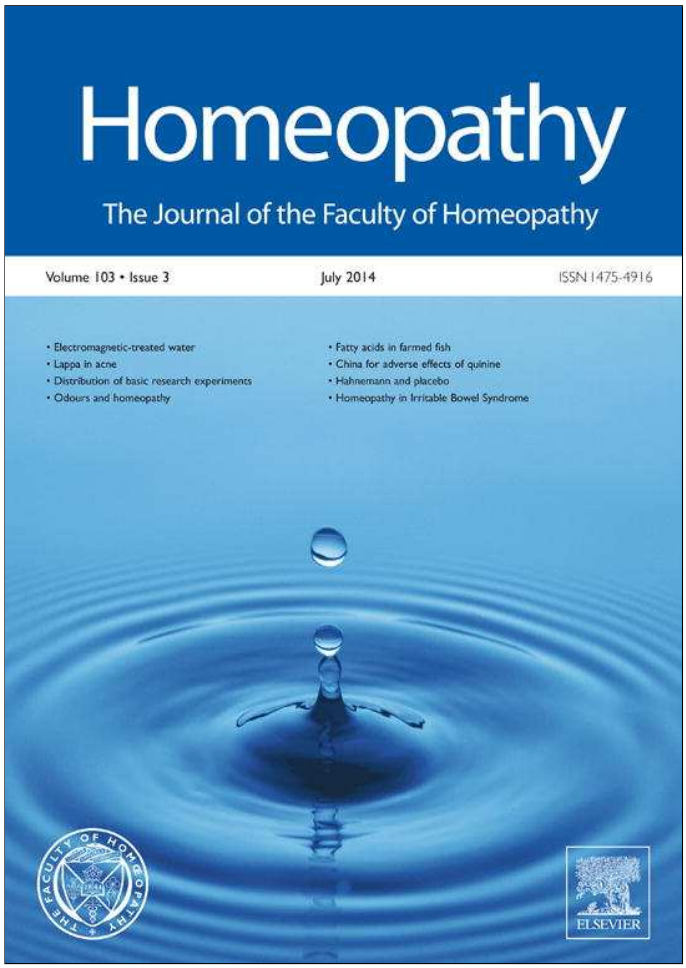

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ORIGINAL PAPER

Diverse biological effects of electromagnetic-treated water

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The effects of water treated with an electromagnetic field (EMF) were investigated on two biological systems, humans and plants. Purified de-ionised water was treated by (1) boiling, (2) exposure to microwave radiation, and (3) low frequency electromagnetic oscillation molecular resonance effect technology (MRET), before being used to prepare media for culturing human peripheral blood mononuclear cells (PBMC) from three healthy females. Our results indicated that PBMC culture in MRET-activated medium showed significantly less oxidative metabolism when compared to media prepared from other types of water. As for the effects on soybean, our results indicated that both MRET- and microwave-treated water greatly enhanced the length of the root. These results suggested that electromagnetic-treated water can have diverse biological effects on both animal and plant cells. Since these effects are related to the 'Memory of Water', hypothesis which has been suggested as an explanation of the action of high homeopathic dilutions, our finding warrant a further investigation on the mechanisms of various types of physically conditioned water on specific cellular activities. *Homeopathy* (2013) **103**, 186–192.

Keywords: Molecular resonance effect technology; Water; Biological effect; PBMC; Soybean; Resazurin

Introduction

Water is essential for life. Each day, humans are exposed to water that has gone through different processes such as boiling or heating with a microwave. Recent issues in the news have mentioned the possible harmful effect of microwaved water.[1] In addition, the beneficial effects of water that has gone through special treatment such as ionisation[2] or activation by low frequency electromagnetic oscillations i.e., molecular resonance effect technology (MRET) is quite popular, especially in Thailand and other countries in Southeast Asia. Even though the claims about the various biological effects of this physically conditioned water are met with scepticism, stories about the health-promoting effects of MRET-treated water have been reported.[3,4] These studies suggested that water treated with low frequency electromagnetic fields (EMF) might have effects on biological systems by a mechanism that is based on a quantum physics model.[5]

Some reports on the effects of magnetic field and EMF on water molecules have been published.[6] These include changes in viscosity, surface tension, index of light refraction, electrical conductivity, and light absorption.[7,8] It has been suggested that long-term changes in water properties mediate the effect of EMFs on biological systems, such as the activation of ion channels.[9] This hypothesis is related to the 'Memory of Water', a hypothesis which has been suggested as an explanation of the action of high homeopathic dilutions.[10]

In this study, we seek to investigate the biological effects of water treated with two kinds of EMFs. The first one was a non-ionising microwave radiation at a frequency around 2.45 GHz, which is used in household microwave ovens. In

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this experiment, boiled water, using regular stovetop, was used as a control for water heated with a microwave. The second treatment was a low frequency, resonant EMF, obtained from a patented technology termed MRET (see Methods section). The experiments were conducted on two complex normal biological systems, human peripheral blood mononuclear cells (PBMC) and soybean seeds. The effects on PBMC bioactivity (indicated by mitochondrial oxidative phosphorylation) and on the growth of soybean (indicated by dry weight, height, and root length) were reported along with appropriate statistical analysis.

Experimental methods

Preparation of physically conditioned water

A diagram describing different treatments of water for the experiments on human cells (A) and soybean growth (B) is shown in Figure 1. Purified and de-ionised water (Milli Q water) was obtained by passing triple distilled water through Millipore machine (model: simplicity, MA, USA). The Milli Q water contained total organic carbon (TOC) < 50 ppb, and resistivity > 1 MΩcm. RO water was a regular drinking water that was purified by reverse osmosis (RO), followed by UV radiation. Tap water was from a standard municipal water system at Suranaree University of Technology (SUT). Boiled water was obtained by boiling the MilliQ- (Figure 1A) or RO-UV treated- (Figure 1B) water on a hotplate for 5 min and cooled down to room temperature. Microwaved water was prepared by heating the water in a cooking microwave at 2.45 GHz (Sharp Thai, Co., Ltd.) for 5 min, and cooled down to room temperature. MRET-treated water was obtained by activating the water with a commercially available MRET activator machine (USA patent number 6022479) for 30 min as recommended by the manufacturer. The device includes a liquid reservoir; an activation body formed of a patented polymeric matrix with the body disposed in a strong (2500−25,000 Oersted) magnetic field and responsive to visible electromagnetic radiation having a frequency of 7.2−7.8 Hz and a wavelength of 400−800 nm, and with one end disposed proximate to the surface of the liquid; and an electrical circuit adjacent at the other end of the body, activating diodes to emit flashes of light having that wavelength and frequency, so that the materials and the polymeric body are energised and emit low frequency oscillations, which activate the liquid contained in the reservoir.[11] MRET is an acronym for molecular resonance effect technology which is claimed to reorganise the structure of water molecules such that it will be more compatible to biological systems, allowing proper function of cellular signalling and metabolism.[3]

Preparation and culture of PBMC

Normal human PBMC were chosen as the representative of human cells in our study because they are abundant in the human body, have rapid turn over rate, and are crucial for the immune system. The biological effects were detected by measuring the overall oxidative activity at the molecular and cellular level that occurred in mitochondria

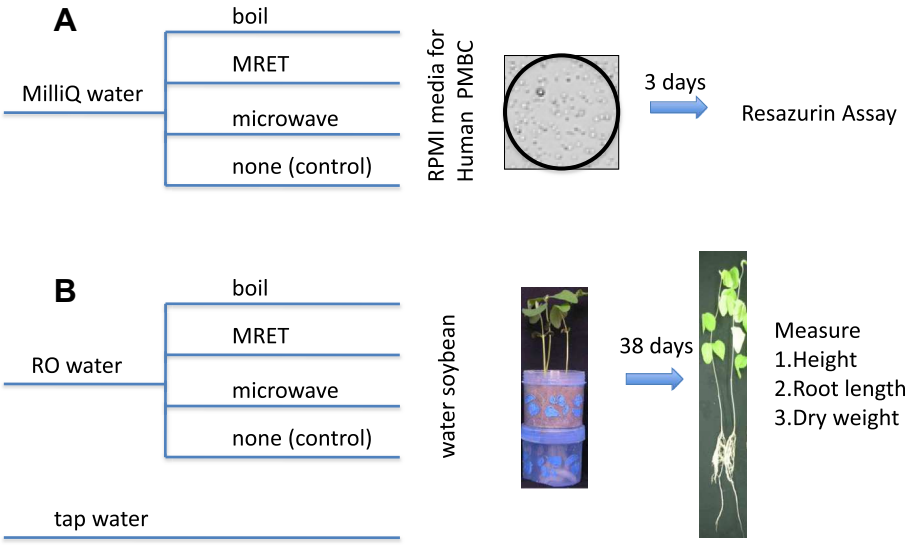

Figure 1 Schematic diagram of the protocols used in this study. ***Panel A: Experiments on human cells***. Four types of water were used to prepare media for culturing peripheral blood mononuclear cells (PBMCs). After culturing for three days, resazurin reagent was added to measure the overall oxidative activity of the cells. All three types of water treatments (boiled, MRET activation, and exposure to microwave radiation) were done on Milli Q water on the same day as described in the Methods section. Medium prepared from none-treated Milli Q water was used as a control; whereas boiled water using a regular stovetop was used as a control for water heated with a microwave. The experiments were done in triplicate wells of PBMC cells obtained from three adult (25−26 year old) females. ***Panel B: Experiments on soybean growth***. Four types of water and tap water were used to water soybean seeds and soybean plants grown in Leonard jars under a strictly controlled room. After the soybean seeds germinated, they were grown for 38 days, before the plants were collected to measure dry weight, root length and height. All three types of water treatments (boiled, MRET activation, and exposure to microwave radiation) were prepared from reverse osmosis (RO) water every week. None-treated RO water was used as a control, and boiled water using a regular stovetop was used as a control for water heated with a microwave.

using resazurin. The measurement was done after culturing for 3 days, when the number of cells in every condition was still equal. The intensity of the pink colour (O.D. value) indicates the degree of metabolic oxidative reaction within the cells.

Four types of water (Figure 1A) were used to prepare RPMI 1640 media (Gibco BRL, MD, USA), which was used to culture PBMCs. All RPMI media were prepared and filter-sterilised using 1000 mL bottle top vacuum filter, 0.22 μm, pore 54.5 cm^2, CA Membrane (Product #430015, Corning®, USA) on the same day, immediately before use. PBMCs were obtained from 3 healthy females (25−26 year old) and isolated by Ficoll−Paque (GE healthcare, UK), according to previously published protocol.[12] Approximately 2×10^5 cells were cultured in triplicate wells of a 96-well plate containing four different RPMI 1640 media, supplemented with 10% (v/v) foetal bovine serum and cocktail antibiotic-antimycotic (penicillin/streptomycin/amphotericin; Gibco BRL, MD, USA). The cells were incubated at 37°C in 5% CO_2 incubator with 95% air. Wells on the outside rows of a 96-well plate were not used for culturing cells, but filled with RPMI media prepared from un-treated water. The experiment was performed according to the ethical guidelines for human research of SUT in an assessor-blind manner.

Cultivation of soybean

Plants are complex eukaryotic organisms that diverged from animal more than a billion years ago along the evolutionary history of life on earth. Since plants and animals evolved multicellularity independently, the molecules and mechanisms used for cellular metabolism and signal transduction could be expected to be highly different; therefore we seek to determine the effects of different physically conditioned waters on plant as well. Soybean was used as the representative of plants in this study because it is a dicotyledonous plant, which is abundant, fast growing and has commercial value. In this assay, regular drinking water, which is purified by RO followed by UV irradiation, was used as the initial water for various treatments. In addition, regular tap water, normally used for watering plants, was also used for comparison.

Soybean seeds (*Glycine max*) were surface sterilised in 95% ethanol for 10 s before addition of 3% sodium hypochlorite to immerse the seeds completely. After 5 min, the seeds were rinsed six times with sterilised water. Sterilised seeds were put on a Petri dish containing sterilised moist tissue paper and kept in a dark place at room temperature for 2 days. Five germinated seeds were grown in each Leonard jar containing sterilised sand and different types of water. Purified water by RO, followed by UV irradiation, was used as a control in this study (RO water). This water was physically treated by boiling, exposure to microwave radiation (2.45 GHz), and MRET activation as described above. In addition, tap water, which is used to prepare the RO water was also tested. Soybeans were cultivated in optimal conditions as previously described.[13] The Leonard jars were put on the shelf inside a light-controlled growth chamber, which was set up under alternating 12-h light and 12-h darkness at controlled room temperature (27−28°C). Three plants from 3 Leonard jars (for a total of 9 plants) were harvested after 38 days, and their dry weight (in grams after incubation at 70°C for 2 days), height (in centimetres), and roots length (in centimetres) were recorded. All three types of physically treated water were prepared weekly and kept at the same condition throughout the entire experiment. The experiments were conducted in a double-blind manner.

Measurement of cellular oxidation reaction

After the cells were incubated for 72 h, 50 μg/ml of resazurin (Sigma, MO, USA) was added into each well and further incubated for 8 h before the absorbance at 595 nm was recorded according to the manufacturer's protocol. Optical density (OD) values that were obtained from the oxidation of resazurin (blue) to resorufin (pink) by electrons generated during mitochondrial oxidative phosphorylation inside the cells.

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the mean O.D. values of the oxidation reaction of PBMC cells from the three female donors that were cultured in different water. For those circumstances where there were statistically significant changes, a protected Fisher's Least Significant Difference (LSD) test was used to compare the mean of one group (various physically treated waters) with the mean of another (multiple comparison). Using this method, the calculations were done only when the overall ANOVA resulted in a P value less than 0.05. Note that other multiple comparison tests such as Tukey Honestly Significant Difference (HSD) does not require this step. If the P value for the ANOVA is greater than 0.05, we concluded that the data are consistent with the null hypothesis that all population means are identical, therefore no further analysis was performed.

Duncan's test, which is commonly used in agronomy and other agricultural research, was used to compare the effects of different physically treated waters on soybean plant's dry weight, height and root length.

Results and discussion

The effects of physically conditioned water on human PBMC

The overall effects of various physically conditioned waters on human PBMC cells were evaluated by multiple comparisons and the result after statistic analysis is illustrated in Figure 2. Our results indicated that cells cultured in medium prepared from MRET-treated water showed significantly less oxidative metabolism when compared to those cultured in media prepared from other types of water. On the contrary, media prepared with water conditioned by microwave or normal boiling did not show significant difference in overall oxidative phosphorylation, when compare with those of un-treated water. Tables of

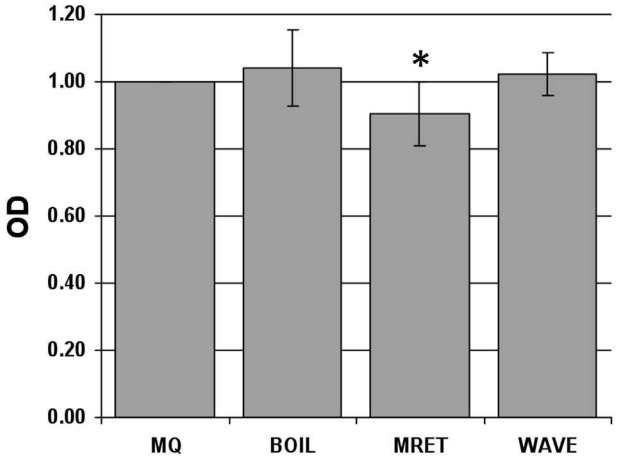

Figure 2 Overall effects on PBMCs. The average of OD values indicates the degree of metabolic oxidative reaction within the cells (pink colour) from triplicate wells of microtiter plates. A protected Fisher's Least Significant Difference (LSD) test was used to compare the mean of each physically treated water. Asterisk indicates a significant difference ($P < 0.05$) compared to control (MQ). **MQ**: ultra-purified de-ionised water using Milli Q system (Milli Q water); **BOIL**, **MRET**, and **WAVE** are MQ water that has been treated by boiling, MRET activation, and exposure to microwave radiation, respectively, as described in the Methods section. Table of multiple comparisons from this experiment can be found in the Supplementary data.

multiple comparisons for statistic analysis of these results can be found in the Supplementary data.

The effects of physically conditioned water on the growth of soybean

Height: A summary of the results of the effects of various physically treated waters on plant height is shown in Figure 3, top panel. After statistical analysis, we found that the height of soybean plant watered with all three physically treated waters and the tap water were slightly, but significantly, shorter than those watered with non-treated purified water. There were no significant differences between the two types of water treated with EMFs.

Dry weight: A summary of the results of the effects of various physically treated waters on plant dry weight is shown in Figure 3, middle panel. After statistical analysis, we found that the dry weight of soybean plants watered with microwaved water were slightly, but significantly, higher that that of other types of water. There were no significant difference among MRET-treated water, stovetop boiled water and tap water.

Root length: A summary of the results of the effects of various physically treated waters on the length of soybean roots is shown in Figure 3, bottom panel. After statistical analysis, we found that the root lengths of soybean plants watered with both types of EMF, i.e., microwave and MRET, were approximately three times longer that that of boiled, non-treated, and tap water (Figure 4). However, there were no significant differences between microwaved and MRET-treated water.

Tables of multiple comparisons for statistic analysis of these results can be found in the Supplementary data.

Discussion

Taken together, our results indicated that no other physically conditioned water has any effect on PBMC, except for MRET-activated water, which could significantly reduce the overall oxidative reaction in normal human cells *in vitro*. The measurement was done using resazurin, a

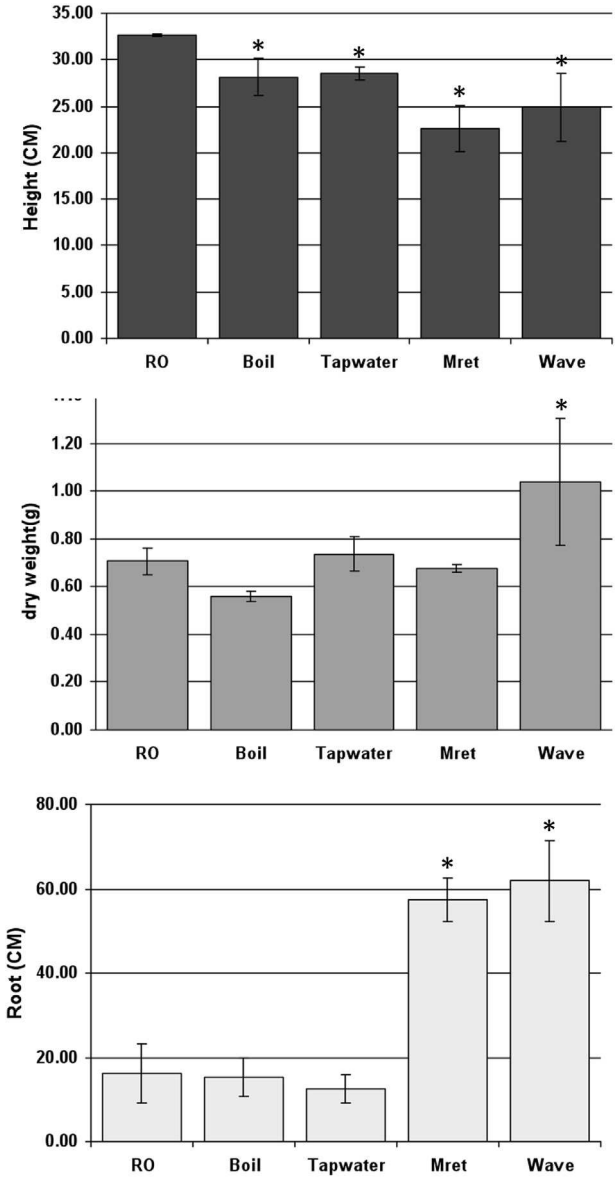

Figure 3 Effects on soybean plants. The effects are reported as three different parameters, i.e., plant height (upper panel), dry weight (middle panel), and root length (bottom panel). The average values from three plants growing in three Leonard jar was reported. Duncan's multiple comparison test was used to determine the significance of the reported data. Asterisk indicates a significant difference with $P < 0.05$ when compared with control (reverse osmosis water). **RO**: reverse osmosis water; **Boil**, **MRET**, and **Wave** are RO water that has been treated by boiling, MRET activation, and exposure to microwave radiation, respectively, as described in the Methods section. **Tap water** was regular tap water from a standard municipal water system at Suranaree University of Technology. Table of multiple comparisons from this experiment can be found in the Supplementary data.

Figure 4 Illustration of soybean plants grown in different water. This picture represents the results demonstrating the effects of various physically conditioned waters on the growth of soybean plant, as described in Figure 2. Plants watered with MRET-activated or microwaved water showed much longer root lengths.

coloured compound, which was reduced and changes the colour from blue (oxidised form; resazurin) to pink (reduced form; resorufin) by electrons generated during the mitochondrial oxidative phosphorylation reaction. This dye has been used to quantify mitochondrial activity in leukocytes to study innate and adaptive immune responses.[14] EMF has been previously shown to alter redox status in a cellular-specific manner.[15] In addition, it has been suggested that long-term changes in water properties mediate the effect of EMFs on biological systems such as activation of ion channels.[9] MRET-activated water is proposed to have a long-term memory effect. 'Memory of Water' was proposed as the basis of action of homeopathic high dilutions suggested by a controversial report published in 1988.[16] Homeopathy is a form of complementary medicine that remains contentious after more than 200 years.[17–19]

A recent report using a specially designed, ultrathin sample cell revealed that liquid water does not maintain ordered networks of molecules longer than 50 fs, yet none of the biological tests were done in this study.[20] Moreover, it has been proposed that water memory effects are much more likely to be dependent on dynamic ordering effects that could be explained by quantum physics.[21] Various mechanisms such as natural water clustering and nanobubbles[22] have been proposed to created water memory, which could diversely effects biological systems.[23]

The molecular mechanism of how MRET-activated water reduces the cellular mitochondrial oxidative phosphorylation reaction is enigmatic since there are so many cellular components involving oxidative reaction in the cells. Physically conditioned water by static magnetic or electromagnetic oscillating fields has been shown to stimulate or inhibit the multiplication of yeast cells, depending on the degree of conditioning.[24] It has been suggested that these effects are due to colloidal impurity in the water. However, since the water used in this study is ultra-purified de-ionised water, the colloidal effect of the activated water

on biological systems can be rule out. It is possible that MRET-activated water could modulate specific cellular signal transduction processes, which could lead to reduction of oxidative phosphorylation reactions in the mitochondria of PBMCs in a resting state. However, when the PBMC cells are activated, such as by LPS, the effects of MRET-activated water on the mitochondrial activity is likely to be dissimilar, because distinct signal transduction pathways are triggered when the cells are stimulated. Since various cells at different states have unique signal transduction pathways, the effect of activated water on particular cell types at myriad states can be expected to be diverse.

Reduction of mitochondrial oxidative phosphorylation reactions could lead to reduction of reactive oxygen species that are harmful to the cell components. This finding provides the first clue to the beneficial effects of MRET-activated water, yet many more experiments have to be done before any conclusion on specific cellular process can be drawn.

Taking together the results from the experiments on soybeans, it can be concluded that physical conditioning of water by EMF both in the form of microwave or MRET could remarkably increase the length of the soybean root, but not the height and the overall growth of the plants (Figure 4), after 38 days of cultivation. This result is in accordance with the previous report from the MRET patent that showed that soybeans irrigated with MRET-activated water could sprout more (13/20) than those irrigated with non-activated water (7/20), and after 15 days, the average length of the sprout treated with MRET-activated water was significantly longer (10 cm versus 23 cm).[11] The mechanisms that regulate root and shoot development in plants at various stages are largely different.[25] Therefore, it is likely that the water activated by an EMF could stimulate specific biological processes that lead to growth and development of the soybean root. The beneficial effects of electromagnetic spectrum on plants have been previously suggested.[26] Even if in this study there were no differences between microwaved or MRET-treated water, detailed investigation at the molecular and cellular level might reveal their specific biological effects on particular plant signalling pathways.

Conclusions

Our results suggested that electromagnetic-treated water can have diverse biological effects on both animal and plant cells. Whether these effects are beneficial, detrimental, or insignificant to the wellbeing of humans remains to be explored. Nevertheless, these findings warrant further investigation on the molecular mechanisms of various physically conditioned waters on specific cellular processes. In the future, experiments on larger sample sizes should be conducted. The effects on human and animal health should be investigated on both male and female from diverse age group. For plants, effects on a wide variety of plants at various stages of growth and developments should be pursued. In particular an investigation on molecular and cellular level should be done in order to decipher the precise mechanism of actions of low frequency electromagnetic-treated water. Exploration of an effect on the growth and differentiation on cancer cell-lines, stem cells, plant tissue culture, which can easily be tested, should also be of interest to the scientific community at large.

Declaration of interest

SC, WS, and KY were supported by postdoctoral and/postgraduate funding of SUT, which is funded by Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission. They declare no conflict of interest.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.homp.2013.11.004.

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