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Assessment of Hair Growth Treatment with the Consciousness Energy Healing Treated Williams Medium E Using Mouse Vibrissae Hair Follicle Organ Culture

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Abstract

Hair is playing an interesting part in human for social and sexual communication. Loss of hair follicle leads to various skin disorders. For this consequence, the present study has investigated the potential of the Biofield Energy Healing (The Trivedi Effect®) Treated test item (William's Medium E) on the vibrissae hair follicle organ culture cells for the assessment of hair cell growth and development *in vitro*. The test item was divided into two parts. One part was defined as the untreated test item, where no Biofield Energy Treatment provided, while the other part was defined as the Biofield Energy Treated test item, which received the Biofield Energy Healing Treatment by renowned Biofield Energy Healer, Mahendra Kumar Trivedi. The study parameters like bulb thickness and formation of telogen were assessed using cell-based assay with the help of UTHSCSA Image tool version 3. The experimental results showed that the untreated test item group showed 20.9% and 28.2% increased bulb thickness on day 5 and 7, respectively compared to the day 1, while did not produce telogen follicles upto day 7. Besides, the percentage of telogen follicle was found as 43%, 57%, and 71% on day 3, 5, and 7, respectively of the Biofield Energy Treated test item group compared to the day 1. The overall results demonstrated that the Biofield Energy Treatment has the potential for hair growth promotion as evident *via* increased the formation of telogen. Therefore, the Biofield Energy Healing (The Trivedi Effect®) Treatment might be useful as a hair growth promoter for various treatment of skin injuries and skin-related disorders like necrotizing fasciitis, actinic keratosis, sebaceous cysts, diaper rash, decubitus ulcer etc.

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Introduction

Lots of assays are routinely used to assess hair growth, while hair follicle organ culture model is one of the most popular and influential *in vitro* systems [1]. Rodent vibrissa follicles are regular, predictable, and relatively short growth cycles. Hence, considering these properties, authors choose mouse vibrissa follicles as a test model for the assessment of hair cycle and compared morphologic changes in culture [2]. With the measurement of follicular activity concerning bulb thickness and improvement of anagen initiation, regression of catagen, and finally shifting of hair bulb *i.e.,* telogen formation is the main criteria for hair growth [3]. The hair follicle is consist of mainly two components one is epithelial components, and the others are dermal components. Hair growth is regulated by the division of the hair follicle matrix cells under control of the dermal papilla. Three different stages of hair growth can be identified, an active phase (anagen) during which hair growth occurs, an intermediate regressive (catagen) stage and a resting phase (telogen) during which no cell proliferation occurs [4]. The positive control used in this experiment *i.e.,* minoxidil because from literature reported that it can directly promote hair growth *via* the stimulation of growth factor release from adipose-derived stem cells dermal papilla and epithelial cells [5]. In recent years, several scientific reports and clinical trials have revealed the beneficial effects of Biofield Energy Treatments, which have shown to enhance immune function in cases of cervical cancer patients *via* therapeutic touch [6], massage therapy [7], 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 (17.7%) 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 efficiently [8]. CAM therapies have been practiced worldwide with reported clinical benefits in different health disease profiles [9]. This energy can be harnessed and transmitted by the experts into living and non-living things *via* the process of Biofield Energy Healing. Biofield Energy Treatment (The Trivedi Effect®) has been published in numerous peer-reviewed science journals with significant outcomes in many scientific fields such as cancer research [10, 11], microbiology [12-15], biotechnology [16, 17], pharmaceutical science [18-21], agricultural science [22-25], materials science [26-29], nutraceuticals [30, 31], skin health, human health and wellness.

Based on the 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 (William's Medium E) for hair cells growth activity with respect to the assessment of different hair growth parameters like bulb thickness and telogen formation using standard assays in vibrissae hair follicle organ culture cells with the help of UTHSCSA Image tool version 3.

Materials and Methods

Chemicals and Reagents

Insulin from bovine pancreas, hydrocortisone, vitamin B_{12}, and glucose were obtained from Sigma Chemical Co. (St. Louis, MO). Minoxidil sulphate (positive control) was purchased from Clearsynth Labs Ltd, Mumbai. L-glutamine and fungisone were procured from Gibco, India. William's Medium E (phenol-free) with growth factors were procured from HiMedia, India. Antibiotics solution (penicillin-streptomycin) and DMEM

(phenol red-free) were procured from HiMedia. All the other chemicals used in this experiment were analytical grade procured from India.

Isolation and Maintenance of Vibrissa Hair Follicles from Mice

Vibrissa hair follicles were isolated from 16 days old C57BL/6 mice by microdissection using the standard method with few modifications [32]. Briefly, both the left and right whisker pads of C57BL/6 mice were excised out and placed in a 1:1 solution of Earle's balanced salts solution and phosphate-buffered saline (PBS) supplemented with 100U penicillin per mL and 100 mg streptomycin per mL. After that, individual anagen follicles were isolated from the whisker pad and were randomized into different groups and transferred on to a 5 cm plastic petri dish containing Earle's balanced salts solution/PBS (1:1) using one dish per animal. Isolated anagen follicles were maintained in a 24-well plate in William's medium E (supplemented with growth factors) for a period of 7 days and maintained at 37 ºC at 5 % CO_2 [33]. William's Medium E (phenol-free) with growth factors was used as a test system in the present study. Vibrissae hair follicle culture was maintained under William's Medium E growth medium for routine culture supplemented with 10% FBS [34].

Experimental Design

Isolated anagen follicles were grouped into following treatment groups. Group 1 was served as untreated control (William's Medium E cells phenol-free supplemented with growth factors). Group 2 was defined as Biofield Energy Treated William's Medium E. Group 3 was denoted as the positive control, minoxidil sulphate (1 mM).

Biofield Energy Healing Approach

The William's Medium E has used a test item in this experiment. The test item was divided into two parts. One part was considered as control, where no Biofield Energy Healing Treatment was provided. Further, the control group were treated with "sham" healer for comparison purpose. The sham healer did not have any knowledge about the Biofield Energy Healing Treatment. The second part of the test item was received Biofield Energy Healing Treatment (known as The Trivedi Effect®) under laboratory conditions for ~3 minutes through Mahendra Kumar Trivedi's unique Biofield Energy Transmission process to the test item. Biofield Energy Healer in this study did not visit the laboratory, nor had any contact with the test samples. After that, the Biofield Energy Treated and untreated test items were kept in similar sealed conditions and used for the study as per the study plan.

Morphological Analysis of Vibrissa Hair Follicles

All the follicles in the well plate were observed daily through a microscope for any morphological changes. Photographs of the individual vibrissae follicles were captured during the study up to day 7. After the completion of the experiment, all the follicles treated with test item and positive control groups were measured for hair bulb thickness and compared to the respective baseline thickness of day 1 using UTHSCSA Image tool version 3.

Statistical Analysis

Data were represented as the Mean ± standard error of mean (SEM). For statistical analysis, Sigma-Plot (version 11.0) was used as a statistical tool. Statistically significant values were set at the level of $p \leq 0.05$.

Results and Discussion

Assessment of Vibrissa Hair Follicles

The hair follicle cycle occurs through a simultaneous sequence of major stages known as the stage of growing (anagen), stage of involuting (catagen), stage of resting (telogen), and finally the stage of shedding (exogen) (phase) [35, 36]. The reference standard used in this experiment, minoxidil is a well-established therapeutic for various types of hair growth-related disorders like alopecia [37]. The vibrissae hair follicle organ culture cells were treated with the positive control (minoxidil sulphate) and the untreated test item (William's Medium E). The bulb thickness of minoxidil sulphate and the untreated test item groups are depicted in Figure 1. Results showed that the bulb thickness in the positive control (minoxidil) group was 1.9 ± 0.29, 2.6 ± 0.37, and 3.3 ± 0.36 mm on day 1, 5, and 7, respectively. Moreover, the bulb thickness in the untreated test item group showed 1.5 ± 0.45, 1.8 ± 0.57, and 1.9 ± 0.60 mm on day 1, 5, and 7, respectively. Follicles treated with the positive control (minoxidil sulphate) showed an increase in hair bulb

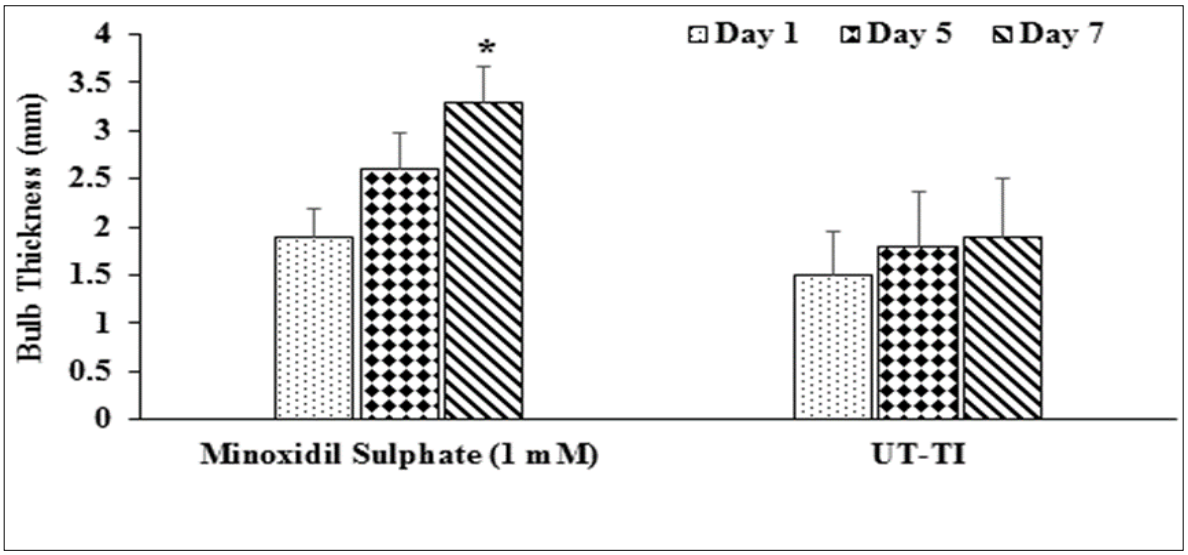

Figure 1. Assessment of hair follicle growth and development in William's Medium E in terms of bulb thickness (mm) on vibrissae hair follicle organ culture cells of minoxidil sulphate and untreated test item groups. UT-TI: Untreated test item (William's Medium E). Values are expressed as Mean ± SEM. *$p \leq 0.05$ *vs.* day 1.

thickness in follicles by 34.5% and 73.2% on day 5 and day 7, respectively compared to day 1. Further, in the untreated test item group, the follicles were restored their integrity to some extent with slightly increased the thickness of hair bulb by 20.9% and 28.2% on day 5 and 7, respectively concerning day 1 (Figure 1). Follicles were observed to have catagen-like changes with increased in hair bulb thickness in both minoxidil and untreated test item groups (Figure 3A).

Besides, the percent of telogen follicles after treatment with the Biofield Energy Treated test item (William's Medium E) on vibrissae hair follicle organ culture cells and are presented in Figure 2. The percentage of telogen follicle was remarkably elevated by 43%, 57%, and 71% on day 3, 5, and 7, respectively in the Biofield Energy Treated test item group compared to day 1 (Figure 2). On day 7, shifting of the hair shaft from its original place was observed in five out of seven follicles *i.e.,* 71%, in the Biofield Energy Treated test item group which is a hallmark of telogen transition shown in Figure 3B.

Overall, the untreated test item group did not show any telogen formation; rather it exhibited catagen formation with increased hair bulb thickness on day 7 compared to day 1. While, the Biofield Energy (The Trivedi Effect®) Treated test item significantly exhibited telogen formation up to day 7 observation, *i.e.* could be able to promote hair growth. Based on that, it is assumed that in this experiment the improvement of hair cell growth and development in terms of telogen formation could be due to the impact of The Trivedi Effect® - Biofield Energy Healing Treatment.

Briefly, the complementary and alternative therapeutic method i.e. the Biofield Energy Therapy (The Trivedi Effect®) possibly work through thought intervention and energy transmission to the molecular level, that reflects the outcomes in the Biofield Treated test item (William's Medium E) group. Here, the William's Medium E can act as a carrier for unique energy. It also assumed that the transition of telogen follicles in the Biofield Treated test item could be due to the stimulation of cell proliferation, or inhibition of collagen synthesis, and/or stimulation of vascular endothelial growth factor (VEGF) and prostaglandin (PG) synthesis, etc.

Conclusions

The study outcomes revealed that the untreated test item group showed 20.9% and 28.2% increased bulb thickness on day 5 and 7, respectively compared to the day 1, while it did not produce telogen follicle up to day 7. Besides, the Biofield Energy Treated test item group exhibited 43%, 57%, and 71% of telogen follicle

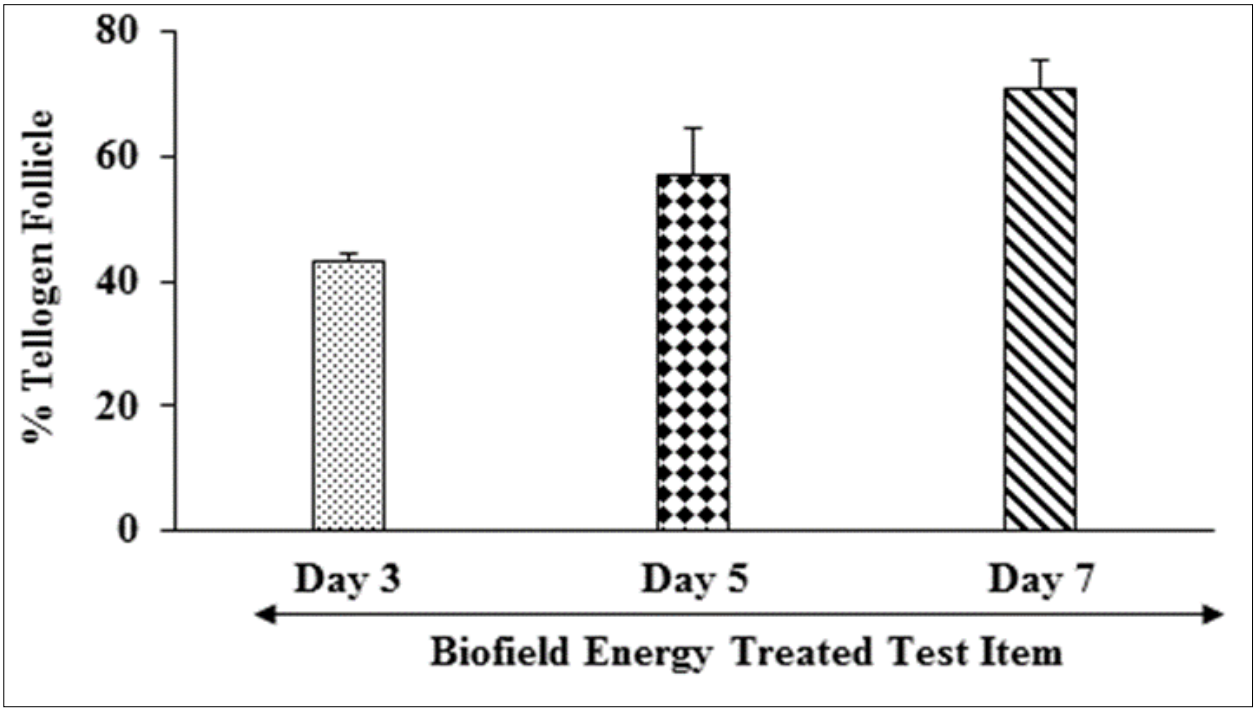

Figure 2. Effect of Biofield Energy Treatment on vibrissae hair follicle organ culture cells for the assessment of hair follicle growth and development in William's Medium E in terms of telogen follicles of Biofield Treated test item (William's Medium E).

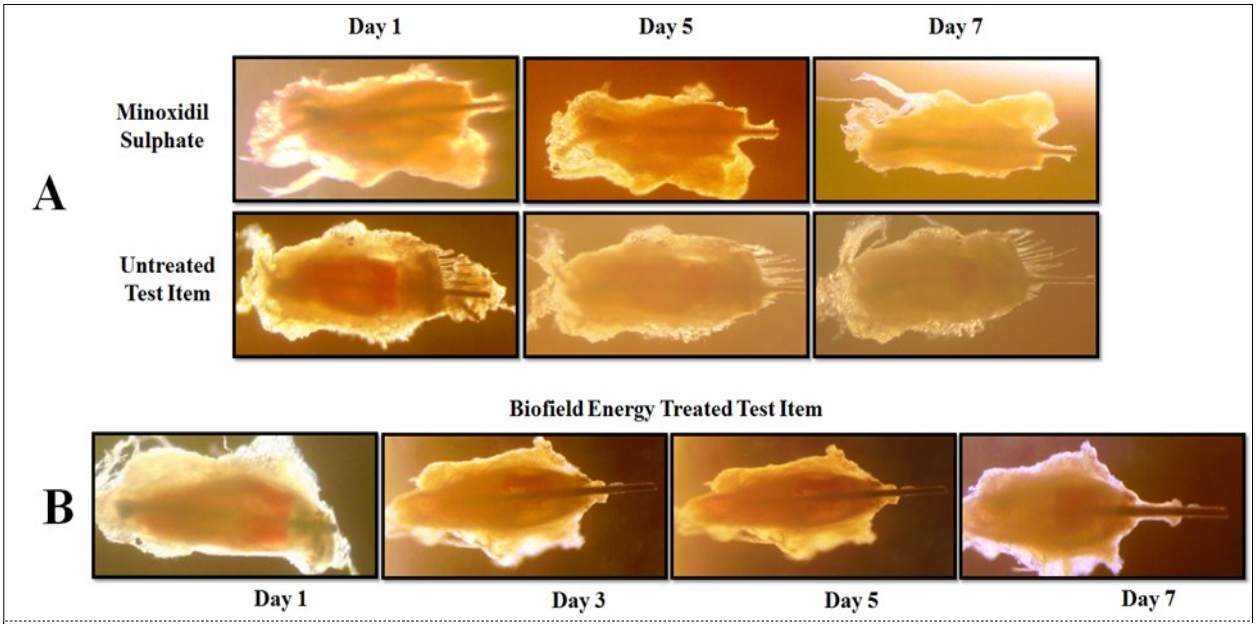

Figure 3. Representative photomicrograph of hair follicle development (anagen - catagen - telogen) of different treatment groups. A: Initiation of anagen follicle (thick hair bulb); B: Transformation of initiation, regression of hair bulb, and shifting of the hair shaft (telogen follicle) in the Biofield Energy

Treated test item (William's Medium E) group .

on day 3, 5, and 7, respectively as compared to day 1. Overall, the Biofield Energy Treated test item significantly enhanced hair follicles in terms of telogen formation compared to the untreated test item group in vibrissae hair follicle organ culture cells derived from mice. In conclusion, The Trivedi Effect® - Consciousness Energy Healing Treatment might act as an effective hair growth promoter and it can be used as a complementary and alternative treatment for the prevention of various types of skin-related disorders *viz.* necrotizing fasciitis, actinic keratosis, sebaceous cysts, diaper rash, decubitus ulcer etc. Besides, it might 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. Besides, it can also be utilized in organ transplants (for example kidney transplants, liver transplants and heart transplants), hormonal imbalance, aging, and various immune-related disease conditions such as Ulcerative Colitis, Alzheimer's Disease, Dermatitis, Irritable Bowel Syndrome, 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, stress, etc. with a safe therapeutic index to improve overall health, and quality of life.

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Abbreviations

CAM: Complementary and alternative medicine;

PBS: Phosphate-buffered saline;

DPCs: Dermal papilla cells;

UT-TI: Untreated test item

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