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The Implication of Biofield Energy
Treated DMEM on Bone-Health
Assessment Using Osteoblast CellsAlice Branton¹ and Snehasis Jana^{2*}¹Trivedi Global, Inc., Henderson, USA²Trivedi Science Research Laboratory Pvt. Ltd., Bhopal, India

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CC-BY 4.0**Keywords** Biofield energy; ALP;
Osteosarcoma cells; DMEM; Bone
health; Mg-63**Abbreviations** CAM: Complementary
and Alternative Medicine; NCCAM:
National Center for Complementary and
Alternative Medicine; MG-63: Human
Bone Osteosarcoma Cells; ALP: Alkaline
phosphatase; DMEM: Dulbecco's
Modified Eagle's Medium; FBS: Fetal
Bovine Serum

Abstract

Nutrition plays a crucial role for healthy growth and development of the skeleton system. Study objective was to find the effect of Biofield Treatment on test item (DMEM medium) for bone health activity in human bone osteosarcoma cell line (MG-63) cells. After divided of the test item into two parts, one part was treated by Alice Branton's Biofield Energy i.e., Biofield Treated DMEM and others part kept as such i.e., untreated DMEM, respectively. MTT assay showed cell viability was observed more than 74%, which signify with safe and non-toxic profile of the test item on MG-63 cells. Further, the bone health parameter, level of ALP was significantly ($p \leq 0.001$) increased by 40.58% in the Biofield Treated DMEM compared to untreated DMEM. In conclusion, the experimental data suggested that the Biofield Energy Treatment have some effect on the growth and development of bone cells, and could be useful against certain bone-related disorders like osteomalacia, osteoma, fractures, rickets, osteoporosis, stress, and aging, etc.

Introduction

Bone is a remarkable tissue with a functional structure (the skeleton), which is capable to withstand the intense physical activity or low to the changes in activity with efficient movement. The skeleton system is one of the major physiological metabolic systems [1]. Skeleton system has dynamic tissue, which protects damage in various vital organs with mechanical forces. It acts as levers that transmit the mechanical force from one area of the body to another through muscle-driven motion. Metabolically, the skeleton contains abundant blood cell types and is the major calcium reservoir of the body. Vitamin D₃ is very essential for the maintenance of a healthy mineralized bone skeleton. However, sunlight, plants, irradiation of other foods cod liver oil, etc. are found to be effective against bone-related disorders, which lead to discovering the active principle-vitamin D [2]. Besides nutritional factors, vitamin D aids in absorption and utilization of calcium, thus regarded as one of the important factors for good bone health. In addition, vitamin D has multiple effects that regulate the functions in different organs such as heart, skeletal, brain, lungs, reproductive systems, liver, immune, kidneys, etc. Moreover, calcium and vitamin D play significant roles as anti-inflammatory, anti-arthritis, anti-osteoporosis, anti-stress, anti-aging, anti-apoptotic, and wound healing, anti-cancer, anti-psychotic, and anti-fibrotic roles [3-4]. The deficiency of calcium and vitamin D is the major health problem, which causes metabolic bone disease in the young and elderly populations [5]. Apart from vitamin and calcium, dietary protein is one of the major constituents in maintain good bone health, however selective deficiency in dietary proteins results to be reported in a significant deterioration in bone mass, microarchitecture and strength, which results in osteoporosis [6]. Besides, nutritional factors physical activity plays a major role in bone health throughout life. It helps to increase or preserve bone mass and to reduce the risk of falling. All types of physical activity can contribute to bone health in different ways. Body weight also plays a major role in maintaining the bone mass and density in bone health throughout life. However, underweight increases the risk of fracture and bone loss [7]. Thus, in order to estimate the bone health using *in vitro* studies in cell lines and its resorbing effects using important key biomarkers, such as alkaline phosphatase (ALP) has been a well-established model. MG-63 cells obtained from juxtacortical osteosarcoma cells in long-term culture. The response of 1, 25-dihydroxyvitamin D₃ (1, 25(OH)₂D₃) on MG-63 cells is similar to normal human osteoblast cells [8]. Hence, MG-63 cells are widely used for studying the potential of any test compounds to improve bone health [9]. A new bone formation is a complex event of differentiation and proliferation of osteoblasts. ALP is a crucial biomarker for the early differentiation and maturation of osteoblast cells. It increases the concentration of local inorganic phosphate that is required for bone mineralization and hence is an important marker for osteogenic activity [10]. In this context, the authors evaluated the effect of Biofield Treatment on the test item (DMEM) for bone health using MG-63 cells ALP as a biomarker.

Every living organism contains subtle types of unique energy known as “Biofield Energy”. The nature of this energy is a para-dimensional, infinite, and an electromagnetic field that is surrounding the human body. Complementary and Alternative Medicine (CAM) therapies are widely used with significant benefits in the healthcare system. Many energy healing practices have been widely demonstrated significant findings in the healthcare system. The CAM therapies includes therapeutic touch, external qigong, yoga, Johrei, Qi Gong, Reiki, Tai Chi, polarity therapy, deep breathing, pranic healing, chiropractic/osteopathic manipulation, meditation, guided imagery, massage, homeopathy, progressive relaxation, hypnotherapy, acupressure, special diets, relaxation techniques, acupuncture, Rolfing structural integration, movement therapy, Ayurvedic medicine, healing touch, pilates, mindfulness, traditional Chinese herbs and medicines in biological systems both *in vitro* and *in vivo* [11]. Biofield Energy contains putative bioenergy, which can be channeled by a renowned practitioner. “Biofield Energy Healing” has been proven as CAM and manifested excellent outcomes in various biological studies [12]. On the other side, the National Center for Complementary and Alternative Medicine (NCCAM) well-defined that the Biofield therapies has been enlisted under the subcategory of Energy Therapies [13]. Biofield Treatment has been demonstrated with a significant revolution in the physicochemical properties of polymers, metals, and ceramics [14-16], improved quality of agricultural crops and yield [17-18], transformed antimicrobial phenotypic as well as genotypic structure [19-21], biotechnology [22,23], improved bioavailability [24-26], skin health [27, 28], nutraceuticals [29,30], cancer research [31,32], human health and wellness, and bone health [33-35]. On the basis of significant results of Biofield Energy Treatment, authors planned to evaluate the effect of the Biofield on DMEM for bone health activity in MG-63 cells.

Material and Methods

Chemicals and Reagents

Penicillin and streptomycin were procured from HiMedia, India, while 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium (MTT), Direct Red 80, and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma, USA. Rutin hydrate was purchased from TCI, Japan. Dulbecco’s Modified Eagle’s Medium (DMEM) and fetal bovine serum (FBS) were purchased from Life Technology, USA. All the other chemicals used in this experiment were analytical grade procured from India.

Maintenance of MG-63 in Culture Medium

Human bone osteosarcoma cell line (MG-63) was used as test system in this experiment. It was maintained in DMEM growth medium for routine culture supplemented with 10% FBS. Growth conditions were maintained at 37°C, 5%CO₂, and 95% humidity and subcultured by trypsinisation followed by splitting the cell suspension into new flasks with new 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 [34].

Experimental Design

The experimental groups consisted of group 1 (G-I) with cells in untreated DMEM. Group 2 (G-II) consisted of positive control (rutin hydrate) at non-cytotoxic concentrations. Further, group 3 (G-III) included Biofield Treated DMEM.

Consciousness Energy Healing Treatment Strategies

The test item, DMEM medium was divided into two parts. One part each of the test item was treated with the Biofield Energy by a renowned Biofield Energy Healer, Alice Branton and coded as the Biofield Energy Treated DMEM, while the second part did not receive any sort of treatment and referred as the untreated DMEM group. This Biofield Energy Healing Treatment was provided by Alice Branton remotely for ~5 minutes. Biofield Energy Healer was located in the USA, while the test item was located in the research laboratory of Dabur Research Foundation, New Delhi, India. This Biofield Energy Treatment was administered through the Healer’s unique Energy Transmission process remotely to the test sample under laboratory conditions. Healer, in this study never visited the laboratory in person, nor had any contact with the test item. Further, the untreated DMEM 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 samples were kept in similar sealed conditions for experimental study.

MTT Assay for the Assessment of Non-cytotoxic Concentration

For the evaluation on non-cytotoxic concentration of the test items (untreated and Biofield Treated DMEM) the MTT cell viability assay was performed in human bone osteosarcoma cell line (MG-63) as per Trivedi et al. 2018 [34-36]. The percentage cytotoxicity of the test items were calculated with the help of Equation (1):

$$\% \text{ Cytotoxicity} = (1 - X/R) * 100 \text{ ----- (1)}$$

Where, X = Absorbance of treated cells; R = Absorbance of untreated cells

The percentage cell viability corresponding to each treatment was calculated with the help of Equation (2):

$$\% \text{ Cell Viability} = (100 - \% \text{ Cytotoxicity}) \text{ ----- (2)}$$

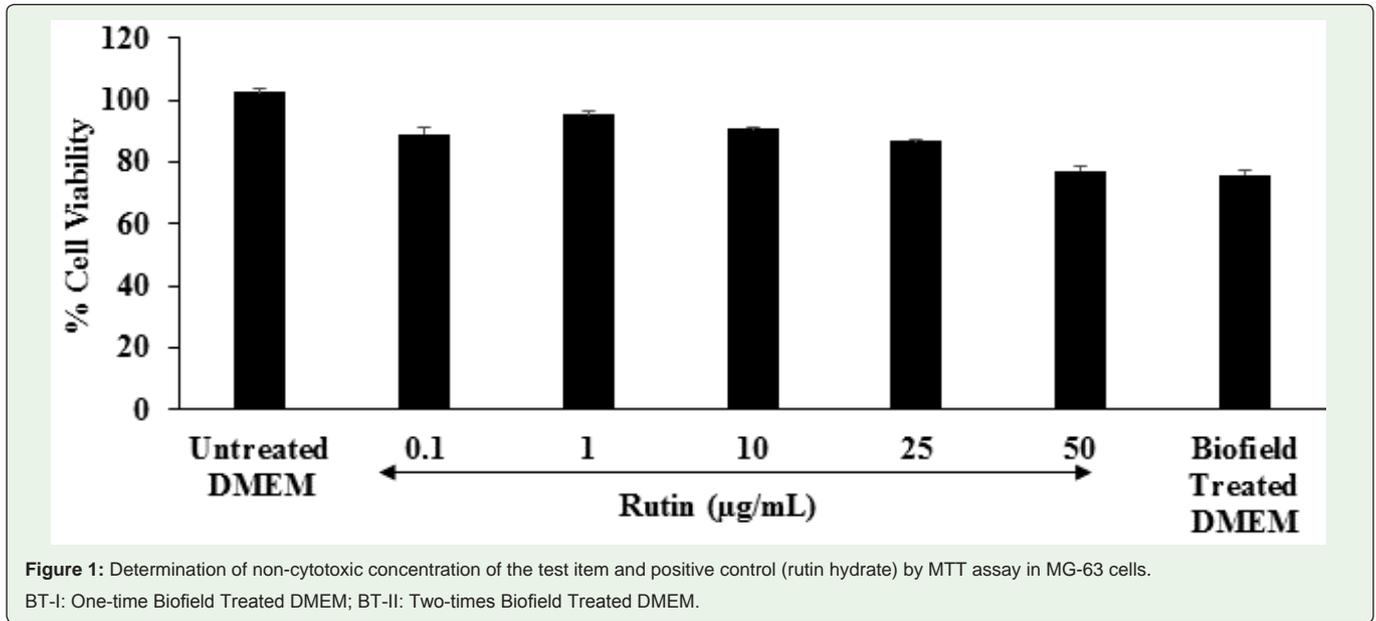
The concentrations $\geq 70\%$ cell viability was considered as safe and non-toxic.

Alkaline Phosphatase (ALP)

Evaluation of alkaline phosphatase (ALP) activity of the untreated and Biofield Treated DMEM in human bone osteosarcoma cell line (MG-63) was conducted as per Trivedi et al. 2018 [33-34]. The level of ALP enzyme was recorded as mg/mL with respect to the untreated DMEM group.

Statistical Analysis

Data were represented as mean \pm standard deviation (SD). One-way analysis of variance (ANOVA) was used for multiple groups comparison 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

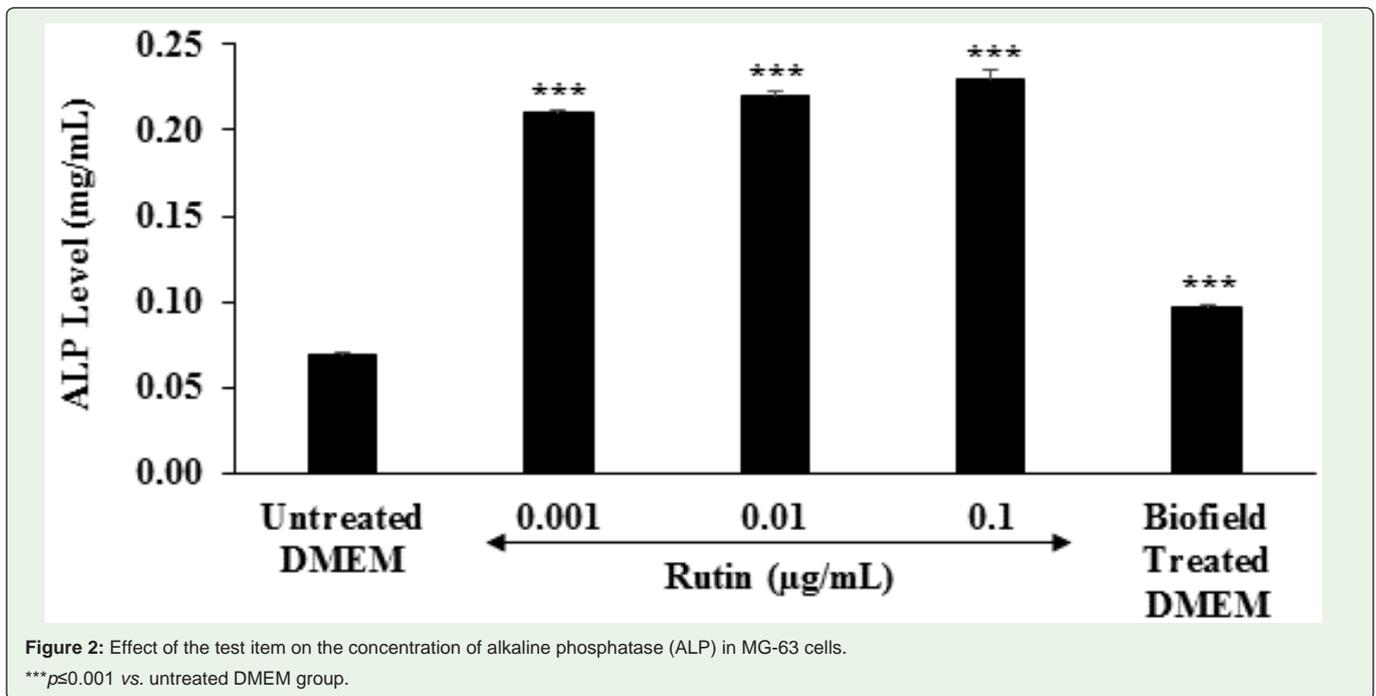
Determination of non-cytotoxic concentration by MTT assay

The percentage of cell viability using MTT assay was performed to test the cell viability of the Biofield Energy Treated test sample (DMEM medium) in MG-63 cells. The percent cell viability of the test items are shown in (Figure 1). The results showed that the test sample was found to have cell viability with more than 74%. Overall, experimental MTT data suggested that the Biofield Energy Treated

DMEM was found safe up against the tested MG-63 cells. Thus, the test sample was used to study the bone health parameter, alkaline phosphatase (ALP) activity in MG-63 cells.

Determination of Alkaline Phosphatase (ALP)

The results of ALP level for Biofield Treated DMEM in MG-63 cells are presented in (Figure 2). The positive control; rutin showed a significant ($p \leq 0.001$) increased value of ALP by 185.71% at 0.001, 0.01, and 0.1 µg/mL compared to untreated DMEM group. The Biofield Treated DMEM group was significantly ($p \leq 0.001$) increased the level of ALP by 40.58% compared with the untreated DMEM group.



Alteration of ALP results in several bone disorders *viz.* Paget's disease of bone, osteogenic sarcoma, osteoporosis, bone cancers, healing fracture, acromegaly, myelofibrosis, leukemia, bone metastases, and rarely myeloma. The reduced ALP level can be overcome using some nutraceutical supplements or vitamin D₃, calcium, etc. [37-39]. Thus experimental data concluded that the Biofield Energy Healing Treatment in the DMEM showed a significant improved level of the ALP, which could be the suitable supplementation to treat different bone and age-related disease conditions like osteoporosis [40]. Overall, the data suggested that The Trivedi Effect[®]-Energy of Consciousness Healing based DMEM could be used to improve the ALP concentration in many bone disorders.

Conclusions

MTT assay for cell viability showed significant improved cell viability with more than 74% among all the tested groups, which suggested that the test sample are safe and non-toxic. The bone health parameter, alkaline phosphatase (ALP) level was significantly increased by 40.58% in the Biofield Treated DMEM group compared with the untreated DMEM group. Thus, the Biofield Treated (The Trivedi Effect[®]) DMEM was found to have a significant impact on bone ALP level, which is very vital to combat against various bone disorders. Thus, with respect to untreated DMEM, Biofield Energy Treated DMEM would be highly significant in growth of MG-63 cells on bone health parameter. It could be utilized for various bone-related disorders *viz.* Paget's disease of bone, osteoma, osteoporosis, fractures, rickets, osteomalacia, etc. Additionally, it might be useful to improve various physiological processes those are involved in the communication from one cell to another cells, cell cycling, growth, proliferation, differentiation, neurotransmission, cardiovascular functions, hormonal balance, and skin health. It also use transplant of vital organs like kidney, heart, and liver transplants, hormonal imbalance, aging, and various immune-related disorders (ulcerative colitis, dermatitis, irritable bowel syndrome, Alzheimer's disease, hashimoto thyroiditis, asthma, multiple sclerosis, pernicious anemia, sjogren syndrome, aplastic anemia, hepatitis, graves' disease, myasthenia gravis, dermatomyositis, diverticulitis, diabetes, atherosclerosis, Parkinson's disease, systemic lupus erythematosus, stress, etc.).

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