

Therapeutic Touch Stimulates the Proliferation of Human Cells in Culture

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ABSTRACT

Objectives: Our objective was to assess the effect of Therapeutic Touch (TT) on the proliferation of normal human cells in culture compared to sham and no treatment. Several proliferation techniques were used to confirm the results, and the effect of multiple 10-minute TT treatments was studied.

Design: Fibroblasts, tendon cells (tenocytes), and bone cells (osteoblasts) were treated with TT, sham, or untreated for 2 weeks, and then assessed for [³H]-thymidine incorporation into the DNA, and immunocytochemical staining for proliferating cell nuclear antigen (PCNA). The number of PCNA-stained cells was also quantified. For 1 and 2 weeks, varying numbers of 10-minute TT treatments were administered to each cell type to determine whether there was a dose-dependent effect.

Results: TT administered twice a week for 2 weeks significantly stimulated proliferation of fibroblasts, tenocytes, and osteoblasts in culture ($p = 0.04, 0.01, \text{ and } 0.01$, respectively) compared to untreated control. These data were confirmed by PCNA immunocytochemistry. In the same experiments, sham healer treatment was not significantly different from the untreated cultures in any group, and was significantly less than TT treatment in fibroblast and tenocyte cultures. In 1-week studies involving the administration of multiple 10-minute TT treatments, four and five applications significantly increased [³H]-thymidine incorporation in fibroblasts and tenocytes, respectively, but not in osteoblasts. With different doses of TT for 2 weeks, two 10-minute TT treatments per week significantly stimulated proliferation in all cell types. Osteoblasts also responded to four treatments per week with a significant increase in proliferation. Additional TT treatments (five per week for 2 weeks) were not effective in eliciting increased proliferation compared to control in any cell type.

Conclusions: A specific pattern of TT treatment produced a significant increase in proliferation of fibroblasts, osteoblasts, and tenocytes in culture. Therefore, TT may affect normal cells by stimulating cell proliferation.

INTRODUCTION

Energy medicine is one of the domains in complementary and alternative medicine that is defined by the National Center for Complementary and Alternative Medicine in Washington, D.C. It encompasses biofield therapies including Reiki, *qigong*, and Therapeutic Touch (TT), performed by trained practitioners who focus themselves and energy to facilitate healing. TT was chosen for our studies because it is a highly disciplined method, and requires ex-

tensive training to become an advanced practitioner. Dr. Dolores Krieger and Dora Kunz pioneered this technique in the 1970s and developed modern-day TT.^{1,2} The practice involves centering, assessment, intervention, and reassessment, and does not involve touching. It is not known what the exact nature of the biofield is and how it is modulated. However, a shift in energy emission by practitioners performing TT was measured in a superconducting quantum interference device magnetometer,³ and biomagnetic fields have been recorded at a frequency from 8 to 10 Hz from

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practitioners' hands.⁴ Some clinical studies have been performed with varying results.^{5–12} Due to the psychosomatic effects of the practitioner's interaction with a patient, which can trigger neural circuits associated with safety, health, and healing¹³ and other mind–body mechanisms that affect clinical outcomes during energy medicine treatments, we chose to perform TT on cell cultures. Few *in vitro* studies have been performed with TT, however, Radin and Yount¹⁴ demonstrated that repeated TT application increased the number of colonies of human astrocytes.

Our expertise in bone cell biology prompted us to use cell cultures to study osteoblast proliferation and bone formation in culture.^{15–17} These osteoblasts cultures are derived from the outgrowth of bone taken from patients undergoing surgery in which bone is discarded. Bone fragments are minced, placed in culture medium, and within 2 weeks osteoblast progenitors grow out from the bone chips, which are then removed.¹⁸ The cells are grown to confluence and used for experiments. This use of human osteoblasts for *in vitro* studies is commonly used by numerous investigators,^{19,20} and more than 95% of these cells express high levels of alkaline phosphatase, a differentiation marker for the osteoblast phenotype. These osteoblast progenitors proliferate and then differentiate into mature osteoblasts, expressing bone matrix proteins in a sequence comparable to those expressed *in vivo*,²¹ with subsequent mineralization forming immature bone.^{18,20,22,23} Our studies were initially designed to test the effect of TT on human osteoblasts.²⁴ Upon finding that TT stimulated osteoblast proliferation, tenocytes from tendon and fibroblasts from skin were obtained for additional studies. Previous work with human tenocytes from our laboratory demonstrated that these tenocytes express the proteins characteristic of tendon.²⁵

MATERIALS AND METHODS

Cell culture

Human bone fragments, discarded during orthopaedic foot surgery, were obtained from healthy patients. The University of Connecticut Health Center Institutional Committee on Human Research approved the use of discarded tissue for cell culture. The bone fragments were minced and cultured in Dulbecco's Modified Eagle Medium (DMEM)/F12 Ham (Sigma, St. Louis, MO) with 15% fetal bovine serum (FBS) and 100 U/mL penicillin G and 100 µg/mL of streptomycin sulfate. Cells were allowed to grow out of the bone chips for 2–3 weeks.

Fibroblast cultures were derived from neonatal foreskin obtained during routine circumcisions. The tissues were digested with 0.25% type I collagenase and the resultant dermal fibroblasts were maintained in humidified 5% CO₂ / 95% air.²⁶ Cultures were fed twice weekly with RPMI medium with 8% FBS and antibiotics.

Fresh human hamstring tendon, discarded from orthopaedic procedures, was cut into small pieces, digested with 2 mg/mL collagenase for 3.5 hours at 37°C, pelleted, resuspended, and plated in DMEM with 10% FBS and antibiotics.^{27,28}

Cells were fed weekly until confluent, harvested with 0.01% trypsin, and replated for experiments at 10,000 cells/cm² in six-well Falcon Primaria dishes (Falcon, Franklin Lakes, NJ). During the experiments, all cells were fed twice a week with the medium described above. Tenocytes, fibroblasts, and osteoblasts were isolated from different patients. The cells from one patient per cell type were used for each experiment. Each experiment was repeated at least three times. Therefore, the results from at least 3 patients are shown, and exhibited comparable results. Two technicians grew the cells and performed the assays described below.

Therapeutic touch

Three TT practitioners participated in this study. They were Registered Nurses, who were trained in TT by passing at least two courses in TT, and had more than 5 years each of TT practice on patients. TT requires that the practitioner focus healing intent on the subject and consists of (1) "centering," in which the practitioner focuses and eliminates external distractions; (2) "assessment" of the subject without touching the plate and remaining at least 4 inches away; (3) "unruffling" by using sweeping motions; and (4) "treatment" involving the directing of positive intentions through the hands. TT is performed by holding the hands 4–10 inches away from the subject and was adapted for our *in vitro* study. Treatments were 10 minutes in length and were administered twice a week with at least 1 day in between treatments, or daily when the treatment exceeded 3 times. Prior to the start of the study, the length of time for TT treatments was set by the practitioners, according to their previous experiences with patients with extrapolation to cell culture.

Control (untreated) and "treatment" tissue cultures plates/dishes were clamped in one of two ringstands on a bench top, and were approximately 15 inches in the air. Each cell type was treated separately. Control and treated plates were positioned at either end of an L-shaped laboratory at set sites. Treatment was alternately performed on either end of the room. After "treatment" or "no treatment," the tissue culture plates/dishes were returned to the same shelf in the incubator. Positioning of the plates in the incubator was random.

To consider the possibility that the effect of TT may originate from a physical or common mechanism shared by all humans, selected experiments were performed with a sham treatment. The sham healer was an individual with no knowledge of TT who stood in front of identically prepared cell culture plates and used similar hand movements at a simi-

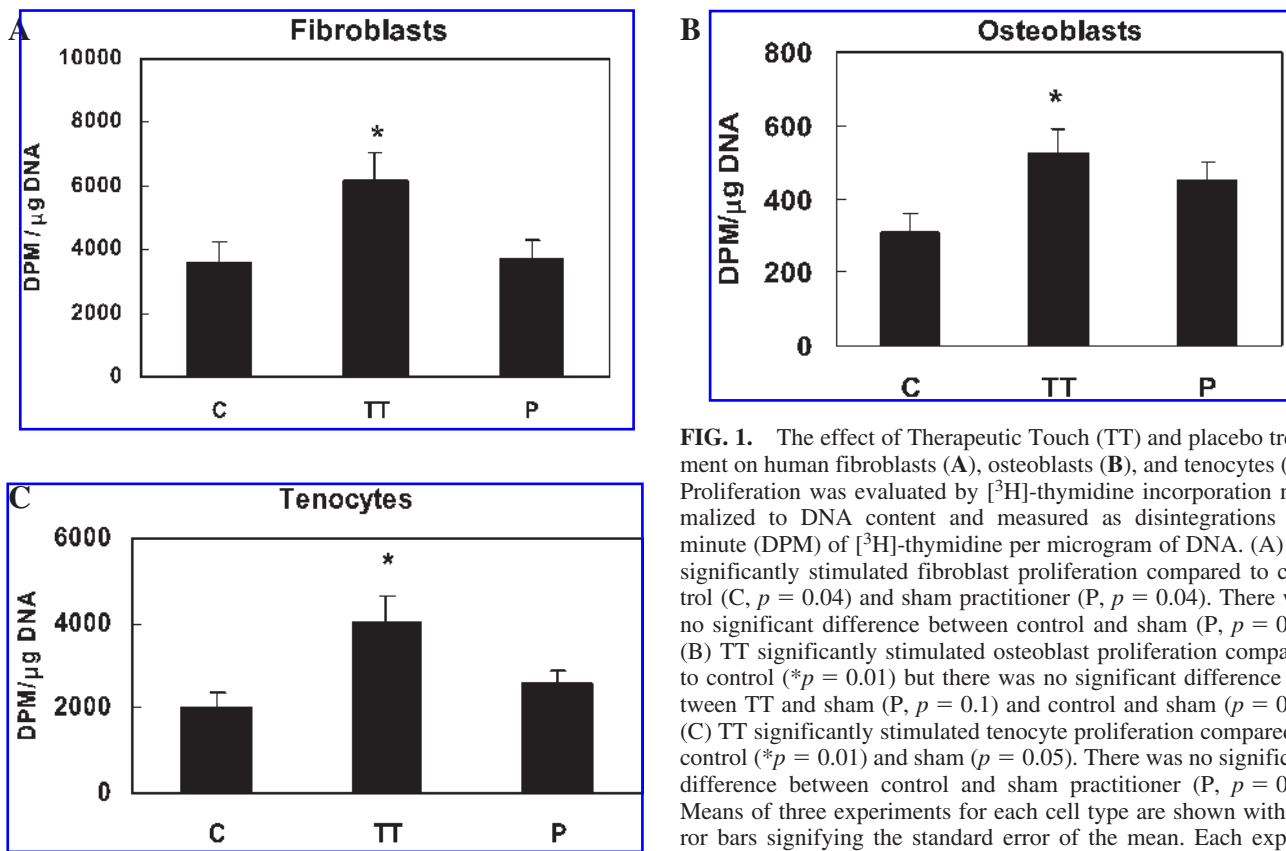


FIG. 1. The effect of Therapeutic Touch (TT) and placebo treatment on human fibroblasts (A), osteoblasts (B), and tenocytes (C). Proliferation was evaluated by [^3H]-thymidine incorporation normalized to DNA content and measured as disintegrations per minute (DPM) of [^3H]-thymidine per microgram of DNA. (A) TT significantly stimulated fibroblast proliferation compared to control (C, $p = 0.04$) and sham practitioner (P, $p = 0.04$). There was no significant difference between control and sham (P, $p = 0.2$). (B) TT significantly stimulated osteoblast proliferation compared to control ($*p = 0.01$) but there was no significant difference between TT and sham (P, $p = 0.1$) and control and sham ($p = 0.1$). (C) TT significantly stimulated tenocyte proliferation compared to control ($*p = 0.01$) and sham ($p = 0.05$). There was no significant difference between control and sham practitioner (P, $p = 0.2$). Means of three experiments for each cell type are shown with error bars signifying the standard error of the mean. Each experiment had 6 replicate samples for a total of 18 samples for each cell type.

lar location in the laboratory. However, the sham treatment individuals counted backwards from 1000 for 10 minutes to prevent any thoughts or intentions. These sham treatments were performed by 4 separate individuals in our laboratory and consisted of graduate students and technicians involved with other projects in the laboratory.

Cell proliferation

During the last 4 hours of culture, 5 μCi of [^3H]-thymidine was added to the cells. Then cells were extracted twice for 5 minutes with 10% trichloroacetic acid and lysed in 0.5 N NaOH for 10 minutes. Liquid scintillation counting was performed to measure radioactivity in the lysates (Packard Instrument Co., Downers Grove, IL). Tritiated-thymidine incorporation was normalized to the DNA content. At least three experiments with six replicates/experiment were performed.

DNA content was measured by fluorimetric analysis at the same time as the assay for [^3H]-thymidine incorporation. Cells were disrupted with 0.01% sodium dodecyl sulfate. Cell lysates were transferred to a 96-well plate, and 10 $\mu\text{g}/\text{mL}$ bisbenzimidazole H33258 (Sigma) was added. DNA content was measured at 360-nm excitation and 460-nm

emission by a microplate reader (Bio-TEK Instruments, Inc., Winooski, VT). DNA content was determined from the same dishes in which [^3H]-thymidine incorporation was performed, so that both received the same TT treatment or no treatment.

To confirm the effect of TT on proliferation determined by thymidine incorporation, an immunocytochemical staining method was used. Cells in replicate wells were fixed in 70% ethanol overnight. Immunocytochemistry for proliferating cell nuclear antigen (PCNA) was performed according to Zymed's PCNA Kit (Zymed, South San Francisco, CA). Fields of cell were photographed without knowledge of treatment group. Then, the number of darkly stained PCNA-positive cells and total number of cells were counted per field.

All assays were performed in a "blinded" manner without knowledge of the groups by the 2 technicians. The dishes were labeled by number, and a schedule of treatment or no treatment was set up at the beginning of the experiment. After treatment or no treatment, the dishes were returned to the same incubator. When the experiment was completed, all dishes were assayed at the same time with the number of the dish and well recorded. Once the data were obtained, the groups were identified as to their treatment group.

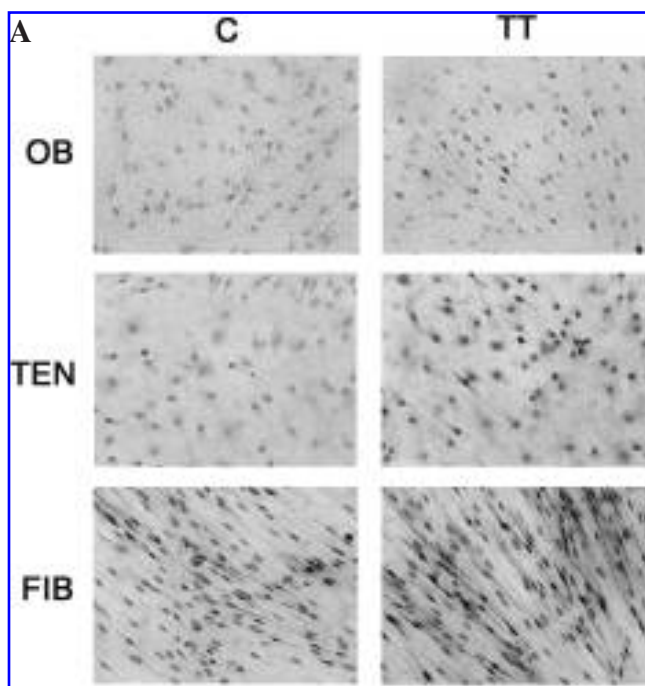
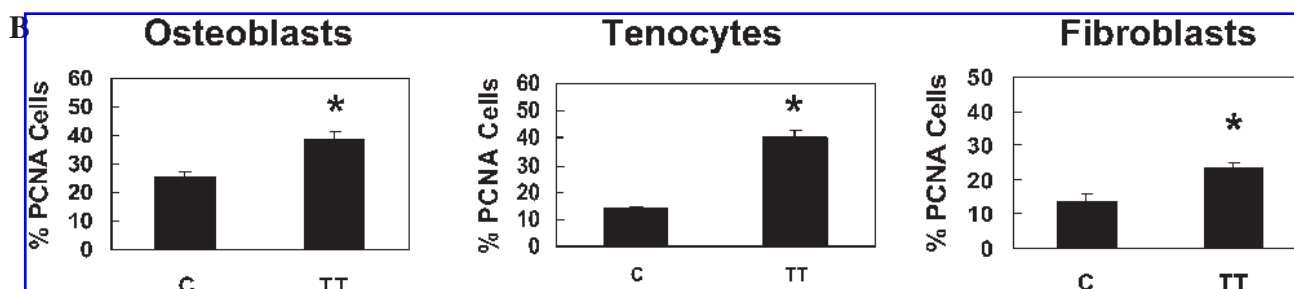


FIG. 2. Proliferating cell nuclear antigen (PCNA) immunocytochemical staining of human osteoblasts (OB), tenocytes (TEN), and fibroblasts (FB) treated with and without TT for 2 weeks twice a week. **A:** A representative field of cells is shown for each cell type; OB, TEN, and FB with untreated panels on the left and TT treatment on the right. Note an increase in dark-stained nuclei for PCNA in the TT-treated cells compared to the untreated cells. **B:** A total of eight random fields of cells were photographed and then quantified. These eight fields had more than 700 cells per group for osteoblasts and fibroblasts, and more than 350 cells in the tenocyte groups. Values are means \pm the standard error of the mean. Asterisks show p values less than or equal to 0.003.



Statistics

Individual values from all experiments were compiled on an Microsoft® Excel® (Microsoft Inc., Redmond, WA) spreadsheet. In the 2 years of the study, no experiments were excluded from the spreadsheets, so that the spreadsheets describe all the experiments that were performed. Upon completion of the data, statistical analysis was performed by a two-way analysis of variance using Microsoft Excel and Student–Newman–Keuls test to determine significance between groups. A p value less than 0.05 represents a significant difference.

RESULTS

The effect of TT and sham healer (P) on the proliferation of human fibroblasts, osteoblasts, and tenocytes was determined by the incorporation of [³H]-thymidine into the DNA at the end of the 2-week culture period (Fig. 1). For fibroblasts (Fig. 1A), TT produced a significant difference in cell proliferation compared to control ($p = 0.04$) and sham treat-

ment ($p = 0.04$) (analysis of variance [ANOVA] p value of 0.01 for source of variance across groups, and 0.06 ANOVA p value for source of variance between the three trials for control, ANOVA p value of 0.3 for source of variance between the three trials for TT, and ANOVA p value of 0.03 for source of variance between the three trials for sham treatment). For osteoblasts (Fig. 1B), TT produced a significant difference in cell proliferation compared to control ($p = 0.01$) but not compared to sham treatment ($p = 0.2$) (ANOVA p value of 0.03 for source of variance across groups and 0.6, 0.2, 0.03 ANOVA p values for source of variance between the three trials for control, TT, and sham treatment, respectively). For tenocytes (Fig. 1C), TT produced a significant difference in cell proliferation compared to control ($p = 0.01$) and sham treatment ($p = 0.05$) (ANOVA p value of 0.02 for source of variance across groups and 0.7, 0.1, 0.3 ANOVA p values for source of variance between the three trials for control, TT, and sham treatment, respectively).

To confirm the [³H]-thymidine results by another technique, immunocytochemical staining for PCNA was performed (Fig. 2A). PCNA-stained nuclei were often found in

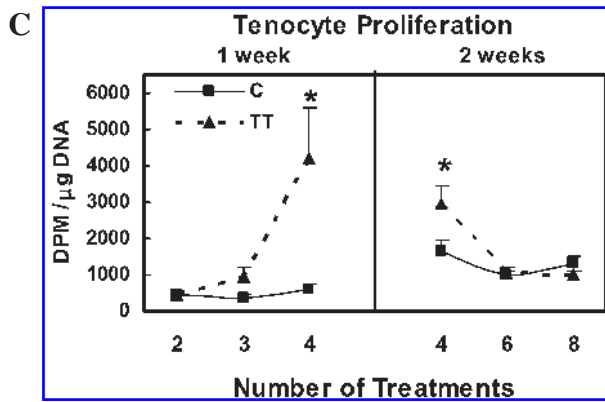
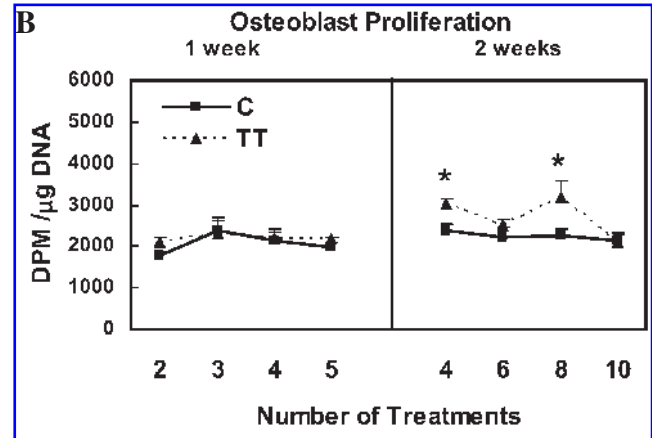
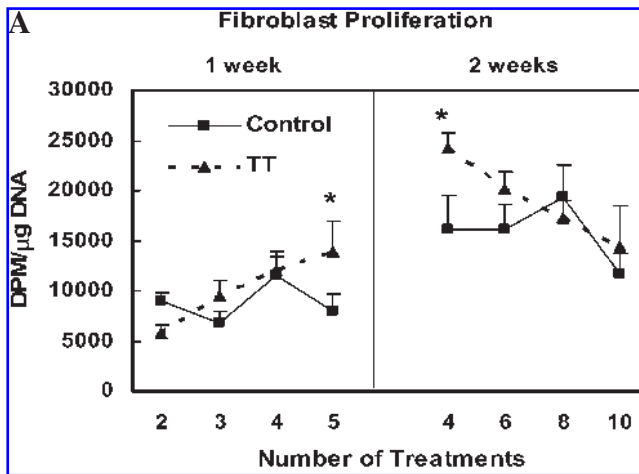


FIG. 3. Therapeutic Touch (TT) dose responses of [^3H]-thymidine incorporation normalized to DNA content for human fibroblast (A), osteoblast (B), and tenocyte (C) cultures. The left panel in each part of the figure shows disintegrations per minute (DPM) of [^3H]-thymidine per μg of DNA after 1 week with (TT) or without (control) two, three, four, or five TT treatments. The right panel in each part of the figure shows DPM/ μg DNA after 2 weeks of treatments. For fibroblasts (A) five TT treatments in 1 week or two treatments for 2 weeks produced significant effects compared to untreated control cultures ($*p < 0.05$). For osteoblasts, no significant differences were found at 1 week, but two and three TT treatments per week for 2 weeks produced more cell proliferation than no treatment ($*p < 0.05$). In tenocyte cultures (C), four TT treatments in 1 week and two TT treatments for 2 weeks stimulated cell proliferation compared to untreated control tenocyte cultures. Values are means \pm the standard error of the mean for three experiments with six replicate samples per experiment.

groups, suggesting that groups of cells were more active in proliferating cells than in cells in other regions, perhaps due to uneven cell density or cell communication. The percentage of dark-stained cells/total number of cells is graphed in Figure 2B and demonstrates that there was a significant increase in PCNA-positive cells in the TT treatment groups compared to the untreated control groups with osteoblasts, tenocytes, and fibroblasts. These results confirm the incorporation of [^3H]-thymidine shown in Figure 1.

To determine the optimal treatment dose and to ensure that we were studying a biologic effect with a reproducible response, the practitioners performed TT on different plates of cells for 10 minutes on separate days so that increasing doses of TT could be administered. Each cell type was treated separately in all experiments. In Figure 3, the left half of the graph, labeled 1 week, describes the number of treatments in the first week. At the end of the first week, cells were processed for [^3H]-thymidine incorporation into the DNA. A second set of plates that were treated for 1 week continued to have TT treatments so that the net number of treatments by the end of the second week were four (two treatments the first week and two the second week), six (three the first week and three the second week), etc. The disintegrations per minute were normalized to the amount of DNA. A significant increase in fibroblast proliferation

was found with five TT treatments in the first week compared to untreated control fibroblasts (Fig. 3A). In the group of fibroblast cultures treated for 2 weeks, a total of four TT treatments were required for a significant effect compared to comparable controls. In three separate osteoblast experiments, only four or eight treatments in 2 weeks produced significant effects with TT (Fig. 3B). The tenocyte experiments showed a significant effect with four treatments in 1 week or a total of four treatments in 2 weeks (Fig. 3C). Other multiple doses did not result in significantly different findings.

DISCUSSION AND CONCLUSIONS

Results demonstrate that TT is able to stimulate proliferation in several different types of human cells; tenocytes, fibroblasts, and osteoblasts compared to untreated cells. In comparison to the sham practitioner control, TT also produced a significant increase in tenocytes and fibroblast proliferation. However, although control and TT treatment groups were significantly different for osteoblasts, there was not a statistically significant difference between sham and TT groups in osteoblasts. Confirming the data using two different assays; tritiated thymidine incorporation into the DNA

and staining for PCNA, which is a factor found in cells undergoing cell division, further strengthens the findings that TT can elicit a significant biologic effect on normal cell growth. PCNA is a 36-kDa protein, also known as a cyclin or the polymerase δ -associated protein, synthesized in the early G1 and S phase of the cell cycle and therefore, is specific for cell proliferation.

Significant effects of TT were found after 2 weeks of TT treatment twice a week for osteoblasts, tenocytes, and fibroblasts, when the proliferation rate decreased. The lack of effect with fewer treatments may be due to too-short treatments, or due to rapid proliferation at early timepoints that overwhelm the small effect of TT or due to as yet unknown properties of the energy field that we are not able to optimize at this point in time. However, tenocytes and fibroblasts but not osteoblasts demonstrated significant increases in proliferation in the first week compared to control when multiple TT treatments were administered. Each treatment was performed on a separate day. Results with multiple treatments per day were not performed but may elicit earlier effects and will be explored in the future. Previous work with Johrei practitioners demonstrated that the number of treatments was important for stimulating the growth of primary human astrocytes compared to control cultures.¹⁴ However, several trials with *qigong* and human astrocytes from the same group produced statistically significant effects in small studies but not in a large replicate study.²⁹ The state of the practitioner has also been shown to influence outcomes. Reiki treatment stimulated the growth of heat-shocked bacteria;³⁰ however, one of the variables for obtaining reproducible and significant results was the practitioner's social and emotional well-being in a healing context.

Sham healer treatment has been known to produce small effects, and in the osteoblast cultures, the sham treatment gave an intermediate value compared to control and TT. In the fibroblast and tenocyte cultures, a sham effect was not apparent when all the experimental data were combined. The sham healer was a person with no knowledge of TT. We switched this person so that they would not repeatedly perform TT and perhaps acquire the ability to affect cells with time. We had no definitive data to suggest that a sham healer would acquire the ability to affect cells, but we decided to control for this possible effect in the original design of the experiments. We do not know whether a person can acquire the ability to perform TT by watching someone perform TT or being in the presence of healers, which should be tested in future experiments. Alternatively, the use of the same room for both TT and sham treatment may influence the results, since the practitioner may have conditioned the room so that the sham treatment cultures were affected. Movaffaghi et al., in a report consistent with Kreiger's early studies,^{31,32} have also reported that their sham therapists appeared to have a small effect on blood hemoglobin and hematocrit levels so that they obtained an intermediate level between untreated and TT treatment.³³

The finding that all three cell types responded to two TT treatments/week for 2 weeks suggests that there is a threshold for TT treatments that affects proliferation in multiple cell types and that there may be common factors involved in proliferation that are the target for energy treatments. Since all three cell types were treated separately, the identical response at similar treatment regimens appear to validate TT as having a significant effect on cells rather than being the result from an artifact of repetitive sampling. Future work will be directed toward finding which molecules involved in cell cycling are modulated by TT. Interestingly, changes in calcium concentrations, important in signal transduction, have been found to be modulated by an external bioenergy specialist.³⁴ A number of complementary and alternative medicine treatments have been shown to affect the proliferative capacity of cells. A human prostate cancer cell line, PC3, was shown to decrease its growth rate with 48 hours of treatment *in vitro* by a Buddhist-Zen master.³⁵ In many studies that were performed in China and reviewed by Dr. Kevin Chen,³⁶ external *qi* has been shown to inhibit cancer cells, derived from breast cancers, liver, lung, and promyelocytic leukemia, etc. Reiki has been shown to improve the growth of heat-shocked bacterial cultures.³⁰ Therefore, there may be differential effects of energy treatments depending on whether the cells have a normal proliferative capacity or abnormal growth rate, such as in cancer. More studies are required to determine and define the effects of the various practices that involve the human biofield, and to identify the mechanisms by which cells may respond.

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