International Journal of Research Studies in Medical and Health Sciences
Volume 3, Issue 9, 2018, PP 18-24
ISSN : 2456-6373

Estimation of Collagen Synthesis in Human Dermal Fibroblast Cells: an Impact of Consciousness Energy Healing Treatment

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ABSTRACT

Human dermal fibroblasts are defined for skin strength and resilience by continuously secreting extracellular matrix (ECM) components, which are responsible for promoting skin rejuvenation and remodeling. Precisely, the branched collagen fibrils provide the skin with its shape and firmness. The objective of the present study was to assess the potential 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 using in vitro model. An increase in cellular proliferation and collagen levels were considered as markers for skin health promoting effects. An aliquot of HFF-1 cells and DMEM were divided into two equal parts, one was treated with the Biofield Energy Treatment and denoted as treated test sample, while other part was coded as the untreated test sample. MTT assay was used to study the cellular proliferation and collagen level was estimated by collagen dye complex formation using Biotek Synergy HT microplate reader. The HFF-1 cellular proliferation rate was significantly increased by 85% in the Biofield Energy Treated DMEM as compared with Biofield Treated cells. Similarly, the collagen level was significantly ($p \leq 0.01$) increased by 44.2% in the Biofield Energy Treated DMEM as compared with control. Hence, the result suggests a significant improvement of collagen level 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 activities in HFF-1 cell line.

Keywords: *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 rhytides [1]. Internal and external aging factors are very complex biological processes that results in skin aging. Intrinsic factors includes genetics factors, hormone, cellular metabolism, and metabolic processes, while external factors includes 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 decreasing 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 to, various photoaging products, antioxidants, pharmacological agents that have anti-aging properties (*i.e.* vitamin B_3, 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, study outcome suggests 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].

The 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 that the 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 acceptance 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

Materials and 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 Hi-Media 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 locally in India.

Cell Culture Preservation (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 DMEM growth medium supplemented with 15% FBS, with added antibiotics penicillin (100 U/mL) and streptomycin (100 µg/mL). The growth condition of cell line was at 37ºC, 5% CO_2, and 95% humidity. The cells were sub-cultured by trypsinisation followed by splitting the cell suspension into fresh flasks and supplementing with fresh cell growth medium. L-ascorbic acid and FBS (positive control) were diluted in DMEM to achieve the working concentration corresponding in cell plate.

Biofield Energy Healing Treatment 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. Further, 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 collagen level. 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 4ºC till cell culture.

Experimental Design

Group I served as the untreated cells in untreated medium (200 µL 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 the positive control (L-ascorbic acid), *i.e.* cells in DMEM with ascorbic acid (10 and 50 µM) 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 Biofield Energy Treated HFF-1 cells in the untreated DMEM.

Assessment of the Effect of Biofield Energy Treatment on Cellular Proliferation

The Biofield Energy Treated HFF-1 cells were trypsinized, counted and plated at density of 5 X 10^3 cells/well/180 µL 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 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. About 20 µL of 5 mg/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 150 µL of DMSO was added to each well to dissolve the formazan crystals followed by the 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 the untreated HFF-1 cells were trypsinized, counted and plated in wells of 48-well plates at a density corresponding to 10 X 10^3 cells/well/0.5 mL 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) and test item (the 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-hoc analysis using GraphPad prism software version 4.01. Statistical significance was considered at $p<0.05$.

Results and Discussion

MTT Assay

The results of percentage change in cellular proliferation after treatment with the Biofield Energy Treated and untreated test samples after 72 hours of incubation showed significantly increased cell growth in HFF-1 cells as compared with the control group as determined by cellular proliferation assay (Figure 1). The percentage of cellular proliferation was calculated with respect to the baseline control group. Positive control groups such as 15% FBS group showed a significantly ($p\leq0.001$) increased the cellular proliferation by 44%. L-ascorbic acid at concentrations 10 and 50 µM were observed with 21% and 16% increased in cellular proliferation, respectively as compared with the baseline control group. Similarly, the Biofield Energy Treated DMEM showed a significant ($p\leq0.001$) increased cell 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 observed with significant cellular proliferation rate as compared with the Biofield Energy Treatment on HFF-1 cell.

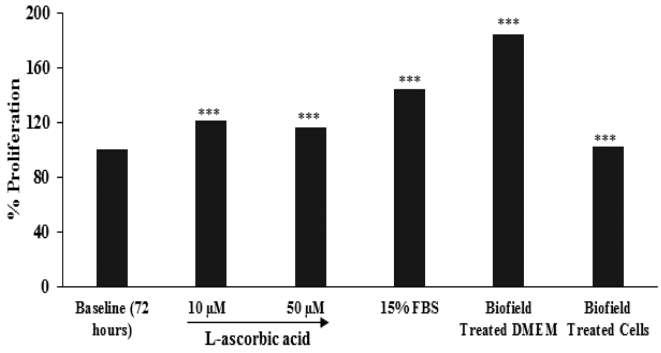

Figure1. *Effect of the Biofield Energy Treatment on HFF-1 cells and DMEM for cellular proliferation assay. Statistical comparison was performed using One-way analysis of variance (ANOVA) with Bonferroni post-hoc analysis (GraphPad prism software version 4.01).* $^{***}p \leq 0.001$ *with respect to baseline.*

These experimental data supports that the cellular proliferation rate after the Biofield Energy Treatment was increased, which can be useful in many skin-related disorders. The significant enhanced of cell proliferation and cell growth rate were reported in the Biofield Energy Treated DMEM group as compared with the Biofield Energy Treated HFF-1 cells. The cell proliferation and cell growth rates are the important parameters, which can be utilized in skin health in terms of wound healing, skin regeneration potential, antiaging, etc.

Estimation of Collagen

Collagen level in the Biofield Energy Treated HFF-1 cells and DMEM were estimated using ascorbic acid as the 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 ± 0.92 µg/mL of collagen level. Ascorbic acid at concentrations 10 and 50 µM showed an increased amount of collagen concentration *i.e.* 27.3 ± 0.32 and 26.9 ± 6.11 µg/mL, respectively. The percentage increase in the collagen level at 10 and 50 µM 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, the Biofield Energy Treated DMEM showed a significantly increased the collagen level by 44.2%, while the Biofield Energy Treated cells showed 15.3% altered in collagen content as compared with the control group.

Table1. *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-tests (GraphPad prism software version 4.01). **p≤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 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 products based on the

supplementation of proline and its precursors such as glutamate reported with significantly increased 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 suggests that vitamin C (ascorbic acid) as reported stimulated the formation of skin collagen possibly by increased epidermal moisture content and an 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 the significant capacity to improve the cellular proliferation rate and collagen content, which would be directly related to maintain the skin health.

CONCLUSIONS

Scientific data suggests that an extensive aging could be prevented, while natural might be genetically determined. The Biofield Energy Healing might be an alternative method to improve the skin health and aging. The present study showed that the 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 along with an improved collagen level. The cellular proliferation rate using MTT assay was significantly increased ($p \leq 0.001$) by 85% in the Biofield Energy Treated DMEM group as compared with the baseline control group. Additionally, a significantly ($p \leq 0.01$) increased the 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 show 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 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.

ACKNOWLEDGEMENTS

Authors are grateful to Dabur Research Foundation, Trivedi Global, Inc., Trivedi Science, Trivedi Testimonials and Trivedi Master Wellness for their support throughout the work.

REFERENCES

[1] Ganceviciene R, Liakou AI, Theodoridis A, Makrantonaki E, Zouboulis CC (2012) Skin anti-aging strategies. Dermatoendocrinol. 4: 308-319.

[2] Farage MA, Miller KW, Elsner P, Maibach HI (2008) Intrinsic and extrinsic factors in skin ageing: A review. Int J Cosmet Sci 30: 87-95.

[3] Naylor EC, Watson RE, Sherratt MJ (2011) Molecular aspects of skin ageing. Maturitas 69: 249-256.

[4] Loesch MM, Somani A-K, Kingsley MM, Travers JB, Spandau DF (2014) Skin resurfacing procedures: New and emerging options. Clin Cosmet Investig Dermatol 7: 231-241.

[5] Baumann L (2007) Skin ageing and its treatment. J Pathol 211: 241-251.

[6] Uitto J (1997) Understanding premature skin aging. N Engl J Med 337: 1463-1465.

[7] Dorai AA (2012) Wound care with traditional, complementary and alternative medicine. Indian J Plast Surg 45: 418-424.

[8] Peck SD (1998) The efficacy of therapeutic touch for improving functional ability in elders with degenerative arthritis. Nurs Sci Q 11: 123-132.

[9] Giasson M, Bouchard L (1998) Effect of therapeutic touch on the well-being of persons with terminal cancer. J Holist Nurs 16: 383-398.

[10] Turner JG, Clark AJ, Gauthier DK, Williams M (1998) The effect of therapeutic touch on pain and anxiety in burn patients. J Adv Nurs 28: 10-20.

[11] Schlitz M, Hopf HW, Eskenazi L, Vieten C, Radin D (2012) Distant healing of surgical wounds: An exploratory study. Explore (NY) 8: 223-230

[12] Jain S, Hammerschlag R, Mills P, Cohen L, Krieger R, Vieten C, Lutgendorf S (2015) Clinical studies of biofield therapies: summary, methodological challenges, and recommendations. Glob Adv Health Med 4: 58-66.

[13] Movaffaghi Z, Farsi M (2009) Biofield therapies: Biophysical basis and biological regulations. Complement Ther Clin Pract 15: 35-37, 31.

[14] Trivedi MK, Patil S, Shettigar H, Gangwar M, Jana S (2015) Antimicrobial sensitivity pattern of *Pseudomonas fluorescens* after biofield treatment. J Infect Dis Ther 3: 222.

[15] Trivedi MK, Patil S, Shettigar H, Bairwa K, Jana S (2015) Phenotypic and biotypic characterization of *Klebsiella oxytoca*: An impact of biofield treatment. J Microb Biochem Technol 7: 203-206.

[16] Trivedi MK, Patil S, Shettigar H, Bairwa K, Jana S (2015) Effect of biofield treatment on phenotypic and genotypic characteristic of *Provindencia rettgeri*. Mol Biol 4: 129.

[17] Patil SA, Nayak GB, Barve SS, Tembe RP, Khan RR (2012) Impact of biofield treatment on growth and anatomical characteristics of *Pogostemon cablin* (Benth.). Biotechnology 11: 154-162.

[18] Nayak G, Altekar N (2015) Effect of biofield treatment on plant growth and adaptation. J Environ Health Sci 1: 1-9.

[19] Shinde V, Sances F, Patil S, Spence A (2012) Impact of biofield treatment on growth and yield of lettuce and tomato. Aust J Basic Appl Sci 6: 100-105.

[20] Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC (2015) Effect of biofield treated energized water on the growth and health status in chicken (*Gallus gallus domesticus*). Poult Fish Wildl Sci 3: 140.

[21] Trivedi MK, Branton A, Trivedi D, Nayak G, Panda P, Jana S (2016) Gas Chromatography-mass spectrometric analysis of isotopic abundance of ^{13}C, ^{2}H, and ^{18}O in biofield energy treated p-tertiary butylphenol (PTBP). American Journal of Chemical Engineering 4: 78-86.

[22] Trivedi MK, Branton A, Trivedi D, Nayak G, Sethi KK, Jana S (2016) Gas chromatography-mass spectrometry based isotopic abundance ratio analysis of biofield energy treated methyl-2-napthylether (Nerolin). American Journal of Physical Chemistry 5: 80-86.

[23] Trivedi MK, Branton A, Trivedi D, Nayak G, Panda P, Jana S (2016) Isotopic abundance ratio analysis of 1,2,3-trimethoxybenzene (TMB) after biofield energy treatment (The Trivedi Effect®) using gas chromatography-mass spectrometry. American Journal of Applied Chemistry 4: 132-140.

[24] Trivedi MK, Branton A, Trivedi D, Nayak G, Sethi KK, Jana S (2016) Determination of isotopic abundance ratio of biofield energy treated 1,4-dichlorobenzene using gas chromatography-mass spectrometry (GC-MS). Modern Chemistry 4: 30-37.

[25] Trivedi MK, Tallapragada RM, Branton A, Trivedi D, Nayak G, Latiyal O, Jana S (2015) The Potential impact of biofield energy treatment on the atomic and physical properties of antimony tin oxide nanopowder. American Journal of Optics and Photonics 3: 123-128.

[26] Trivedi MK, Tallapragada RM, Branton A, Trivedi D, Nayak G, Latiyal O, Jana S (2015) Characterization of physical and structural properties of aluminium carbide powder: impact of biofield treatment. J Aeronaut Aerospace Eng 4: 142.

[27] Trivedi MK, Nayak G, Patil S, Tallapragada RM, Latiyal O, Jana S (2015) Impact of biofield treatment on atomic and structural characteristics of barium titanate powder. Ind Eng Manage 4: 166.

[28] Trivedi MK, Tallapragada RM, Branton A, Trivedi D, Nayak G, Latiyal O, Jana S (2015) Evaluation of physical and structural properties of biofield energy treated barium calcium tungsten oxide. Advances in Materials 4: 95-100.

[29] Junqueira LC, Bignolas G, Brentani RR (1979) Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. Histochem J 11: 447-455.

[30] Junquiera LC, Junqueira LC, Brentani RR (1979) A simple and sensitive method for the quantitative estimation of collagen. Anal Biochem 94: 96-99.

[31] Kligman LH (1989) Photoaging. Manifestations, prevention, and treatment. Clin Geriatr Med 5: 235-251.

[32] Takema Y, Hattori M, Aizawa K (1996) The relationship between quantitative changes in collagen and formation of wrinkles on hairless mouse skin after chronic UV irradiation. J Dermatol Sci 12: 56-63.

[33] Baumann L (2007) Skin ageing and its treatment. J Pathol 211: 241-251.

[34] Karna E, Miltyk W, Wolczynski S, Palka JA (2001) The potential mechanism for glutamine-induced collagen biosynthesis in cultured human skin fibroblasts. Comp Biochem Physiol B Biochem Mol Biol 130: 23-32.

[35] Shuster S, Black MM, McVitie E (1975) The influence of age and sex on skin thickness, skin collagen and density. Br J Dermatol 93: 639-643.

[36] Fusco D, Colloca G, Lo Monaco MR, Cesari M (2007) Effects of antioxidant supplementation on the aging process. Clin Interv Aging 2: 377-387.

[37] Marini A (2011) [Beauty from the inside. Does it really work?] Hautarzt 62: 614-617.

Citation: *Mahendra Kumar Trivedi et.al, "Estimation of Collagen Synthesis in Human Dermal Fibroblast Cells: an Impact of Consciousness Energy Healing Treatment". International Journal of Research Studies in Medical and Health Sciences. 2018; 3(9):18-24.*