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## Elasticity Profile of Skin, Neuronal, Cardiac, and Skeletal Muscle Cells after Treatment with the Biofield Energy Healing-Based Proprietary Test Formulation

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#### Abstract

The present study aimed to evaluate the effect of the Trivedi Effect<sup>®</sup>- Biofield Energy Treated/Blessed Test formulation/item (TI) composed of minerals (magnesium, zinc, copper, calcium, selenium, and iron), vitamins (ascorbic acid, pyridoxine HCl, alpha tocopherol, cyanocobalamin, and cholecalciferol), Panax ginseng extract, CBD isolates, and  $\beta$ -carotene on elasticity of skin, heart, muscle, and neuronal cells in the H9C2 (rat cardiomyocytes), C2C12 (mouse myoblast cells), HaCaT (human keratinocytes), and SH-SY5Y (human neuroblastoma cells) cell line in DMEM medium. The test formulation constituents were divided into two parts; one section was defined as untreated test formulation (UT), while the other portion of test formulation received Biofield Energy Healing/Blessing Treatment (BT) by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi. The test items were treated with Biofield Energy Healing/Blessing Treatment and divided as Biofield Energy Treated/Blessed (BT) and untreated (UT) test items. MTT data showed that the test formulation in various concentrations was found as safe and nontoxic in the tested concentrations with viability range from 73% to 307%. Young's modulus (YM) is a measure of cell stiffness, a decrease in YM value indicates increase elasticity of the cells and vice-versa. YM in H9C2 cells were decreased by 9.6% and 66.1% in the BT-DMEM + UT-TI group at 0.1 and 1 µg/mL respectively, as compared with untreated test group. However, C2C21 cells showed increased YM by 443.9% at 1 µg/mL in the UT-DMEM + BT-TI group, while 869.6% increased YM in the BT-DMEM + UT-TI group at 1 µg/mL as compared with untreated test group. However, 314% increased YM was reported in the BT-DMEM + BT-TI group at 1  $\mu$ g/mL as compared with the untreated test group. However, the value of YM was significantly decreased in the HaCaT cell line by 247.7% (at 1  $\mu$ g/mL), 225.8% (at 0.1  $\mu$ g/mL), and 97.9% (at 1 µg/mL) in the UT-DMEM + BT-TI, BT-DMEM + UT-TI, and BT-DMEM + BT-TI group respectively, as compared with the untreated group. In addition, YM was significantly decreased in the SH-SY5Y cell line by 92.6%, 18.1%, and 26.6% at 1 µg/mL in the UT-DMEM + BT-TI, BT-DMEM + UT-TI, and BT-DMEM + BT-TI group respectively, as compared with the untreated group. Overall, the results showed the significant decreased YM among the SH-SY5Y, HaCaT, and H9C2 cells, while it was increased in the C2C21 cell line. Thus, the mechanical properties of cells such as cellular function, including shape, motility, differentiation, division, and adhesion to its surrounding extracellular matrix were improved. Overall, it can be useful in many disease progressions with improved cellular elasticity and its associated complications/symptoms.







Corresponding author:Snehasis Jana, Trivedi Science Research Laboratory Pvt. Ltd., Thane (W),<br/>Maharashtra, India, Tel: +91-022-25811234Keywords:Biofield Treatment, Elasticity, The Trivedi Effect<sup>®</sup>, SH-SY5Y, HaCaT, and H9C2, C2C12.Received:Apr 20, 2021Accepted: Jul 12, 2021Published: Jul 13, 2021Editor:Fuwen Yuan, Duke University, USA.

## Introduction

Elastic properties of cells play crucial role in normal physiological processes in living organisms. Cell differentiation is one of such example. In addition, aging of the cells causes changes in the cross linking of extracellular matrix components which further alters the cytoskeletal organization [1,2]. These changes are characterized by alterations in mechanical properties of the cells such as elasticity and rigidity. Elasticity of the cells can be measured by investigating deformability of the cells using techniques such as Atomic Force Microscopy (AFM) [2]. AFM is used to determine the Young's modulus (YM) which is a measure of the stiffness of the biological sample in response to an applied load [1,3, 4]. Cell and tissue stiffness (elasticity) are considered as an important characteristic of normal and diseased cellular states [5]. The present study focused on the detection of cellular elasticity using evaluation of YM using H9C2, C2C12, HaCaT, and SH-SY5Y cell lines. Thus, a novel test formulation was designed with the combination of vital minerals (Ca, Zn, Mg, Se, Fe, Cu), vitamins (B<sub>12</sub>, E, D<sub>3</sub>, C, B<sub>6</sub>), and some biological active plant-based extracts such as β-carotene, ginseng, and cannabidiol isolate (CBD). All the minerals and vitamins used in the test formulation have significant functional role to provide vital physiological role [6-8]. Besides, cannabidiol itself has wide range of pharmacological profile and was reported to role in different disorders [9, 10], while ginseng extract is regarded as the one of the best immune booster for overall immunity [11]. Vitamins are the major source that builds cellular immunity using different pathways to boost energy level. Vitamin D boosts elasticity of skin, joints, and others parts of body [12, 13]. In addition, vitamin D has also been reported to improve arterial stiffness and neuronal plasticity [14]. Cannabidiol and *Panax ginseng* are also known to increase skin elasticity [15-17]. In the present study,

effect of Biofield Energy Treatment was determined in cardiac, muscle, neuronal and skin cells.

Novel test formulation was evaluated to study the effect on the level of YM, which in terms represents cellular elasticity. Further, the test formulation and cell line media (DMEM) were treated with Biofield Energy Healing/Blessing Treatment by a renowned Biofield Energy Healer. Biofield Energy Healing Treatment, as a Complementary and Alternative Medicine (CAM) is successfully reported and accepted by National Center for Complementary/Alternative Medicine (NCCAM) against various disorders [18-21]. Various CAM therapies have been accepted by the National Centre of Complementary and Integrative Health (NCCIH) along with the Biofield Energy Healing/Blessing, such as deep breathing, Tai Chi, yoga, therapeutic touch, Reiki, chiropractic/osteopathic manipulation, relaxation techniques, pranic healing, meditation, homeopathy, Avurvedic medicine, movement therapy, mindfulness, traditional Chinese herbs and medicines in biological systems, etc. [22, 23]. However, the Trivedi Effect<sup>®</sup>-Consciousness Healing/Blessing Energy Treatment to have beneficial impact on various living and non-living things in different disciplines like materials science [24, 25], agriculture science [26], antiaging [27], gut health [28], nutraceuticals [29], pharmaceuticals [30], and overall human health and wellness. In this study, the authors sought to study the impact of the Biofield Energy Treatment (the Trivedi Effect<sup>®</sup>) on the given novel test formulation on the level of cellular elasticity in H9C2, C2C12, HaCaT, and SH-SY5Y cell lines in terms of YM.

#### **Material and Methods**

## Chemicals and Reagents

Pyridoxine hydrochloride (vitamin  $B_6$ ), calcitriol, zinc chloride, magnesium (II) gluconate, and  $\beta$ -carotene (retinol, provit A) were purchased from TCI,



Japan. Copper chloride, cyanocobalamin (vitamin B<sub>12</sub>), vitamin E (Alpha-Tocopherol), calcium chloride, cholecalciferol (vitamin D<sub>3</sub>), iron (II) sulfate, and sodium carboxymethylcellulose (Na-CMC) were procured from Sigma-Aldrich, USA. Ascorbic acid (vitamin C) and sodium selenate were obtained from Alfa Aesar, India. Cannabidiol isolate and panax ginseng extract were obtained from Panacea Phytoextracts, India and Standard Hemp Company, USA, respectively. Imipramine Hydrochloride was purchased from Sigma, USA. H9C2 (rat cardiomyocytes), C2C12 (mouse myoblast cells), HaCaT (human keratinocytes), and SH-SY5Y (human neuroblastoma cells) cell lines were procured from NCCS, Pune. Positive control, calcitriol purchased from TCI Chemicals, However, cell line medium such as DMEM, DMSO, FBS, EDTA, and MTT were procured from Genexlife, Protag Biomedical, Parshuram and Parshuram Genexlife, traders, respectively.

#### Cell Culture

H9C2, C2C12, HaCaT, and SH-SY5Y cell lines were used as test system in the present study. The cell lines were maintained in DMEM growth medium for routine culture supplemented with 10% FBS. Growth conditions were maintained as 37°C, 5%CO<sub>2</sub>, and 95% humidity and subcultured by trypsinisation followed by splitting the cell suspension into fresh flasks and supplementing with fresh cell growth medium. Three days before the start of the experiment (*i.e.*, day-3), the growth medium of near-confluent cells was replaced with fresh phenol-free DMEM, supplemented with 10% charcoal-dextran stripped FBS (CD-FBS) and 1% penicillin-streptomycin [31].

#### Experimental Design

The experimental groups consisted of cells in baseline control, vehicle control group (0.05% DMSO with Biofield Energy Treated/Blessed and untreated DMEM), positive control group (Calcitriol) and four different experimental test groups. The experimental groups included the combination of the Biofield Energy Treated/Blessed and untreated test formulation/Medium (DMEM). It consisted of four major treatment groups on specified cells with Untreated (UT)-DMEM + UT-Test item (UT-TI), UT- DMEM + Biofield Energy Treated/ Blessed test item (BT-TI), BT- DMEM + UT-TI, and



#### BT- DMEM + BT-TI.

#### Consciousness Energy Healing Strategies

The novel test formulation was consisted of zinc chloride, iron (II) sulfate, copper chloride, vitamin B<sub>6</sub>, vitamin B<sub>12</sub>, vitamin D<sub>3</sub>, sodium selenate, calcium chloride, ascorbic acid, vitamin E, beta carotene, Panax *ginseng* extract, cannabidiol isolate and magnesium (II) gluconate. Each ingredient of the test formulation was divided into two parts, one part of the test compound was not received any sort of treatment and were defined as the untreated or control sample. The second part of the test formulation was treated with the Trivedi Effect<sup>®</sup> - Energy of Consciousness Healing/Blessing Treatment (Biofield Energy Treatment) by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi under laboratory conditions for ~3 minutes in the research laboratory, Dabur Research Foundation, New Delhi, India without touching the samples. After that, the Biofield Energy Treated/Blessed samples was kept in the similar sealed condition and used as per the study plan. In the same manner, the control test formulation group was subjected to "sham" healer for ~3 minutes, under the same laboratory conditions. The "sham" healer did not have any knowledge about the Biofield Energy Treatment/Blessing. The Biofield Energy Treated/Blessed test medium was also taken back to experimental room for further culture methods.

## Determination of Non-cytotoxic Concentration

The single cell suspension of H9C2, C2C12, HaCaT, and SH-SY5Y cells were prepared in DMEM with 10% FBS. The cells were counted on a hemocytometer, while the cells were seeded with specific cell density ((5000) cells in H9C2, while 10,000 C2C12, HaCaT, and SH-SY5Y cells/well/180 µL in DMEM + 10% FBS in 96-well plates). The cells were incubated in a CO<sub>2</sub> incubator for 48 hours. After 24 hours, medium was removed, and following treatments were given in medium along with the 10% FBS in various experimental groups. After incubation for 24 hours, the effect of the test formulation on cell viability was assessed by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. About 20 µL of 5 mg/ mL of MTT was added to all the wells and incubated at 37°C for 3 hours. The cells were centrifuged to obtain the pellet. The supernatant was removed and 150 µL of



DMSO was added to all wells to dissolve formazan crystals. Further, all the wells were reported using optical density (OD) values at 540 nm using Synergy HT microplate reader. The effect of the test formulation on viability of cells was determined using equation 1.

% Cell viability = 100 - % Cytotoxicity - (1)

Where; % Cytotoxicity = {(O.D. of Control cells – O.D. of cells treated with test formulation)/ OD of Control cells} \*100

## Estimation of Cellular Elasticity

The single cell suspension of H9C2, C2C12, HaCaT, and SH-SY5Y cells were prepared in DMEM and 10% FBS using a hemocytometer. The cells were seeded density of 30,000 cells/well/0.5 mL in 48-well plates and incubated in a CO<sub>2</sub> incubator for 24 hours and 95% humidity. The cells were centrifuged to obtain the pellet. Supernatants were removed and cells were resuspended in DMEM with 10% FBS. After treatment in all the experimental test groups, the cells were I ncubated in a 5% CO<sub>2</sub> incubator for 48 hours, while H9C2 cells were incubated for 72 hours. After 24 hours of incubation, culture supernatants were collected from each well and stored at -20°C until analysis. The cells were fixed using 1% glutaraldehyde for 20 minutes, RT and processed for elasticity measurement by AFM at IISC, Bangalore. Young modulus was calculated from the force/ distance indentation curves generated during AFM analysis using XEI data processing and analysis software.

## Statistical Analysis

The data were represented as mean  $\pm$  standard error of mean (SEM) and subjected to statistical analysis using Sigma-Plot statistical software (Version 11.0). For multiple comparison One-way analysis of variance (ANOVA) followed by post-hoc analysis by Dunnett's test and for between two groups comparison Student's *t*-test was performed. The *p*≤0.05 was considered as statistically significant.

## **Results and Discussion**

## MTT Assay- Non-cytotoxic Effect of the Test Formulation

The cytotoxic effect of the test formulation was evaluated on H9C2, C2C12, HaCaT, and SH-SY5Y cells using MTT assay. The results were compared with respect to defined positive control such as calcitriol. The



## Assessment of Cellular Elasticity in H9C2 Cells

Cellular elasticity in H9C2 cells was determined using percentage change in young's modulus (YM). The cells were co-treated with the test formulation and stimulated with 0.01  $\mu$ g/mL to 10  $\mu$ g/mL for 72 hours. The level of YM was calculated in all the groups, which were compared and presented graphically (Figure 2). The decreased values of YM were calculated on the basis of the untreated test group of DMEM and test formulation. Calcitriol data showed a significant decreased value of YM by 11.1%, 48.3%, and 56.9% at 10, 100, and 500 nM concentrations, respectively. YM in all the treated experimental groups were significantly decreased as compared with the H9C2 cells in DMEM control group, which suggest an improved cellular elasticity of H9C2 cells. Furthermore, the data of the Biofield Energy Treated/untreated test formulation/ DMEM combination was compared with respect to the untreated test formulation and DMEM group. Thus, the data suggested that UT-DMEM + BT-TI group showed increase YM by 44.9% and 25.5% in the 0.01 and 0.1 µg/mL, respectively; while decreased YM by 48.8% at 1  $\mu$ g/mL as compared with the UT-DMEM + UT-TI group. Moreover, 66.1% increased the value of YM was also reported in the BT-DMEM + UT-TI group at 1 µg/mL as compared with untreated test group. Besides, BT-DMEM + BT-TI group showed a increased YM value by 96.8% and 24.9% at 0.01 and 0.1 µg/mL, respectively; while YM value was decreased by 11.5% at 1  $\mu\text{g/mL}$  as

















compared with the UT-DMEM + UT-TI group. Overall, H9C2 cells (human cardiac muscle cells) treated with Biofield Energy Treated/Blessed medium and TI showed a modulation of elasticity. Modulation in elasticity of cardiac cells (cardiomyocytes) is important for normal heart functions (contractile functions) [32, 33].

## Assessment of Cellular Elasticity in C2C12 Cells

The C2C21 cells were evaluated for cellular elasticity, which was determined using percentage change in young's modulus (YM) and the data was compared. The cells were co-treated with the test formulation and stimulated with 0.01 µg/mL to 10 µg/ mL for 48 hours. The level of YM was calculated in all the groups, which are compared and presented graphically (Figure 3). The increased values of YM were calculated on the basis of untreated test group of DMEM and test formulation. Calcitriol data showed a significant increased the value of YM by 203.8%, 525.3%, and 733.7% at 50, 100, and 500 nM concentrations, respectively as compared with the C2C21 cells in DMEM. The data of Biofield Energy Treated/untreated test formulation/DMEM combination was compared with respect to the untreated test formulation and DMEM group. The increased values of YM were calculated on the basis of untreated test group of DMEM and test formulation. Thus, the data suggested that UT-DMEM + BT-TI group showed decreased value of YM by 103.8% and 84.2% at 0.01 and 0.1 µg/mL, respectively while increased the value of YM by 443.9% at 1 µg/mL as compared with untreated test group (UT-DMEM + UT-TI group). However, 74.1% and 869.6% increased the value of YM was also reported in the BT-DMEM + UT-TI group at 0.1 and 1 µg/mL, respectively as compared with the untreated test group. Besides, BT-DMEM + BT-TI group showed an increased YM value by 314% at 1 µg/ mL as compared with the UT-DMEM + UT-TI group. Therefore, in present study increase in YM with Biofield Energy Treatment suggests an increase of differentiation of skeletal muscle cells. Muscle cells have different mechanical properties depending on the physiological processes (cell differentiation, growth, adhesion, etc.). In muscle cells, an increase in YM has been observed which correlates with the extent of differentiation [34]. Overall, the present study increase



(s) in YM after Biofield Energy Treatment/Blessing suggested increase of differentiation of skeletal muscle cells.

#### Assessment of Cellular Elasticity in HaCaT Cells

The cellular elasticity in HaCaT cells was determined using percentage change in young's modulus (YM). The cells were co-treated with the test formulation and stimulated with 0.1 µg/mL to 10 µg/ mL for 48 hours. The level of YM was calculated in all the groups, which are compared and presented graphically (Figure 4). The altered values of YM were calculated on the basis of untreated test group of DMEM and test formulation. The values of YM were calculated on the basis of the untreated DMEM and test formulation. Calcitriol data showed a significant decreased value of YM by 7.4%, 28.5%, and 52.7% at 10, 50, and 100 nM concentrations, respectively as compared with the HaCaT cells in DMEM control group. YM in all the treated experimental groups were decreased as compared with the HaCaT significantly cells in DMEM control group, which suggest an improved cellular elasticity of HaCaT cells. Furthermore, the data of Biofield Energy Treated/ untreated test formulation/DMEM combination was compared with respect to the untreated test formulation and DMEM group. Thus, the data suggested that UT-DMEM + BT-TI group showed a significant reduced the value of YM by 247.7% at 1 µg/ mL as compared with the untreated test group. However, 225.8% and 59.7% decreased the value of YM was also reported in the BT-DMEM + UT-TI group at 0.1 and 1 µg/mL, respectively as compared with the untreated test group. Besides, BT-DMEM + BT-TI group showed a significant decreased YM value by 25.9% and 97.9% at 0.1 and 1  $\mu\text{g/mL},$  respectively as compared with the UT-DMEM + UT-TI group. Thus, present study concluded that when cells were treated with Biofield Energy Treated/Blessed medium and test formulation, decrease in YM was observed, which indicates an increase in cellular elasticity. In the present study parameter, decrease in YM with Biofield Energy Treatment/Blessing suggests an increase in elasticity of keratinocytes. However, it was reported that modulation in elasticity of skin cells is important for maintenance of skin firmness and elastic properties [35], which was significant improved after Biofield







Figure 3. The effect of the test item on the level of young's modulus (YM) in C2C21 cell line after 48 hours. Vehicle control (DMSO-0.05%), UT: Untreated; BT: Biofield Energy Treated/Blessed; TI: Test Item.



Figure 4. The effect of the test item on the level of young's modulus (YM) in HaCaT cell line after 48 hours. UT: Untreated; BT: Biofield Energy Treated/Blessed; TI: Test Item



# Freely Available Online Energy Treatment/Blessing. Assessment of Cellular Elasticity in HaCaT Cells

The cellular elasticity in HaCaT cells was determined using percentage change in young's modulus (YM). The cells were co-treated with the test formulation and stimulated with 0.1  $\mu$ g/mL to 10  $\mu$ g/mL for 48 hours. The level of YM was calculated in all the groups, which are compared and presented graphically (Figure 5). The altered values of YM were calculated on the basis of untreated test group of DMEM and test formulation. The values of YM were calculated on the basis of the untreated DMEM and test formulation.

Calcitriol data showed a significant decreased value of YM by 7.4%, 28.5%, and 52.7% at 10, 50, and 100 nM concentrations, respectively as compared with the HaCaT cells in DMEM control group. YM in all the treated experimental groups were significantly decreased as compared with the HaCaT cells in DMEM control group, which suggest an improved cellular elasticity of HaCaT cells. Furthermore, the data of Biofield Energy Treated/untreated test formulation/ DMEM combination was compared with respect to the untreated test formulation and DMEM group. Thus, the data suggested that UT-DMEM + BT-TI group showed a significant reduced the value of YM by 247.7% at 1  $\mu$ g/ mL as compared with the untreated test group. However, 225.8% and 59.7% decreased the value of YM was also reported in the BT-DMEM + UT-TI group at 0.1 and 1 µg/mL, respectively as compared with the untreated test group. Besides, BT-DMEM + BT-TI group showed a significant decreased YM value by 25.9% and 97.9% at 0.1 and 1 µg/mL, respectively as compared



with the UT-DMEM + UT-TI group. Thus, present study concluded that when cells were treated with Biofield Energy Treated/Blessed medium and test formulation, decrease in YM was observed, which indicates an increase in cellular elasticity. In the present study parameter, decrease in YM with Biofield Energy Treatment/Blessing suggests an increase in elasticity of However, it was reported keratinocytes. that modulation in elasticity of skin cells is important for skin maintenance of firmness and elastic properties [36], which was significant improved after Biofield Energy Treatment/Blessing.

In this research plan, the results showed the significant improved level of cellular elasticity in H9C2, C2C12, HaCaT, and SH-SY5Y cell-lines, which helps in slowdown of the disease progression, disease-related all other symptoms/complications and also reduced the chances of disease susceptibility. This improved cellular differentiation, contractile functions, exonal extensions, and skin elasticity and firmness of the cell lines used in the study after treatment was very significant. Based on the overall data, it suggests that the Biofield Energy Healing/Blessing Therapy was found to be most effective and benefited in order to prevent and protect from the occurrence of any type of diseases. It can also be used as significant way for energy boosting in various disease states that ultimately improve the overall health and guality of life in human.

#### Conclusions

Cellular elasticity of the cell lines such as H9C2, C2C12, HaCaT, and SH-SY5Y were used and the effect of test formulation was evaluated at different





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concentrations. Young's modulus (YM) was calculated using AFM analysis using XEI data processing and analysis software. YM showed a measure of cell stiffness, a decrease in YM value indicates increase elasticity of the cells and vice-versa. MTT analysis for evaluation the non-toxic concentrations were evaluated and data showed that the test formulation in various concentrations was found as safe and nontoxic in the tested concentrations with viability range 73% to 307%. Cellular elasticity using YM was calculated in H9C2 cells, which showed a significant decreased YM values by 9.6% and 66.1% in the BT-DMEM + UT-TI group at 0.1 and 1 µg/mL respectively, as compared with untreated test group. However, C2C21 cells showed increased YM by 443.9%, 869.6%, and 314% at 1 µg/mL in the UT-DMEM + BT-TI, BT-DMEM + UT-TI, and BT-DMEM + BT-TI group, respectively as compared with the untreated test group. Similarly, YM in the HaCaT cell was significantly decreased by 247.7% (at 1 µg/mL), 225.8% (at 0.1 µg/mL), and 97.9% (at 1 µg/mL) in the UT-DMEM + BT-TI, BT-DMEM + UT-TI, and BT-DMEM + BT-TI group respectively, as compared with the untreated group. Furthermore, SH-SY5Y cell line also showed a significantly decreased YM by 92.6%, 18.1%, and 26.6% at 1 µg/mL in the UT-DMEM + BT-TI, BT-DMEM + UT-TI, and BT-DMEM + BT-TI group respectively, as compared with the untreated group. Overall, the Biofield Energy Treated (the Trivedi Effect<sup>®</sup>) test formulation showed a significant improved cellular elasticity in the tested cell lines, which play a vital role in maintaining various immune and life style related disorders, increase alertness, energy, attention, allergy, Alzheimer, cardiovascular, cancer, diabetes, eye, immune, inflammatory, or Parkinson. Therefore, the Consciousness Energy Healing based test formulation might be suitable alternative nutritional supplement, which could be useful for the management of various immune related disorders. Thus, in conclusion this therapy might also reduce the severity of many acute/ chronic diseases and its progression rate.

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