

Enhancement of Hair Growth through Stimulation of Dermal Papilla Cells in Human Follicular Dermal

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Abstract

Hair gives one of the most memorable images of a person's identity. Hair loss has an enormous impact on quality of life. Although various treatment strategies are available to prevent hair loss, unfortunately not adequate. Hence, in this context authors performed this study to evaluate the impact of Consciousness Energy Healing (The Trivedi Effect®) Treatment on the test item (DMEM) in human follicular dermal papilla culture cells for the assessment of hair cell growth and development *in vitro*. The test item, DMEM was divided into three parts. First part did not receive any sort of treatment and defined as the untreated DMEM group. The second and third parts were treated with the one-time and two-times Biofield Energy Treatment by a renowned Biofield Energy Healer, Mahendra Kumar Trivedi and coded as the one-time Biofield Energy Treated DMEM (BT-I) and two-times Biofield Energy Treated DMEM (BT-II) groups, respectively. Results showed that BT-I group exhibited 14.8%, however BT-II group was significantly ($p \leq 0.001$) increased cellular proliferation of dermal papilla cells by 124.3% compared to the untreated DMEM group. The results demonstrated that the Biofield Energy Healing Treatment significantly increased the proliferation of human hair follicle dermal papilla cells. Therefore, the Consciousness Energy Healing (The Trivedi Effect®) Treatment could be useful as a hair growth enhancer against different skin injuries like actinic keratosis, necrotizing fasciitis, sebaceous cysts, diaper rash, decubitus ulcer and hair-related disorders like androgenetic alopecia (male and female pattern baldness), alopecia totalis or alopecia universalis, traction alopecia, telogen effluvium, trichodystrophy, scarring alopecias, alopecia areata, etc.

Keywords: The Trivedi Effect®; Biofield Energy Healing; Cell proliferation; Dermal papilla cells; Skin health; Hair health; Alopecia; BrdU assay

Abbreviations: CAM: Complementary and Alternative Medicine; DPCs: Dermal Papilla Cells; BT-I: One-time Biofield Energy Treated DMEM; BT-II: Two-times Biofield

Energy Treated DMEM; DMEM: Dulbecco's Modified Eagle Medium; FBS: Fetal Bovine Serum; BrdU: Bromodeoxyuridine; POD: Peroxidase.

Introduction

Hair plays many vital biological activities, which includes protection from the elements and distribution of sweat-gland products. In addition, it has an important psychosocial role in society. Hair life-cycle may be subject to abnormal changes due to seasons, fatigue, stress, hormonal imbalance, hair treatment, treatment of medication, and pollution or aging [1]. Hair loss has a great negative impact on Quality of Life. Traditional plant remedies have been used for centuries in the treatment of hair loss, but only a few have been scientifically evaluated [2]. The huge numbers of population (male and female) are suffering from baldness and also increasing day-by-day due to various factors like stress, pollution, smoking, drinking, changes of lifestyle, fast globalization, indiscriminate grow of industrialization, and advance medical therapies. Hence, it is very essential to establish new therapies that inhibit baldness or increase hair proliferation [3,4]. Alternative medical systems are the systems of theory and practice that are different from conventional therapy, both in Western cultures (*e.g.*, homeopathy, naturopathy) and in non-Western cultures (*e.g.*, Ayurvedic medicine, traditional Chinese medicine). Due to lack of scientific data and information mechanism these systems have not yet been incorporated in the conventional medical practice [5]. A lot of scientific work of literature including United State - Food and Drug Administration (US-FDA) indicated that minoxidil has potent hair growth promotion activity both animal studies as well as cell-based assay [6-8].

In recent years, several scientific reports and clinical trials have revealed the useful effects of Biofield Energy Treatment, which have shown to enhance the immune function in cases of cervical cancer patients *via* therapeutic touch [9], massage therapy [10], etc. Complementary and Alternative Medicine (CAM) therapies are now rising as preferred models of treatment, among which Biofield Therapy (or Healing Modalities) is one approach that has been reported to have several benefits to enhance physical, mental, and emotional human wellness. However, as per the data of 2012 from the National Health Interview Survey (NHIS), which indicated that the highest percentage (~18%) of the Americans used dietary supplements as a complementary health approach as compared with other practices in past years. The National Center of Complementary and Integrative Health (NCCIH) has recognized and accepted Biofield Energy Healing as a CAM health care approach in addition to other therapies, medicines and practices such as natural products, deep breathing, yoga, Tai Chi, Qi Gong, chiropractic/osteopathic

manipulation, meditation, massage, special diets, homeopathy, progressive relaxation, guided imagery, acupressure, acupuncture, relaxation techniques, hypnotherapy, healing touch, movement therapy, pilates, rolfing structural integration, mindfulness, Ayurvedic medicine, traditional Chinese herbs and medicines, naturopathy, essential oils, aromatherapy, Reiki, and cranial sacral therapy. Human Biofield Energy has subtle energy that can work effectively [11]. CAM therapies have been practiced worldwide with reported clinical benefits in different health disease profiles [12]. This energy can be harnessed and transmitted by the practitioners into both living and non-living objects *via* the process of Conscious Energy Healing Treatment. The Trivedi Effect®-Consciousness Energy Healing Treatment has been reported with a significant revolution in the physicochemical properties of metals, chemicals, ceramics, and polymers [13-15], an improved agricultural crop yield, productivity, and quality [16,17], transformed antimicrobial characteristics [18-20], biotechnology [21,22], an improved bioavailability [23-25], skin health [26,27], nutraceuticals [28,29], cancer research [30,31], bone health [32-34], human health and wellness. Considering the promising importance of alternative natural therapies-based literature information and importance of Biofield Energy Healing Treatment on various fields, the authors sought to evaluate the impact of the Biofield Energy Treatment (The Trivedi Effect®) on the test item (DMEM) for hair cells growth activity using standard assay in human follicular dermal papilla cells (HFDPC).

Materials and Methods

Chemicals and Reagents

Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco, India. 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) and ethylenediaminetetraacetic acid (EDTA) were obtained from Sigma Chemical Co. (St. Louis, MO). Minoxidil sulphate (positive control) was purchased from Clearsynth Labs Ltd., Mumbai. Antibiotics solution (penicillin-streptomycin) was procured from HiMedia, India. Other chemicals used in this study were analytical grade obtained from India.

BrdU Incorporation Cell Proliferation Assay in HFDPCs

The human follicular dermal papilla cells (HFDPCs) in DMEM supplemented with 10% FBS were counted using an hemocytometer and a single cell suspension was prepared. The single cell suspension was seeded at a

density of 800 cells/well in a fresh DMEM supplemented with 10% FBS in 96-well plates. Then, the cells were incubated in a CO₂ incubator for 24 hours at 37°C, 5%CO₂, and 95% humidity. After 24 hours of incubation, the medium was replaced with a fresh DMEM supplemented with 0.1% FBS. Further, after 24 hours, cells were treated with the test items and positive control (minoxidil sulphate). After incubation for 48 hours, the effect of the test items on cell proliferation was assessed by bromodeoxyuridine (BrdU) incorporation using colorimetric ELISA kit. For that, 10 µL of BrdU solution was added per well and the cells were incubated for 90 minutes at 37°C. After incubation, the medium was removed from each well by gentle pipetting. About 200 µL of a FixDenat solution was added to each well. After incubation, cells were incubated for 30 minutes at room temperature (RT) (15-25°C). The FixDenat solution was removed by gentle pipetting. After incubation, 100 µL of anti-BrdU-POD (peroxidase) solution was added to each well. Then, the cells were incubated for 90 minutes at RT (15-25°C). After incubation, the anti-BrdU-POD solution was removed by gentle pipetting. Each well was washed 3 times using 200 µL of washing solution. About 100 µL of substrate solution was added to each well. Cells were incubated for 30 minutes at RT (15-25°C). After incubation, the absorbance of each well was measured at 370 nm.

Cellular proliferation was determined as following Equation (1):

$$\% \text{ Cellular proliferation} = \{(B - A)/A\} \times 100 \quad (1)$$

Where, A = OD of Untreated DMEM wells

B = OD of cells treated with the test items/positive control

Experimental Design

The experimental groups composed of group 1 (G-I) with DMEM medium defined as the untreated DMEM group. Group 2 (G-II) contained positive control (minoxidil sulphate) at various concentrations. Further, group 3 (G-III) included one-time Biofield Energy Treated DMEM and denoted as BT-I, while group 4 (G-IV) included the two-times Biofield Energy Treated DMEM and denoted as BT-II.

Biofield Energy Healing Strategy

The test item, DMEM was divided into three parts. First part did not receive any sort of treatment and defined as the untreated DMEM group. The second and third parts were treated with the one-time and two-times Biofield Energy Treatment by a renowned Biofield Energy Healer (The Trivedi Effect®), Mahendra Kumar Trivedi

remotely for ~3 minutes under laboratory conditions and coded as the one-time Biofield Energy Treated DMEM (BT-I) and two-times Biofield Energy Treated DMEM (BT-II) groups, respectively. Biofield Energy Healer was located in the USA, while the test items were located in the research laboratory of Dabur Research Foundation, New Delhi, India. Healer in this study never visited the laboratory in person, nor had any contact with the test items (DMEM medium). Further, the untreated DMEM group was treated with a "sham" healer for comparative purposes. The "sham" healer did not have any knowledge about the Biofield Energy Treatment. After that, the Biofield Energy Treated and untreated test items were kept in similar sealed conditions for experimental study.

Statistical Analysis

All the values were represented as Mean ± SEM (standard error of mean) of three independent experiments. The statistical analysis was performed using SigmaPlot statistical software (v11.0). For two groups comparison student's t-test was used. For multiple group comparison, one-way analysis of variance (ANOVA) was used followed by post-hoc analysis by Dunnett's test. Statistically significant values were set at the level of $p \leq 0.05$.

Results and Discussion

BrdU Incorporated Cell Proliferation of Dermal Papilla Cells

The effect of the test items after treatment with the Biofield Energy and the results in terms of percent change in the proliferation of DPCs is shown in Figure 1. Based on the literature information the hair follicles start to form during the embryonic and matured in postnatal phase. The hair follicles are undergoing various periodic growths over lifetime. The hair cycle consists of three stages *viz.* anagen, catagen and telogen [35]. Androgens can control the proliferation of human hair based on secretion of hormone in different location. Androgen target cells are very important for the assessment of hair growth in terms of proliferation of Dermal papilla cells (DPCs) in various location such as beard, armpit, and scalp, etc. [36]. The human follicular dermal papilla cells suspension were treated with the positive control and test item (DMEM). Topical application of minoxidil is a well-established therapeutic for various types of hair growth-related disorders like alopecia [37]. The untreated DMEM group showed 100% proliferation of DPCs. Additionally, the positive control, minoxidil showed 68.57%, 187.14%, and 230.95% increased the proliferation of DPCs at 0.001, 0.01, and 0.1 µM, respectively in a concentration-

dependent manner compared to the untreated DMEM group. Moreover, the one-time Biofield Energy Treated DMEM group (BT-I) showed 14.8% and two-times Biofield Energy Treated DMEM group (BT-II) showed significantly ($p \leq 0.001$) increase the percent proliferation of DPCs by 124.3% with respect to the untreated DMEM group (Figure 1). Here, the study outcomes clearly signify that Biofield Energy Treatment significantly enhanced the percent proliferation of DPCs. The dermal papilla (DP) is a unique mesenchymal component of the hair follicle (HF) responsible for morphogenesis and regeneration of HF [38]. Hair loss (alopecia) is a general and distressing problem for both men and women. The ability of the differentiated and highly specialized fibroblasts of the DP to induce neighboring epidermal cells is a suitable approach to treat alopecia [39]. Hair follicles undergo different cycles of growth (anagen), regression (catagen), quiescence (telogen), and regeneration [40]. The hair follicle consisted of two types of cells like DP and dermal sheath (DS) that can regenerate *vis-a-vis* and possess a high level of plasticity [41]. The current experimental results revealed that Biofield Energy Treatment significantly increased the proliferation of dermal papilla cells (DPCs). From the literature point of view, it is reported that thrombin can stimulate phosphoinositide 3-kinase (PI3K)-Akt pathway-dependent acquisition of DS-like properties by DP cells *in vitro*, responsible for an increased proliferation rate [42]. Based on the literature and findings of this study, it is concluded that the high growth of DPCs is due to the impact of The Trivedi Effect® and which could be due to activation of thrombin-mediated (PI3K)-Akt pathway signaling pathway and regulation of keratinocytes during hair growth and development.

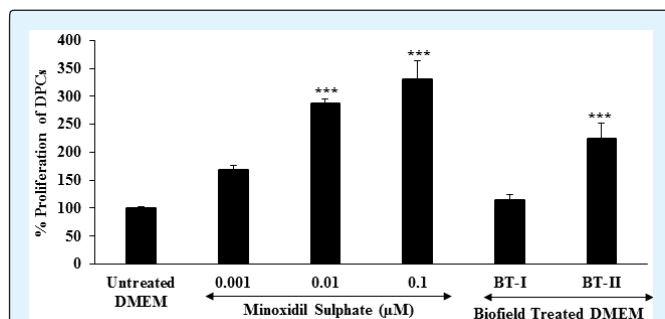


Figure 1: Effect of the test samples on hair growth regarding dermal papilla cells (DPCs) proliferation after 48 hours of treatment in immortalized human follicular dermal papilla cell line (HFDPC). BT-I: One-time Biofield Energy Treated DMEM; BT-II: Two-times Biofield Energy Treated DMEM. All the values are represented as mean \pm SEM of three independent experiments. *** $p \leq 0.001$ vs. untreated DMEM group.

The representative images are indicating the intensity of proliferative DPCs after treatment with the test item (DMEM) in HFDPC (Figure 2). Overall, data suggested that two-times Biofield Energy Treated DMEM significantly improved the growth and proliferation of human dermal papilla cells compared to the one-time Biofield Energy Treated DMEM reflected in the photograph, which might be due to the Biofield Energy Healing (The Trivedi Effect®) and the Consciousness Energy Therapy could be helpful in proliferation of hair follicles.

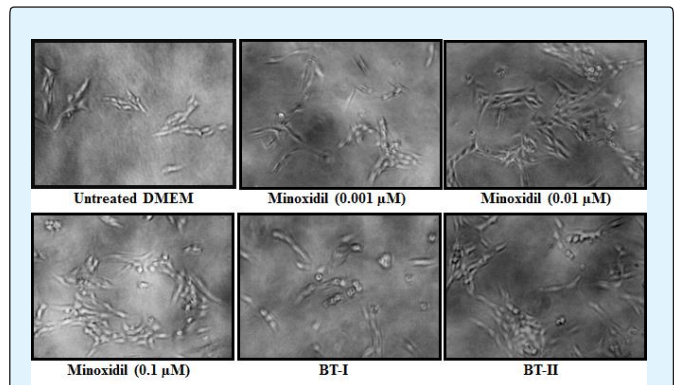


Figure 2: Effect of Biofield Energy Treated DMEM on the proliferation of dermal papilla cells (DPCs) in human follicular dermal papilla cell line (HFDPC) and the representative images of different treatment groups. BT-I: One-time Biofield Energy Treated DMEM; BT-II: Two-times Biofield Energy Treated DME.

Conclusions

The study results showed that the percent of dermal papilla cells (DPCs) was significantly elevated by 14.8% and 124.3% in the one-time Biofield Treated DMEM (BT-I) and two-times Biofield Energy Treated DMEM (BT-II) groups, respectively compared to the untreated DMEM group in human follicular dermal papilla cells (HFDPC). Overall, the Biofield Energy Treated test items significantly increased the DPCs. In conclusion, The Trivedi Effect® - Consciousness Energy Healing Treatment might act as a hair growth promoter, and it can be used as a complementary and alternative treatment for the prevention of various types of skin and hair-related disorders *viz.* necrotizing fasciitis, actinic keratosis, sebaceous cysts, diaper rash, decubitus ulcer, androgenetic alopecia, telogen effluvium, trichodystrophy, alopecia areata, etc. Besides, it could be useful to improve cell-to-cell communication, normal cell growth, cell differentiation, neurotransmission, cell

cycling and proliferation, hormonal balance, skin health, immune and cardiovascular functions. Moreover, it can also be utilized in organ transplants (i.e., kidney transplants, liver transplants and heart transplants), hormonal imbalance, aging, and various immune-related disease conditions such as Ulcerative Colitis (UC), Alzheimer's Disease (AD), Dermatitis, Irritable Bowel Syndrome (IBS), Asthma, Hashimoto Thyroiditis, Pernicious Anemia, Sjogren Syndrome, Multiple Sclerosis, Aplastic Anemia, Hepatitis, Diverticulitis, Graves' Disease, Dermatomyositis, Diabetes, Myasthenia Gravis, Parkinson's Disease, Atherosclerosis, Systemic Lupus Erythematosus (SLE), Stress, etc. with a safe therapeutic index to improve overall health and the Quality of Life.

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