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Beneficial Health Effects of Alpha Omega (AO) Water® Crystal

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ABSTRACT

Question of the study: In the present study, cell biological methods were used to investigate whether tap water, after passing through an Alpha Omega (AO) Water® Crystal instrument, has received and stored vibrational energy that possesses beneficial health effects. The effects of this Alpha Omega (AO) Water® on cultured cells were directly compared with the corresponding initial tap water without activation.

Experimental: By using cultured connective tissue fibroblasts we examined the basal metabolism, long-term vitality and the regeneration process of initial tap water after treatment with the Alpha Omega (AO) Water® Crystal instrument in comparison to untreated initial tap water. In addition, the endogenous generation of superoxide anion radicals by inflammation-mediating cells (functional neutrophils) was investigated.

Results: The basal metabolism of the connective tissue fibroblasts was stimulated by the tap water after Alpha Omega (AO) Water® Crystal instrument treatment in a concentration-dependent manner with a maximum of more than 50% in direct comparison to untreated cells. In accordance with the stimulation of cell metabolism were the results of the long-term cultivation of connective tissue fibroblasts with 25 vol% of Alpha Omega (AO) Water® Crystal vs. untreated initial tap water for 8 days. Here we found that the cell density was significantly increased by the Alpha Omega (AO) Water® Crystal suggesting that cell proliferation and vitality was increased by the treated water. In addition, also the regeneration of connective tissue fibroblasts was markedly stimulated by the tap water after Alpha Omega (AO) Water® Crystal instrument treatment when compared with the untreated initial tap water. Even though the basal cell metabolism of functional neutrophils as inflammation-mediating cells was significantly increased by the tap water after Alpha Omega (AO) Water® Crystal instrument treatment in comparison to the untreated initial tap water, we observed a significant inhibition of endogenous superoxide anion radical generation by the Alpha Omega (AO) Water® Crystal by more than 30%.

Conclusions: The Alpha Omega (AO) Water® Crystal instrument seems to be a very potent instrument for the activation of local tap water by vibrational energy which restores healthy resonance in the body. From our current in vitro studies with cultured cells, we can recommend the use of Alpha Omega (AO) Water® Crystal for the improvement and maintenance of health and well-being.

Keywords

Alpha Omega Water® Crystal
Connective tissue fibroblasts
Functional neutrophils
Cell metabolism
Regeneration
Oxidative burst
Inflammation
Tissue repair
Well-being
Health

INTRODUCTION

Water is present in all of its three phases in our natural world. It is a tasteless, odorless, and nearly colorless chemical substance that is the main constituent of most living organisms. From the strictly scientific point of view, water is composed of two hydrogen atoms covalently bonded to one oxygen atom

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(H_2O). Water exhibits unique properties that make it vital for biological processes. Water is a polar molecule which contributes to its ability to dissolve a wide range of substances as a universal solvent. This property is crucial for biochemical reactions, nutrient transport, and waste removal in living organisms. Moreover, water posesses a number of unique properties which can be explained by natural science only insufficiently. Water is much more than just hydrating the body. Under specific circumstances water molecules can organize themselves into structures, layers or clusters of energy which carry information [1-6].

Moreover, the resonance principle, one of the omnipresent and omniactive phenomena in nature, can easily reason that vibration carried in water must act on anybody which intakes or submerges oneself in it. If these vibrations are beneficial, they may be able to restore healthy resonance in the human body [7,8].

The basic principle of the Alpha Omega (AO) Water® Crystal instrument is the application of resonance to bring specific vibrations into the water which is passed through the instrument. For the maintenance of health, the beneficial qualities of the vibrational levels stored in water were applied by different natural inorganic materials as well as physical qualities such as flow pattern geometry, vortexes, electromagnetic fields and others. The "crystal" version is equipped with gold and silver-coated water pipes as well as with a 99.99% pure crystal housing instead of the traditional acrylic housing.

In the present scientific study, cell biological methods were used to investigate whether tap water, after passing through an Alpha Omega (AO) Water® Crystal instrument, has received and stored vibrational energy that possesses beneficial health effects. The effects of this Alpha Omega (AO) Water® on cultured cells were directly compared with the corresponding initial tap water without activation.

MATERIALS AND METHODS

Alpha Omega Water® Crystal

The Alpha Omega (AO) Water® instrument is manufactured and distributed by SIA JL Ventures, AO Water Technologies, LV-2111 Kekavas Novads, Latvia. According to the information of the manufacturer, the Alpha Omega (AO) Water® instrument can be regarded as a water recorder. The basic principle of the instrument is the application of the resonance principle, one of natures omnipresent and omniactive phenomena, that vibration carried in water must act on anybody which intakes or submerges oneself in it. For the maintenance of health, the beneficial qualities of the vibrational levels stored in water were applied by different natural inorganic materials like lava, quarz sand, amethyst, mountain crystal as well as physical qualities such as flow pattern geometry, vortexes, electromagnetic fields and others. The "crystal" version is equipped with gold and silver-coated water pipes as well as with a 99.99% pure crystal housing instead of the traditional acryl housing. This means that the provided frequency range within the Alpha Omega (AO) Water® instrument is projected into and through the crystal housing which creates a natural fullness of overtones which are oscillating through the instrument and within. Thus the frequency richness rises impressively compared to the acrylic version of the Alpha Omega technology.

For the experiments, local tap water from Latvia as well as local tap water from Germany was converted to Alpha Omega (AO) Water® Crystal and used within the next four weeks. The untreated corresponding initial tap water without activation was taken as control.

Test concentrations of Alpha Omega Water® Crystal

Although the daily intake of water of about 1.5 liters is equivalent to a percentage of only 3% of the whole body fluid, the continuous daily intake of Alpha Omega (AO) Water® Crystal might cause an accumulation within the body. This explains the use of higher test concentrations of water as performed in this study. Concentrations of the added water in the tests varied from 0 vol% (= culture medium/internal control) up to a maximum of 40 vol%.

Cell culture

The studies were performed with two different cell lines: (1) Connective tissue fibroblasts (cell line L-929, ACC-2; Leibniz Institute DSMZ, Braunschweig). (2) Human promyelocytes (cell line HL-60; ACC-3; ECACC 98070106; Leibniz Institute DSMZ, Braunschweig, Germany). The non-adherently growing promyelocytes were differentiated over a period of 6 days by the addition of 1.5 % dimethylsulfoxide to the culture medium to inflammation-mediating cells (functional neutrophils), which are capable to generate superoxide anion radicals after stimulation by a phorbol ester *in vitro* [9-11]. Both cell lines were routinely cultured in RPMI 1640 supplemented with 10% growth mixture (FBS) and 0.5% gentamycin in an incubator at 37°C and an atmosphere of 5% CO_2 and 95% air at approximately 95% to 99% humidity. Cells were subcultured twice a week.

Basal metabolism of connective tissue fibroblasts

Cells from mass cultures were seeded in 96-well culture plates (200 µl culture medium/well) at a cell density of 50,000

cells/well and incubated for 24 hours until the cells were completely attached and spread. The culture medium was exchanged and 0 vol% (= internal control) up to 40 vol% of tap water with and without treatment with the Alpha Omega (AO) Water® Crystal instrument was added. Cells were incubated for another 24 hours. Thereafter, a reaction mixture consisting of phosphate buffered saline with 10 mM glucose as an energy source and the cell impermeant tetrazolium dye WST-1 (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt; Sigma-Aldrich, Taufkirchen, Germany) was added to the cells. The reduction of this tetrazolium dye into its highly coloured formazan is directly proportional to the mitochondrial dehydrogenase activity and the cellular energy metabolism [12,13]. Finally, the optical density was measured as a difference measurement ΔOD = 450-690 nm at definite time points by an Elisareader (BioTek ELx808 with software Gen 5 version 3.00) and analyzed using Microsoft Excel. Four independent experiments with 4 to 6 replicate wells per experiment were performed (n=4).

Long-term cultures of connective tissue fibroblasts

Cells from mass cultures were seeded into 75 cm^2 cell culture flasks at a density of 10,000 cells/flask and incubated for 24 hours until the cells were completely attached and spread. Thereafter, 25 vol% of tap water with and without treatment with the Alpha Omega (AO) Water® Crystal instrument was added to the culture medium and the cells were continuously cultured for 8 days without further culture medium exchange. This represents a period of several weeks in vivo. Finally, cells were washed with phosphate buffered saline, fixed with methanol p.a., stained with Giemsa methylene blue solution and air-dried. The colonized area was documented micrographically under the microscope of at least 6 points per cell culture. Analysis of the data was done using a software with artificial intelligence (confluence assay; Ikosa AI, KML Vision, Graz, Austria). A total of four independent experiments was performed (n=4).

Regeneration/wound healing of connective tissue fibroblasts

Connective tissue fibroblasts were seeded at a density of 100,000 cells/ml into the four compartments of a silicone frame (4 well-culture inserts; ibidi, Gräfelfing, Germany). The individual compartments are separated from each other by a 500 µm thick silicone bar. The silicone frame adheres firmly to the bottom of a culture dish and forms a defined cell-free space that the cells can colonize by proliferation and migration after the frame has been removed.

After reaching confluency within 48 hours after cell seeding, the silicone frames of the cell cultures were removed with tweezers. A sharp wound edge was obtained between the four compartments of the frame. Immediately after removing the silicone frame, the cell cultures were washed several times with culture medium and, finally, fresh culture medium containing 25 vol% of tap water with and without treatment with the Alpha Omega (AO) Water® Crystal instrument was added. After a continuous regeneration period of 24 hours the cells were washed with phosphate buffered saline, fixed with methanol p.a., stained with Giemsa methylene blue solution and air-dried. The width of the remaining cell-free area was documented micrographically under the microscope of at least 4 points per cell culture. Analysis of the data was done using a software with artificial intelligence (scratch assay; Ikosa AI, KML Vision, Graz, Austria). A total of three independent experiments with duplicate wells was performed (n=3).

Endogenous superoxide anion radical generation by functional neutrophils

For the examination of endogenous superoxide anion radical formation, the culture flasks with the functional neutrophils were collected and washed by centrifugation at 190 x g and repeated washings in phosphate buffered saline. Thereafter, the functional neutrophils were stimulated to generate superoxide anion radicals by adding a phorbol ester to the reaction mixture. The reaction mixture also contained 0 vol% (= control) up to 40 vol% of tap water with and without treatment with the Alpha Omega (AO) Water® Crystal instrument. The radicals caused a cleavage of the tetrazolium dye WST-1 (Sigma-Aldrich, Taufkirchen, Germany), which was also added to the reaction mixture. The cleavage of the dye was directly related to the amount of oxygen radicals, i.e. the more reactive radicals were present in the reaction mixture, the more pronounced was the cleavage of the dye and the change in optical density (= color change of the dye). The optical density was recorded at t=0 and at definite time points with the Elisareader (BioTek ELx808 with software Gen 5 version 3.00) and analyzed using Microsoft Excel. A total of three independent experiments with triplicate wells was performed (n=3).

STATISTICAL ANALYSIS

Statistical analysis was done using the parameter-free two-tailed Wilcoxon-Mann-Whitney rank sum test.

RESULTS

The basal metabolism of the connective tissue fibroblasts was stimulated by the tap water after Alpha Omega (AO) Water® Crystal instrument treatment in a concentration-

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dependent manner. The maximum stimulation of activated water versus untreated controls was 51.6 ± 9.5% at 20 vol% and 49.8 ± 10.8% at 30 vol% (mean values ± standard deviations; $p \leq 0.01$). At higher water concentrations the difference between activated and untreated tap water decreased to about 20.5 ± 6.9%, which was still statistically significant (mean value ± standard deviation; $p \leq 0.01$).

In accordance with the stimulation of cell metabolism by the Alpha Omega (AO) Water® Crystal, were the results of the long-term cultivation of connective tissue fibroblasts with 25 vol% of tap water after Alpha Omega (AO) Water® Crystal instrument treatment in comparison to 25 vol% of initial tap water. After 8 days of continuous cultivation, the colonized cell area was 91.7 ± 2.2% of total area, whereas untreated cells resulted in a colonized cell area of only 84.9 ± 1.4% of total area (mean values ± standard deviations). Thus, the proliferative activity and vitality of the cells was significantly higher after Alpha Omega (AO) Water® Crystal instrument treatment ($p \leq 0.01$). This difference became also apparent in the direct visualization of the cell cultures (Figure 1).

The regeneration of connective tissue fibroblasts was also markedly stimulated by the tap water after Alpha Omega (AO) Water® Crystal instrument treatment when compared with the untreated initial tap water. The totally colonized area after 24 hours was 92.9 ± 1.8% (mean value ± standard deviation) for the cell cultures with the Alpha Omega (AO) Water® Crystal and only 86.8 ± 1.6% for cell cultures with the initial tap water (mean values ± standard deviations). Again, the difference was statistically significant ($p \leq 0.01$). For a microscopic visualization of the results, see Figure 2.

Even though the basal cell metabolism of functional neutrophils was significantly increased by 25.9 ± 8.4% (mean ± standard deviation; $p \leq 0.01$) by the addition of water treated with the Alpha Omega (AO) Water® Crystal instrument in comparison to the untreated initial tap water, we observed a significant inhibition of endogenous superoxide anion radical generation by the Alpha Omega (AO) Water® Crystal by 33.3 ± 3.6% (mean ± standard deviation; $p \leq 0.01$) in comparison to the untreated initial tap water.

DISCUSSION

Connective tissue fibroblasts play a crucial role in the maintenance and homeostasis of connective tissue integrity and function throughout the body. These specialized cells are primarily responsible for the synthesis and remodeling of the extracellular matrix, which provides structural support and biochemical signaling to surrounding cells. The metabolic activity of fibroblasts is central to their function, influencing tissue repair, inflammation, and the overall health state of connective tissues. Their ability to adapt metabolically allows them to perform essential functions in extracellular matrix synthesis and remodeling, while also presenting challenges when their activity becomes dysregulated. In response to injury or inflammation, fibroblasts become activated, exhibiting increased metabolic activity [14,15]. This activation is characterized by enhanced proliferation and the upregulation

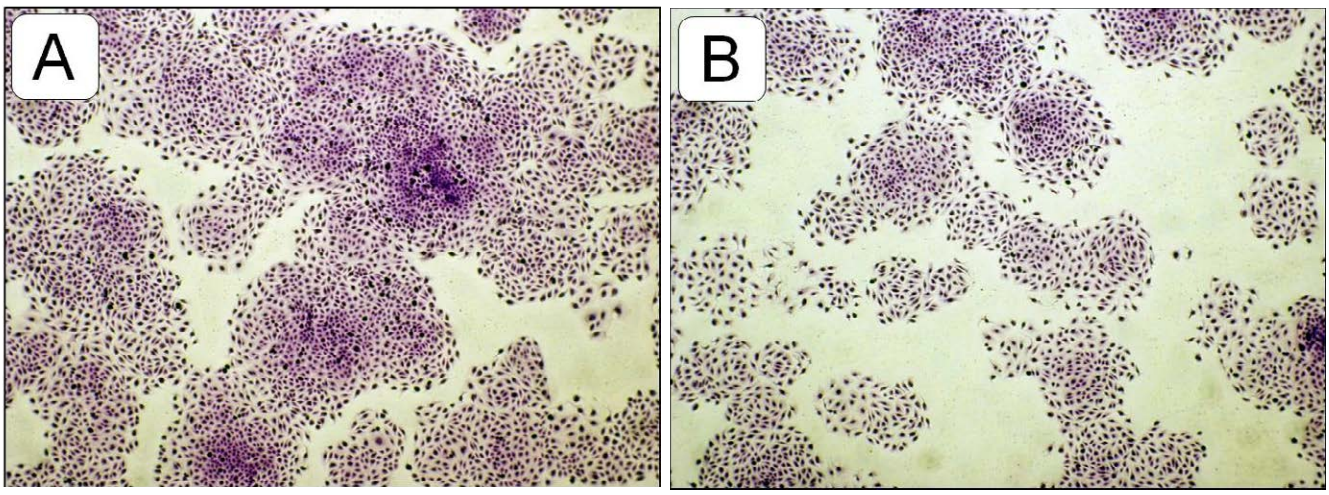

Figure 1: Representative micrographs of the fixed and stained connective tissue cell cultures after 8 days of continuous cultivation with 25 vol% of tap water treated with the Alpha Omega (AO) Water® Crystal instrument (A) and 25 vol% of initial tap water without treatment (B). The different colonized areas between both cell cultures are clearly visible. Olympus IX 50 inverted microscope at brightfield illumination with an Olympus achromate 4x and an Olympus E-20P digital camera at 5 megapixel resolution.

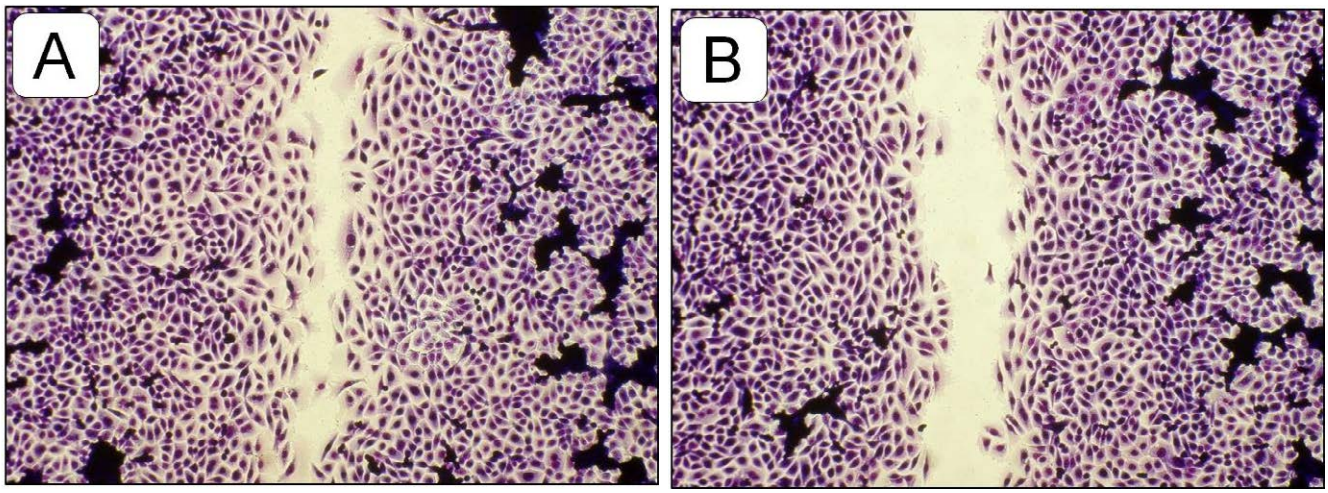

Figure 2: Representative micrographs of the fixed and stained connective tissue cell cultures after 24 hours of regeneration by colonization of the cell-free area. (A) 25 vol% of tap water after treatment with the Alpha Omega (AO) Water® Crystal instrument; (B) 25 vol% of initial tap water without treatment. The difference in the residual uncolonized area between both cell cultures is clearly visible. Olympus IX 50 inverted microscope at brightfield illumination with an Olympus planachromate 10x and an Olympus E-20P digital camera at 5 megapixel resolution.

of extracellular matrix production, which is a prerequisite for wound healing and tissue regeneration [16,17].

The multifaceted process of wound healing can be broadly divided into four overlapping phases: haemostasis, inflammation, proliferation and remodelling. In the in vitro model used here, we emulated the proliferation phase, which is characterized by the formation of new tissue. Connective tissue fibroblasts migrate and proliferate into the denuded/wounded area and synthesize collagen and extracellular matrix, providing structural support and accelerating the regeneration process [18].

Water can be activated by a variety of different methods [19-22]. In this current study we used Alpha Omega (AO) Water® Crystal which has been activated by the beneficial qualities of the vibrational levels stored in water which were applied by different natural inorganic materials like lava, quarz sand, amethyst, mountain crystal as well as physical qualities such as flow pattern geometry, vortexes, electromagnetic fields and others.

Our results have demonstrated that the Alpha Omega (AO) Water® Crystal is able to promote some of the essential physiological functions of connective tissue fibroblasts, namly basal cell metabolism and the regeneration/wound healing process in a significant manner when compared with untreated initial tap water. However, the basal cell metabolism is closely related to cell vitality and refers to the overall health and functional capacity of cells within an organism. It is a critical aspect of biological systems, as the vitality of cells directly impacts tissue function, organ health, and overall well-being. Maintaining high cell vitality is essential for processes such as growth, repair, and regeneration. Healthy cells exhibit optimal metabolic activity, efficient energy production, and robust responses to environmental changes.

One of the essential aspects of cell vitality, namly the basal cell metabolism of connective tissue fibroblasts, was stimulated by more than 50% by the Alpha Omega (AO) Water® Crystal in comparison to untreated initial tap water. Also in long-term cultures and the regeneration assays the stimulation in cellular activity was present and caused a higher colonized cell area in long-term cultures (= cell proliferation) as well as a reduced cell-free area in the regeneration assay (= cell proliferation and migration).

Neutrophilic granulocytes (also called polymorphonuclear neutrophils or PMN) constitute the largest group of leukocytes and form the first line of defence against pathogenic microorganisms. They combat these pathogens through phagocytosis, the release of antimicrobial molecules, and the generation of reactive oxygen species via an oxidative burst [23]. Attracted by chemical substances such as specific chemokines or cytokines released during the inflammatory process, these cells can migrate from the blood into the inflamed tissue and produce superoxide anion radicals [24,25]. These radicals contribute to tissue destruction (necrosis) in the inflamed area and may cause a progression of the inflammatory process, potentially slowing wound healing. For an overview of the role of neutrophils in health and disease, see [26].

We utilized an in vitro model representing one specific aspect of the inflammatory process, namely the generation of reactive superoxide anion radicals. A reduced radical generation by functional neutrophils is comparable to a local anti-inflammatory effect in the tissue. The results of the study demonstrate that Alpha Omega (AO) Water® Crystal shows a marked potential in reducing endogenous radical generation in comparison to untreated initial tap water. However, the effect is within a range that should not significantly impact the innate immune system's ability to defend against microbial pathogens *in vivo* [27].

Finally, a very remarkable aspect should be mentioned. Although the initial tap water samples were of completely different local origin (Latvia and Germany), the observed beneficial effects of the tap water after treatment with the Alpha Omega (AO) Water® Crystal instrument were in the same range. This suggests that this kind of water activation seems to be universal and is not influenced by the origin of the initial tap water.

As a conclusion, the Alpha Omega (AO) Water® Crystal instrument seems to be a very potent instrument for the activation of local tap water. From our current in vitro studies with cultured cells we can recommend the use of Alpha Omega (AO) Water® Crystal for the improvement and maintenance of health and well-being.

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