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Effects of intentionally treated water on the growth of mesenchymal stem cells: An exploratory study

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ABSTRACT

Objective: This study explored if human primary mesenchymal stem cells (MSCs), derived from two donors and cultivated in a medium made with intentionally treated water, would exhibit more growth and pluripotency than MSCs from the same source but grown in untreated (control) water.
Design: To create the treated water, three Buddhist monks directed their attention toward commercially bottled water while holding the intention that the water would enhance the growth of MSCs. Under double-blind conditions, cell culture growth mediums were prepared with the treated and untreated water, which was in turn used to grow the primary MSCs. Primary cells obtained from two donors were designated as Cells #1 and Cells #2. The prediction was that treated water would result in increased cell proliferation, that more cells would enter the cell cycle growth phase, and that there would be increased expression of genes (NANOG, OCT4 and SOX2) associated with improved cell growth and decreased expression of genes (p16, p21, and p53) associated with a decline in cell growth. The improved growth hypothesis was directional, thus one-tailed p-values were used to evaluate the results.
Results: Proliferation averaged across Cells #1 and #2 showed overall increased growth in treated as compared to control water ($p = 0.0008$). Cells #1 and #2 considered separately had differences in the same direction but only Cells #2 showed a significant difference on day 6 ($p = 0.01$). For cell cycle, there was a significantly greater percentage of Cells #2 in the S interphase with treated vs. control water ($p = 0.04$). For the gene expression analysis, when considering the average across the two donor cells, only the NANOG gene expression was in the predicted direction ($p = 0.01$); by contrast, the p16 gene expression was significantly opposite to the predicted direction ($p = 0.005$, one-tailed, post-hoc). For Cells #1 considered separately, no differences were significant except for p16, which resulted in an effect opposite to the predicted outcome ($p = 0.05$). For Cells #2, three genes were significantly in the predicted directions: NANOG ($p = 0.0008$), OCT4 ($p = 0.005$), and P53 ($p = 0.05$); p16 was significantly opposite to the prediction ($p = 0.001$).
Conclusion: Intentionally treated water appeared to have some biological effects on the growth, pluripotency and senescence of human MSCs. This was especially the case in one of the two donor cells tested, but the effects were not consistently in the predicted direction. As an exploratory study, caution is warranted in interpreting these outcomes, and adjustment for multiple testing would likely reduce some of the weaker effects to nonsignificant. But given the double-blind protocol, as well as several more significant outcomes in the predicted directions, further research is warranted.

Introduction

The hypothesis proposed in the present experiment was that water influenced by human intention would enhance the growth and pluripotency of cultured human stem cells. The motivation to perform this study was based on previously reported double-blind experiments that explored the effects of intentionally treated water on enhancing the aesthetics of frozen water crystals,[1,2] improving mood in people who drank oolong tea brewed from treated water,[3]

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and producing more robust growth of the mustard plant, Arabidopsis thaliana.[4,5] Other studies have explored whether samples of wine and chocolate, also treated by focused intention, would produce subjective improvements in mood.[6,7] All of these studies reported significant effects in the predicted directions, supporting an "intention hypothesis."

A common element in many of these studies was water, so it is noteworthy that several other studies have explored whether the molecular structure of water would be affected when healers focused their intentions toward water, or even if the water was merely in proximity while they performed various "energy medicine" modalities on clients. Using FTIR-ATR spectroscopy to analyze the water, those studies found significant changes in hydrogen-oxygen stretching bonds at the primary infrared absorption band.[8,9]

The present study continued this line of research with human mesenchymal stem cells (MSCs). This is of interest not only for basic scientific reasons, but because of its possible pragmatic value. That is, there are three types of stem cells that have been rigorously studied due to their potential for medical applications: embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and postnatal or adult stem cells. Both ESCs and iPSCs are pluripotent and are highly immature stem cells capable of giving rise to all cell types, whereas adult stem cells are more prone to lose stem cell properties (called "stemness") and can only differentiate into a small number of cell types. However, because adult stem cells are relatively easier to obtain and maintain than ESCs and iPSCs, they are considered a useful source of cells for therapeutic and regenerative purposes. They are also safer to use than ESCs or iPSCs and are less likely to develop tumors after transplantation. However, adult stem cells have limited expansion capacity and can be difficult to sustain long-term as stem cells in culture. Various methods have been tested to increase the stemness of these cells to help maximize their potential use.[10]

Human dental pulp stem cells (DPSCs) are a type of MSC isolated from human dental pulp tissue. DPSCs have demonstrated many clinical potentials, including facilitating cardiac angiogenesis and differentiating into neurogenic cells.[11-14] Besides their capacity for dental tissue regeneration, subpopulations of MSCs have also shown a capacity to regulate immune reactions.[15,16] Therefore, investigating ways to enhance the growth and pluripotency of MSCs, including unconventional ways involving focused intention as in the present study, are eminently worthwhile.

Methods

Intentional treatment

The distilled water used to culture the human stem cells were bottles purchased from Vedan, a commercial water bottling plant in Taiwan. A total of 8 bottles (800 ml/bottle) were used for this test, and they were randomly assigned by a research assistant into two groups, A and B, using a truly random number generator available at www.random.org (Randomness and Integrity Services Ltd., Dublin, Ireland). The intentional treatment was provided by a respected monk of the Bliss Wisdom Buddhist Foundation in Taiwan, along with two other senior monks from the same Foundation. The untreated bottles and treated bottle were placed in the same room. The intention they were asked to "imprint" into the treated water was as follows: "The human stem cells that absorb this water will manifest optimal growth; in particular, they will have increased nutrition, energy, vigor and well-being. This intended enhancement is only for this batch of water," explicitly referring to the treated bottles to avoid including the untreated water in the intentional process. These same monks, and this type of intention, had successfully participated in several previous studies.[3-5]

Procedure

Only a research assistant and the three monks who were present during the intentional treatment knew which bottles of water were treated and which were not. None of those individuals were involved in any of the other experimental procedures in this study, and none of the authors of this study were aware of the blinding assignments at this stage of the experiment. After the intentional treatment, the first author shipped the coded bottles of treated and control water (labeled A or B) in the same container to the second and last authors, who used the water to create the cell culture mediums used to grow the stem cells. These three investigators were blind to the treated or control condition of the water. After all measurements and analyses by the first and second authors were completed, the first author contacted the research assistant to break the blinding code, and then the data and codes were forwarded to the third author to double-check the analyses. All experiments were performed in triplicate, except one as noted.

Materials

To obtain the DPSCs used in this experiment, freshly extracted teeth were collected from healthy donors (aged 16–24 years) in the Oral Surgery Clinics or Periodontic Clinics at University of Tennessee Health Science Center (UTHSC) based on exempt protocols approved by the UTHSC Medical Institutional Review Board (#12–01937–XM); no patient consents were required. The pulp was obtained after the tooth was split–opened. Collected tissues were digested in a solution of 3 mg/ml collagenase type I and 4 mg/ml dispase for 30–60 min at 37°C. Single cell suspensions were obtained by passing the cells through a 70 μM strainer and seeded into culture plates. Cells were grown in media containing α–modification of Eagle's medium supplemented with 10% fetal bovine serum, 2 mM L–glutamine, 100 U/ml penicillin–G, 100 mg/ml streptomycin, and 0.25 mg/ml fungizone (Gemini Bio–Products, Inc., West Sacramento, CA, USA) and incubated at 37°C in 5% CO2. Cells were passaged at a 1:3 ratio expansion when cells reached ~80% confluence. Cell passages $\leq$ 5 (considered early passages, as these cells in general demonstrate more stemness at low passages) were used for the studies. DPSCs from two donors were cultured separately and are designated as Cells #1 and Cells #2.

Medium preparation

Growth culture medium was made using treated (labeled B) and control (labeled A) water. According to the protocol, every 100 ml of medium contained 1.017g α-Minimum Essential Medium, 0.22g $NaHCO_3$, 10% fetal bovine serum (FBS), 2mM L-glutamine and 100 units/mL pen-strep antibiotics. Both groups of medium were sterilized by filtering with 0.22 αm filters before being aliquoted into 50 ml and kept in 4°C or frozen in -20°C. Before being used, each medium was warmed in a 37°C-water bath.

Cell proliferation assay

Primary DPSCs from two separate donors were seeded at a density of 3,500/well (Cells #1) or 2500/well (Cells #2) in 12-well plates and cultured in a medium created from control or treated water, each in 0.5ml/well. Fresh medium for all experimental cultures was replaced every 2-3 days to supply sufficient nutrition. The DPSCs were seeded into wells in triplicate, and the total number of cells/well were counted on days 3, 6 and 8. For counting cells, they were trypsinized and detached from the well and suspended in fresh medium as single cells. Portion of the cells were mounted onto a cell counting chamber and counted manually under the microscope to obtain cell concentration and calculate the total number of cells per well. The two donor cells seeded at different densities on different days was due to the donor primary cells having different cell growth rates; e.g. Cells #2 grew faster. For proliferation studies, cells in the wells need to stay subconfluent, so we seeded less cells such that on day 8 they were

still subconfluent. Because we were comparing two different culture media for the same cells and same conditions, the number of cells does not affect the objective of the experiment. The days of cell counting were consistent for cells in both growth media and for both Cells #1 and #2, at days 3, 6, and 8.

Cell cycle analysis

Low passage of DPSCs were seeded in two 10-cm dishes at a density of 1×10^5/dish, and then cultured in treated or control medium for 6 days (Cells #1) or 5 days (Cells #2). The medium was replaced every 2–3 days. When reaching about 70-80% confluence, cells were harvested into single cell suspensions, collected by centrifuge (330 x g, 5min), and washed twice with PBS (phosphate-buffered saline). The cells were re-suspended in 0.5 mL PBS, fixed by adding 5 mL ice-cold 70% ethanol dropwise with gentle vortex, and incubated at 4°C for 48h. Cells were then collected by centrifuge (900 x g, 5min) and washed twice with PBS. Cell pellets were re-suspended and stained with 0.5 ml propidium iodide staining solution (100 mg/mL RNAse A, 50 mg/mL propidium iodide; Sigma) at 37°C for 40 min and subjected to flow cytometry for cell cycle analysis. Cell cycle distributions were analyzed in the form of percentage of cells in the G1, S, and G2 phases. Cell cycle analysis used propidium iodide (PI) to label DNA of cells cultured either in control or treated water at 6 days (Cells #1) or 5 days (Cells #2). Cell cycle distributions were analyzed using flow cytometry.

Gene expression analysis

Purification of total RNA from cells

Low passage of DPSCs were seeded in two 10-cm dishes at a density of 1×10^5/dish and cultured in either treated or control medium for 6 days (Cells # 1) or 5 days (Cells # 2). When reaching 70% confluence, cells were harvested and total cellular RNA was isolated using an RNeasy mini kit (Qiagen) with DNase I (Invitrogen) to remove the genomic DNA contaminant according to the manufacturer's instructions. Pelleted cells were lysed in 350μl Lysis Buffer RLT and were homogenized by vortexing for 1-2 min. Then 350 μl of 70% ethanol was added to the homogenized lysate and mixed well by pipetting. The resulting sample was transferred to a RNeasy spin column placed in a 2 ml collection tube and centrifuged for 15 s at 8000 x g. After being washed with 350 μl Wash Buffer RW1, DNase I incubation mix (80 μl) was directly added to the RNeasy spin column membrane, and the spin column was placed on the benchtop (20–30°C) for 15 min to remove DNA. After being washed with another 350 μl Buffer RW1 and 500 μl Buffer RPE, the spin column membrane was dried by centrifugation (2 min at 8000 x g). Subsequently 50 μl RNAse-free water was directly added to the spin column membrane, and RNA was eluted by centrifuge for 1 min at 8000 x g. The concentration of RNA was measured by a nanodrop (Thermo).

RT-PCR and qRT-PCR

The extracted RNA (1 ug) was reverse transcribed to synthesize the first-strand cDNA using Superscript IV (Invitrogen) according to the manufacturer's instructions. Oligo d(T) was used as the primer and RNase H was applied to remove remaining RNA. The produced cDNA was used as a template for each reverse transcription polymerase chain reaction (RT-PCR). cDNA (100 ng) gene-specific primers (OCT4 / NANOG / SOX2 / p16 / p53 / p21 / GAPDH, 200 nM final concentration), and 45 mL of Platinum Blue SuperMix containing 1 U Taq DNA Polymerase (Invitrogen) in a 50 mL final volume underwent PCR steps with the following program with the Thermocycler (AB Applied Biosystems): Initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 57°C for 30 s, 72°C for 60 s, and a final extension of 72°C for 5 min. The resulting RT-PCR products were run on a 1.5% agarose gel with ethidium bromide to test the effectiveness of cDNA and primers.

The same cDNA was used for quantitative real-time PCR (qRT-PCR). The qRT-PCR was carried out in triplicate in a MicroAmp Fast 96-Well Reaction Plate using the Lightcycler 480 SYBR Green I Master 2X Mix (Roche). Each reaction well contained 10μL Mater Mix, 0.5μL forward/reverse primers (10 μM) to the target genes, 8μL dH2O, and 1μL of cDNA in a final reaction volume of 20μL. The PCR was performed using 7500 Real-Time PCR System (AB Applied Biosystems) with the following thermal cycling conditions: Initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 10 s, 60°C for 20 s, and 72°C for 30 s. The housekeeping gene GAPDH was used as an internal control to normalize the Ct values of target genes. Primers used for qRT-PCR are listed in Table 1.

The expression of NANOG, OCT4, and SOX2 genes indicate the maintaining of cell stemness, and the expression of P16, P53, and P21 genes are associated with cell senescence.[17]

Hypotheses

Hypothesis 1 predicted that cell proliferation would be greater in cells grown in treated vs. control water. To evaluate this, a repeated measures ANOVA was employed with three levels of time (3, 6, and 8 days) and two levels of condition (treated vs. control). This and all subsequent analyses were performed using JASP 0.14.1 (https://jasp-stats.org/), and then confirmed with TIBCO Statistica 13.5.0.17 (https://www.statistica.com/en/).

Hypothesis 2 predicted that the G1, S, and G2 phases of the cell cycle would show improved activity in the treated water than the control water. To analyze these data a t-test was used for each of the phases.

Table 1
Primer sequence.

Gene function	GenBank Accession	Target gene	Primer sequence (5′–3′)	Product size
Pluripotency associated	NM_024865	NANOG	F: TAATAACCTTGGCTGCCGTCTCTG R: GCCTCCCAATCCCAAACAATACGA	150
	NM_002701	Oct-04	F: CAGTGCCCGAAACCCACAC R: GGAGACCCAGCAGCCTCAAA	161
	NM_003106	SOX-2	F: ACACCAATCCCATCCACACT R: GCAAACTTCCTGCAAAGCTC	224
Senescence associated	NM_000077	P16	F: CCCAACGCACCGAATAGTTAC R: CACGGGTCGGGTGAGAGT	153
	NM_000389	P21	F: GACACCACTGGAGGGTGACT R: CAGGTCCACATGGTCTTCCT	172
	NM_000546	P53	F:CCAGGGCAGCTACGGTTTC R: CTCCGTCATGTGCTGTGACTG	205
Housekeeping	NM_002046	GAPDH	F:CAAGGCTGAGAACGGGAAGC R:AGGGGGCAGAGATGATGACC	194

Hypothesis 3 predicted that the NANOG, OCT4, SOX2, p16, p21, and p53 genes would express differently for cells grown in treated and control water, with NANOG, OCT4 and SOX2 showing improved expression, and p16, p21, p53 showing reduced expression. To evaluate these effects a t-test was used for each gene. In all of these analyses checks for sample independence and homogeneity of variance were performed to ensure that parametric assumptions were satisfied.

Results

Cell proliferation

DPSCs from two donors were blindly seeded and cultured in triplicate in either control or treated water, and the total number of cells were counted on days 3, 6 and 8. Considering the average of both Cells #1 and #2, a significant difference was found between the treated and control conditions ($p = 0.0008$), with overall proliferation in the treated water greater than in the control water, as predicted (see Tables 2 and 3). Cell proliferation was significantly enhanced with treated water on days 6 ($p = 0.05$) and 8 ($p = 0.03$) (see Table 4 and Figure 1). No overall or per-day significant effects were observed in Cells #1, but significant differences were observed in Cells #2 on day 6 ($p = 0.01$) (see Figure 2).

Cell cycle

Only one sample of Cells #1 was obtained, and three samples of Cells #2, thus a statistical test was only available for Cells #2. In those cells there was significantly more activity in the S interphase in the treated vs. control condition, as predicted ($p = 0.04$). The G1 and G2 phases were not significant (see Tables 5 and 6).

Gene expression

The efficiency of gene-specific primers evaluated by RT-PCR showed that all genes except SOX2 were adequate for further analysis (Figure 3). Therefore, we measured the expression of NANOG, OCT4, p16, p21 and p53 in a further qPCR analysis. GAPDH was used as the housekeeping gene. Tables 7 and 8 show the results.

For Cells #1 and #2 averaged together, gene expression of NANOG was significantly in the predicted direction ($p = 0.01$). For Cells #1, none of the gene expressions differed from chance expectation (see Table 9). For Cells #2, there were three significant differences in the predicted directions: NANOG ($p = 0.0008$) and OCT4 ($p = 0.005$) were predicted to show positive differences and p53 was predicted to show a negative difference ($p = 0.05$). Interestingly, gene p16 was also predicted to show a negative difference, but it was observed (post-hoc, one-tailed) to be in the positive direction in both Cells #1 ($p = 0.05$) and #2 ($p = 0.001$), as well as on average ($p = 0.01$).

Table 3
Means and standard deviation (SD) for averaged cell counts.

Time	Condition	Mean	SD	N
Day3	Control	1.24	0.08	3
	Treated	1.14	0.16	3
Day6	Control	8.03	0.33	3
	Treated	9.64	0.93	3
Day6	Control	17.37	0.87	3
	Treated	19.35	0.47	3

Table 4
Simple main effects for the factor of condition.

Time	SS	df	MS	F	p
day 3	0.015	1	0.02	0.91	0.39
day 6	3.89	1	3.89	8.06	0.05
day 8	5.888	1	5.89	11.99	0.03

Note. Type II Sum of Squares

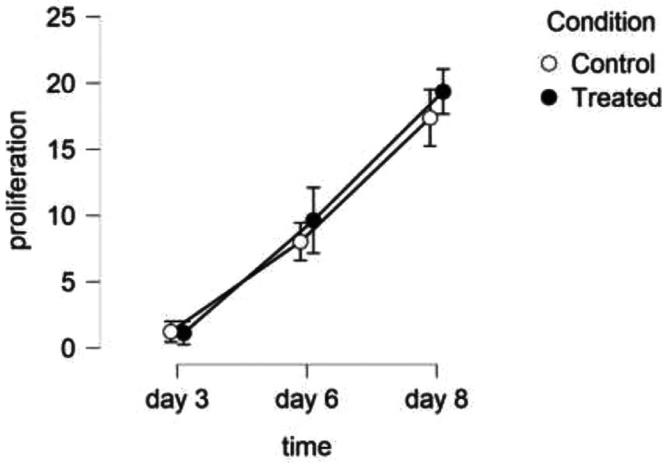

Fig. 1. Cell proliferation for cells grown in treated vs. control water, tested on days 3, 6, and 8, with 95% confidence intervals. Data shown are averaged over Cells #1 and #2. The overall treated vs. control difference is significant ($p = 0.0008$), and individually significant at day 6 ($p = 0.05$) and day 8 ($p = 0.03$).

Discussion

Intentionally treated water apparently affected the growth of adult stem cells derived from human dental pulp in predicted directions. Cells #1 considered separately did not show the predicted effects, but Cells #2 did show several significant outcomes, as did the data averaged over both cells.

This outcome is intriguing given that only three replications of the experimental tests were performed (with cell cycle being the one exception), so the statistical power to identify potential differences

Table 2
Repeated measures ANOVA for cell counts averaged across Cells #1 and #2.

Within Subjects Effects Cases	SS	df	MS	F	p
Time	888.16	2	444.08	968.1	<< .001
Time x Condition	3.67	2	1.85	4.03	0.06
Residuals	3.67	8	0.46		

Between Subjects Effects Cases	SS	df	MS	F	p
Condition	6.1	1	6.1	84.06	.0008
Residuals	0.29	4	0.07		

Note: Type III Sum of Squares

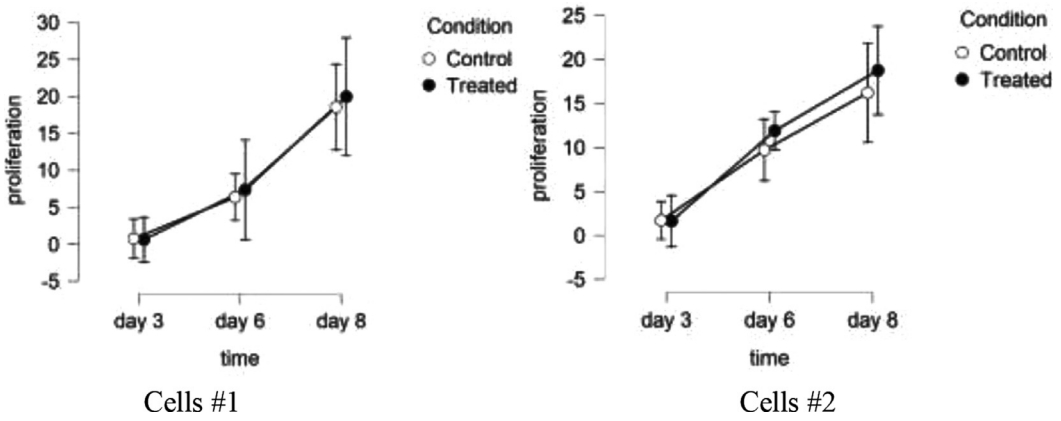

Fig. 2. Proliferation means and 95% confidence intervals for cells grown in treated vs. control water, tested on days 3, 6, and 8. (Left). Cells #1. No statistically significant differences. (Right) Cells #2. Significant difference on day 6, p = 0.01.

Table 5
Cell cycle t-tests for Cells #2.

	t	df	p
G1	-0.85	4	0.54
S	2.26	4	0.04
G2	-2.74	4	0.95

Note. Student's t-test.

Table 6
Group means and standard deviation for cell cycle test for Cells #2.

	Group	N	Mean
G1	Control	3	68.65
	Treat	3	68.04
S	Control	3	16.25
	Treat	3	18.54
G2	Control	3	14.6
	Treat	3	13.42

Table 7
Student t-tests for gene expression differences averaged across Cells #1 and #2.

Independent Samples T-Test	t	df	p
NANOG	3.62	4	0.01
Oct-04	-1.12	4	0.84
p16	4.56	4	0.99
p21	-1.81	4	0.07
p53	-0.5	4	0.32

Note. Student's t-test. Average of Cells #1 and #2

was minimal. Still, as mentioned in the Introduction, this outcome is in alignment with a growing number of previously reported studies studying similar intentional effects involving water.[3-5] Thus, while these results remain anomalous due to lack of an adequate theory about the underlying mechanisms, based on the prior literature this outcome is not especially unexpected.

Limitations

Other than an intentional effect in water, what else might have accounted for the results observed in this present study? False positives are always a possibility when data require statistical evaluation, especially when results are at or near the p = 0.05 threshold and multiple statistical tests are conducted. Countering that explanation is that overall cell proliferation, as well as the enhanced results with the NANOG gene in Cells #2, were quite far from chance (p = 0.0008), and in both cases the observed differences were in predicted directions. In addition, several apparently significant deviations were opposite to the predicted direction. Those results might eventually provide clues about how intentional effects influence stem cells, or from a more mundane perspective perhaps they too were false positives. Only future replications can help elucidate the most likely reasons for these unexpected reversals.

A double-blind protocol was employed to prevent obvious handling or measurement biases, so those were deemed implausible explanations of the observed results. It is conceivable that there may have been chemical differences among the bottles of water that were used for the treatment and controls, and that those differences were responsible for the observed effects. Countering that possibility is that the bottles were randomly selected from a pool of water bottles produced by the same commercial bottling plant.

After all measurements and analyses by the first and second authors were completed, the first author contacted the research assistant to break the blinding code, and then the data and code were forwarded to the third author to double-check the analyses. The third author was not blind to the code while double-checking the analyses, but his results were consistent with the first and second authors' findings. This reduces the possibility that the statistical findings were due to an analytical bias.

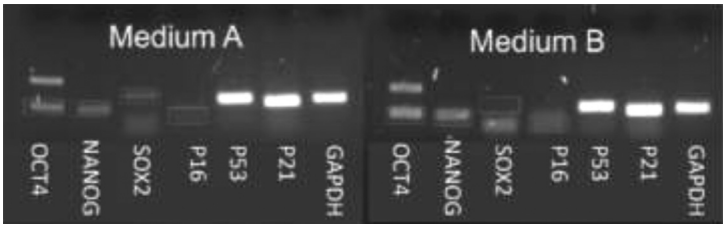

Fig. 3. RT-PCR gel images of amplified target genes.

Table 8
Group means and standard deviation for gene expression differences averaged across Cells #1 and #2.

Group Descriptives	Group	N	Mean	SD	SE
NANOG	control	3	0.64	0.1	0.06
	treat	3	1.01	0.14	0.08
Oct-04	control	3	1.1	0.11	0.07
	treat	3	1.01	0.09	0.05
p16	control	3	0.45	0.08	0.05
	treat	3	1.02	0.2	0.12
p21	control	3	1.7	0.56	0.33
	treat	3	1.03	0.3	0.17
p53	control	3	1.24	0.3	0.17
	treat	3	1.1	0.41	0.24

Table 9
T-tests for gene expression differences in Cells #1 and #2.

Independent Samples T-Test	t	df	p
NANOG_1	1.83	4	0.07
OCT_4_1	-1.72	4	0.92
p16_1	-2.06	4	0.95
p21_1	1.79	4	0.07
p53_1	0.29	4	0.39
NANOG_2	7.66	4	0.0008
OCT_4_2	4.54	4	0.005
p16_2	-6.66	4	0.99
p21_2	1.63	4	0.09
p53_2	2.19	4	0.05

Note. NANOG and OCT predict treat > control.
p16, p21, p53 predict control > treat; p-values are shown one-tailed according to the prediction.

It cannot be excluded that cell culture variations and inherent heterogeneity may have created spurious differences during the duplication and separation of the cell culture wells. Additional systematic errors, such as manual pipetting during the experimental processes, may have also contributed to the observed outcomes. Such possibilities may be resolved by using larger sample sizes in future replications.

Interpretation challenges

As often occurs in studies with statistically significant, if anomalous, outcomes, these results raise more questions than provide answers. For example, the treated and control water bottles used in these tests were mailed nearly 8,000 miles from Taiwan to the US State of Tennessee, where the stem cells were grown. And yet, some aspect of the treated water apparently maintained its intentional qualities. Given the significant environmental influences the water would have been subjected to during the journey, what sort of physical properties could the water have retained during that journey? For example, could the intentional "ingredient" have caused a molecular alteration in the treated water? A few previous studies have shown anomalous, intention-related bond-stretching in intentionally treated water[8,9], but could such molecular changes have persisted after an 8,000 mile trip?

Another question involves unknown differences between stem Cells #1 and #2. The former showed almost no effects, but the latter show numerous differences. Could there be idiosyncratic properties in stem cells that make some more sensitive to intentional treatments than others?

Future studies

Besides conducting a straight replication of this study, future experiments could investigate many interesting variations. For example, the "dose" of intention could be varied by asking those who provide the intentional treatment to influence the treated bottles, say once, three, and five times. Bottles could be prepared from a single large batch of water, then refilled and randomized to ensure that the contents were truly identical before the treatment took place. Bottles could be stored separately in heavily insulated containers to limit environmental influences. Treated bottles could be intentionally influenced while they were inside special containers to see if the intention could be "blocked" by, say, mu-metal or other shielding materials. A systematic negative control could be employed, whereby three bottles of water would be used. Within that design, say A and B were controls, and C was treated. In that case, under blinded conditions null results could be predicted for the comparison of A vs. B, but A vs. C and B vs. C would both be predicted to show positive differences. These experiments could also be tested using higher passages (such as passages >15) of the stem cells to see if the treated water has a greater observable influence.

Finally, Shiah[18] proposed that the cryptochrome protein (CRY) might be a possible "transducer" of intention because it is present in all living systems and is suspected to have quantum biological properties.[18-20] Two previous double-blind studies showed that the treated water resulted in objectively healthier Arabidopsis plants, especially in a genetic CRY "gain-of-function" mutation known as His-CRY2, which over-expresses in blue light as compared to the wild type.[4,5] Thus, future studies could investigate if the effect observed in human mesenchymal stem cells was also related to changes in the CRY protein.

Conclusion

As an exploratory study, it would be imprudent to offer a firm conclusion about the effects of intentionally treated water on the growth of stem cells. But the intriguing results observed here, especially in light of previous positive outcomes using similar designs, justify further research.

Declaration of Competing Interest

The authors declare no conflict of interests. The first author secured funding for this study and wrote the first draft, the second and fourth authors conducted the cell biology components of the experiment and contributed to writing the manuscript, and the third author double-checked the statistical analyses and contributed to writing the manuscript.

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