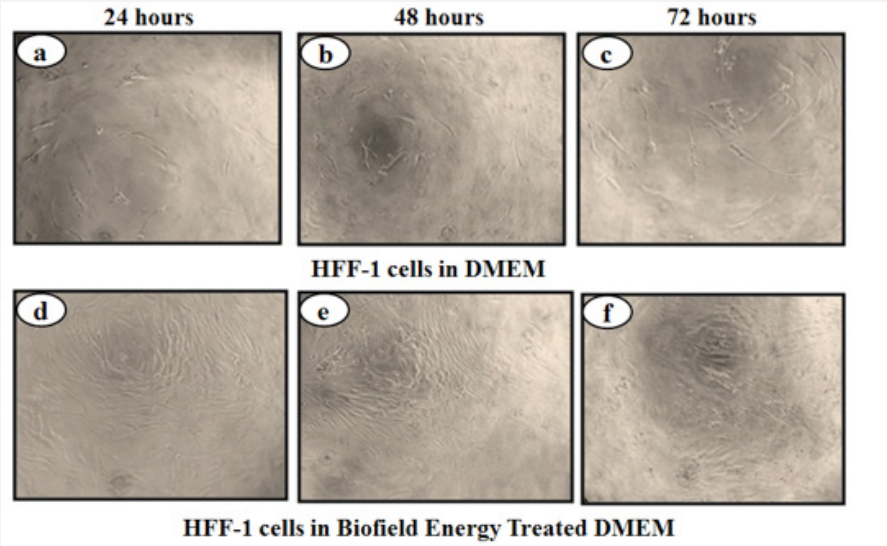

Figure 1: Representative images of HFF-1 cells growth rate in presence of DMEM at (a) 24 hours; (b) 48 hours; and (c) 72 hours; and in the presence of Biofield Energy Treated DMEM at (d) 24 hours; (e) 48 hours; and (f) 72 hours.

Dermal human fibroblast cells (HFF-1) grown in the Biofield Energy Treated DMEM demonstrated greater confluence indicating higher cell number as compared to cells grown in untreated medium (DMEM) at all the three time points. Represented images of the cells in the presence of the Biofield Energy Treated and untreated DMEM are shown in Figure 1. The results suggested that the number of cells were increased at 48 and 72 hours as compared with 24 hours interval in the presence of untreated DMEM (Figure 1a-1c). Similar pattern of growth was observed in the Biofield Energy Treated DMEM, which suggest a significant increased number of cells at 48 hours (Figure 1e) and 72 hours (Figure 1f). This suggests that Mr. Trivedi's Biofield Energy Treatment has the significant capacity to improve the cellular proliferation, which might be useful as a skin health or would work as antiaging therapy.

Cellular Proliferation Assay

After 72 hours of incubation, positive control L-ascorbic acid (10 and 50µM) and 15% FBS significantly increased the cell growth in HFF-1 cells as compared with the control group as determined by cellular proliferation assay (Figure 2) and (Table 1). The results of absorbance in different experimental groups are presented in Table 1. The positive control (10µM, L-Ascorbic acid) and 15% FBS showed a significant ($p\leq0.001$) absorbance value as 0.409 ± 0.00 and 0.453 ± 0.03 at 540 nm. Similarly, the Biofield Energy Treated DMEM showed a significant ($p\leq0.001$) change in absorbance i.e. 0.536 ± 0.01, which suggest a significant increased cellular proliferation rate. Each value represented as mean ± SD of triplicate wells. Statistical comparison was performed with untreated was conducted using One-way analysis of variance (ANOVA) with Bonferroni post-test (Graphpad prism software version 4.01). $p\leq0.001$ with respect to baseline at t=0 hour. The percentage of cellular proliferation was calculated with respect to the baseline control group value. The results of cellular proliferation in all the experimental groups are represented in (Figure 2).

Table 1: Effect of the Biofield Energy Treated DMEM and HFF-1 cells on cellular proliferation.

Group	Description	Absorbance (540 nm)
Baseline (0 hours)	Cells + DMEM	0.168 ± 0.01
Baseline (72 hours)	Cells + DMEM	0.367 ± 0.01
Positive control	10 µM L-Ascorbic acid	0.409 ± 0.00***
	50 µM L-Ascorbic acid	0.399 ± 0.01***
	15% FBS	0.453 ± 0.03***
Test	Biofield treated DMEM	0.536 ± 0.01***
	Biofield treated Cells	0.370 ± 0.01***

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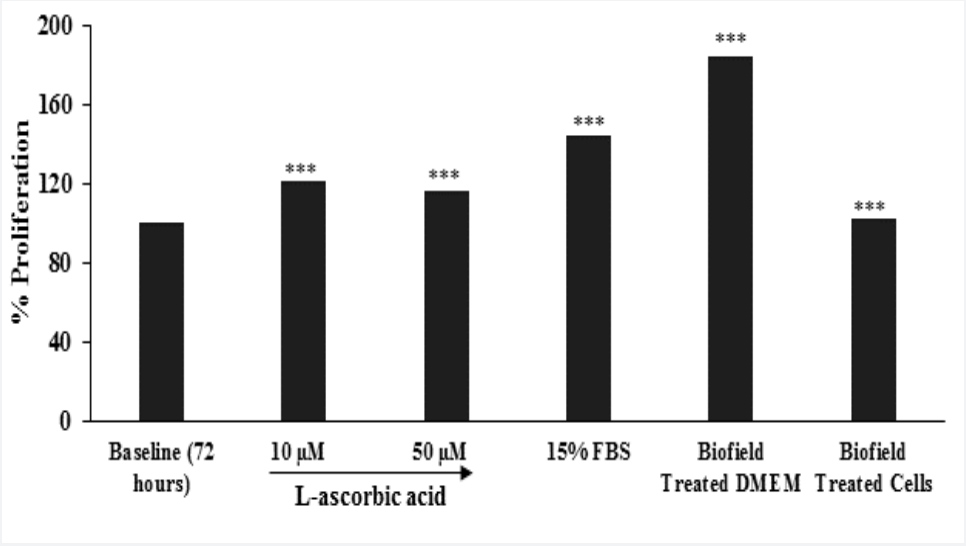

Figure 2: Effect of the Biofield Energy Treatment on HFF-1 cells and DMEM for cellular proliferation assay. Statistical comparison was performed using One way analysis of variance (ANOVA) with Bonferroni post-test (Graphpad prism software version 4.01). ***$p \leq 0.001$ with respect to baseline.

The baseline control group value on the basis of respective absorbance was tabulated as 100%, while 15% FBS groups showed a significant ($p \leq 0.001$) increase (44%) in proliferation rate. L-ascorbic acid at concentrations 10 and 50µM were observed as 21% and 16% increase in proliferation as compared with the baseline control group. Similarly, the Biofield Energy Treated DMEM showed a significant ($p \leq 0.001$) increased proliferation by 85%, while the Biofield Energy Treated cells did not show cellular proliferation, as reported with 2% increased as compared with the baseline control group. Hence, it can be concluded that the Biofield Energy Treatment (The Trivedi Effect®) on DMEM would observed with significant cellular proliferation rate as compared with the Biofield Energy Treatment on HFF-1 cell. These findings suggested that the cellular proliferation rate after the Biofield Energy Treatment was significantly increased. The significant enhanced cell proliferation and migration were reported in the Biofield Energy Treated DMEM as compared with the Biofield Energy Treated HFF-1 cells. Cell proliferation and migration are the important parameters, which can be utilized in skin health in terms of wound healing, skin regeneration potential, antiaging, etc.

Estimation of Collagen Level

Collagen level in the Biofield Energy Treated HFF-1 cells and DMEM were estimated using ascorbic acid as positive control. The results of collagen level and the percentage increase with respect to the baseline control group are summarized in Table 2. The control group cells in presence of DMEM was reported with 21.8 ± 0.92µg/mL of collagen level. Ascorbic acid at concentrations 10 and 50**µ**M showed an increased amount of collagen concentration i.e. 27.3 ± 0.32 and 26.9 ± 6.11µg/mL, respectively. The percentage increase in the collagen level in 10 and 50**µ**M ascorbic acid group was 25% and 23%, respectively as compared with the control group. Similarly, the collagen level in the Biofield Energy Treated HFF-1 cells and DMEM was found as 18.5 ± 0.64 and 31.5 ± 3.08 ($p \leq 0.01$), respectively and the change was significant in the Biofield Energy Treated DMEM as compared with the Biofield Energy Treated HFF-1 cells. However, Biofield Energy Treated DMEM showed a significant increased collagen level by 44.2%, while the Biofield Energy Treated cells showed 15.3% decreased in collagen content as compared with the baseline control group.

Table 2: Collagen level analysis in the Biofield Energy Treated HFF-1 cells and DMEM for potent anti-aging or anti-fibrotic potential.

Group	Description	Collagen (µg/mL) at 540 nm	% Increase in Collagen levels
Baseline	Cells + DMEM	21.8 ± 0.92	0
Positive control	10 µM L-Ascorbic acid	27.3 ± 0.32	25
	50 µM L-Ascorbic acid	26.9 ± 6.11	23
Test	Biofield Treated cells	18.5 ± 0.64	-15.3
	Biofield Treated DMEM	31.5 ± 3.08**	44.2

Each value represents mean ± SD of quadruplet wells. Statistical comparison with untreated was conducted using One way analysis of variance (ANOVA) with Bonferroni post-test (Graphpad prism software version 4.01). $p \leq 0.01$ with respect to the baseline values Collagen is very important in order to maintain integrity, firmness and elasticity of the body structures. The main role of collagen is to sustain the tendons, skin and cartilage. Maximizing the collagen levels would help to maintain the skin supple [31]. Collagen type 1 is present in the skin structure and one of the major constituent of dermis [32]. The important function of collagen is to provide skin

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strength and elasticity, and it was found that its degradation leads to the wrinkles results in aging. Decreased level of dermal collagen content might be due to UV exposure, which results in skin aging due to stimulated factors such as AP-1, EGF, TGF-β, IL1, and TNF-α, that affect collagen metabolism [33]. In order to maintain the skin health, various amino acids have been recommended that may prevent the skin aging by increasing the dermal collagen synthesis. Many product based on supplementation of proline and its precursors such as glutamate reported with significantly increase in collagen synthesis in human dermal fibroblast cells [34]. However, as per standard data, overall dermal collagen per unit area of the skin surface is declining approximately by 1% per year [35].

The experimental data suggest that vitamin C (ascorbic acid) as reported stimulated the formation of skin collagen possibly by increased epidermal moisture content and improved skin hydration tendency [36,37]. However, the Biofield Energy Treated DMEM significantly improved the collagen level, which could be useful in improving skin health, anti-ageing, improves skin hydration capacity, etc. Overall, it can be concluded that The Trivedi Effect® has significant capacity to improve the cellular proliferation rate and collagen content, which would be directly related to maintain the skin health. According the report of Golberg et al. [38] some specific pulsed low intensity electric field parameters was tested in rats, which results in prominent proliferation of the epidermis, microvasculature formation, and collagen secretion [38]. Biofield Energy Treatment, an energetic matrix surrounds the human body, also known as complex, dynamic, weak energy field. It might be expected that Biofield Energy Treatment might stop the partial cell necrosis due to apoptosis, cell membrane electroporation, control the oxidative damage to the skin membrane, maintain local pH changes, or by some other mechanisms and results in improved cell proliferation and collagen content. It can be assumed that The Trivedi Effect®-Consciousness Energy Healing Treatment can be used as a potent anti-aging or anti-fibrotic agent to promote the skin health.

Conclusion

Scientific data suggests that an extensive aging could be prevented, while natural might be genetically determined. The Biofield Energy Healing Treatment might be an alternative method to improve the skin health and aging. The present study showed experimental *in vitro* skin health in HFF-1 cell model, which elucidate the significant role of Biofield Energy Healing (The Trivedi Effect®) Treatment on DMEM and HFF-1 cells in cellular proliferation with improved collagen level. Cell growth rate was significantly improved in the Biofield Energy Treated DMEM at 24, 48, and 72 hours study period. Besides, cellular proliferation percentage was significantly increased ($p \leq 0.001$) by 85% in the Biofield Energy Treated DMEM as compared with the baseline control group. In addition to, significant ($p \leq 0.01$) increased collagen level by 44.2% was reported in the Biofield Energy Treated DMEM group as compared with the baseline control group. However, the Biofield Energy Treated HFF-1 cells did not showed any significant change in proliferation rate and collagen level. Hence, it can be concluded that the Biofield Energy Treatment (The Trivedi Effect®) has the potential to have significant action as wound healing, antiaging, and skin regeneration. Overall, the (The Trivedi Effect®) and the Biofield Energy Treated HFF-1 cells and DMEM can be used as a Complementary and Alternative Medicine (CAM) against skin irregularities that are typically symptoms of a skin disorders such as eczema, diaper rash, chickenpox, measles, warts, acne, hives, wrinkles, ringworm, 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.

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