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Water structure changes induced by ceramics can be detected by increased permeability through aquaporin

Tadao Kozumi [a], Yoshichika Kitagawa [b,c,*]

[a] Cosmic Co., Ltd., Hiroshima 723-0015, Japan
[b] Graduate School of Bioresource Sciences, Akita Prefectural University, Akita 010-0195, Japan
[c] Kitagawa Scientific General Research Institute LLC., Hita 877-0039, Japan

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ABSTRACT

Aquporins are intrinsic membrane proteins that function as water channel to transport water and/or mineral nutrients across biological membranes. In this study, we aimed to clarify whether water structure can be changed by the presence of ceramics and whether such a change can be determined by aquaporin. First, we confirmed that ceramics could transform tap water into active tap water by increasing water permeability through aquaporin. We also found that this change in water permeability by treatment with ceramics occurred in distilled water. The distilled water was determined to exhibit the same aquaporin permeability as the original tap water. Our data indicate that the aquaporin permeability of water can be changed by severe physical shocks, such as slapping and sonication, which is consistent with the implication that the aquaporin permeability is closely related to the structure of the water. In this study, using aquaporins, we first reported that the treatment of water with ceramics can affect the structure of water, and the water can retain the structure for a given period under certain condition

1. Introduction

Water is a critical component in all living cells. Drinking water greatly affects human health [1]. Increasingly, science is providing evidence linking the human health with water quality [2]. For example, certain mineral waters could enhance immune activity in humans and anti-cancer immunity in mice by increasing the activity of natural killer cells [3]. Because natural mineral water contains various solutes, it is believed that mineral water is likely to be good for human health. Conversely, water treated with special ceramics and stone is anecdotally reported to be good for human health and good for the growth for animals and plants, even when it is confirmed that minerals do not elute into the water from the ceramics or stone [4]. If this finding is observed, it is considered that the water molecules themselves have changed. The structure of water has not been sufficiently demonstrated scientifically to date. Furthermore, the consideration that the water structure affects human health or the growth of animals and plants has not been scientifically accepted to date. In the present study, we measured water permeability of ceramic-treated water through human aquaporins and attempt to clarify whether the structure of water is changed by exposure to ceramics. Aquaporin was first discovered as water channel in human blood cells [5]. Aquaporins are present in the plasma membrane of the cells and have a narrow hole of approximately 3 Å which contributes directly to water entrance [6,7] and exit to maintain cellular water balance [8,9]. We have already demonstrated that aquaporin permeability differs depending on the type of water [10]. The factors affecting the gating behavior may include phosphorylation, pH, Ca^{2+}, pressure, solute gradients, temperature and nutritional conditions [11]. If these factors can be excluded completely, the difference in aquaporin water permeability depends on the nature of the water molecule itself.

2. Materials and methods

2.1. Water samples and treatments

Distilled water was prepared from the tap water of Hita city. The ceramics, referred to as “Tadanoumi ceramics®”, were produced by Cosmic Co. Ltd., Hiroshima, Mihara, 723-0015, Japan. This solid ceramic was made by melting and mixing the iron and clay. We use another sample consisting of the clay only and yet another consisting of the iron and clay without melting. Fifty milliliters of tap water and distilled water were treated with 4 g of ceramic for

* Corresponding author at: Graduate School of Bioresource Sciences, Akita Prefectural University, Akita 010-0195, Japan.
E-mail addresses: tadaokozumi@yahoo.co.jp (T. Kozumi), kitagawa@kitagawainst.com (Y. Kitagawa).

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24 h at room temperature. As the controls, the water samples were treated with microwaves (Toshiba ER-501S) for 3–5 min at high power and treated with sonication (Branson 2510) for 3–5 min at normal power at room temperature. Degassing was carried out for 3 h using a vacuum pump. Heating at 100 °C was performed in a boiled water bath for 10 min. The flower Begonia was used for the long shelf life test: the stem of the flower was cut and placed in a glass bottle containing tap water or ceramic-treated tap water.

2.2. In vitro transcription of aquaporin genes, microinjection of Xenopus oocytes and measurement of water permeability

The human aquaporin genes (AQP1, AQP2, AQP3 and AQP5) inserted in the pXβG-ev1 vector were furnished by Dr. Ishibashi, K., Dr. Yasui, M., and Dr. Sasaki, S. The capped complementary RNA (cRNA) was synthesized using T3 RNA polymerase of the mMESSAG EmMACHINE High Yield Capped RNA Transcription Kit (Cat no.: AM1348, Ambion, USA) after linearization of the aquaporin pXβG-ev1 constructs [10]. The synthesized RNA samples were purified, and the concentrations were measured. Oocytes (1.0–1.2 mm in diameter) of stage V–VI were incubated in 0.1% collagenase (*Clostridium histolytium*, Type II, Sigma) solution [100 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, and 5 mM Hepes-Tris (pH 7.5)] at 20 °C for 2 h. The exfoliated oocytes were washed with Modified Barth's Saline (MBS) medium [88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.4 mM $Ca(NO_3)_2$, 0.4 mM $CaCl_2$, 0.8 mM $MgSO_4$, 100 μg ml^{-1} Na-penicillin, and 100 μg ml^{-1} Streptomycin, 15 mM Tris–HCl (pH 7.4)]. 50 nl of cRNA (10–50 ng) or 50 nl water was injected into oocytes using a Nanoject injector (Narishige, Tokyo, Japan). The injected oocytes were cultured in 1× MBS (200 m Osm_{in}) prepared by Milli-Q water for 48 h at 20 °C. For determination of water permeability, the injected and cultured oocytes were transferred into water (0 m Osm_{out}), and the oocyte swelling velocity was measured with a digital camera (Shimazu, Kyoto, Japan) and calculated with Motic Images Plus 21S software (Shimazu, Kyoto, Japan) [10]. Osmotic water permeability (Pf) was determined from the initial slope of the time course of V/V_0 ($d(V/V_0)/dt$), initial oocyte volume ($V_0 = 9 \times 10^{-4}$ cm^3), initial oocyte surface area ($S = 0.045$ cm^2), and molar volume of water ($V_w = 18$ cm^3 mol^{-1}) [7]:

$$Pf = V_0\left[d(V/V_0)/dt \right] / \left[S \times V_w (Osm_{in} - Osm_{out}) \right]$$

2.3. Proliferation test of cultured cells

Normal skin cells (YK-cell) were originally isolated from human skin. Dulbecco's Modified Eagle Medium (DMEM) was purchased from Wako Co. Ltd. (Osaka Japan). DMEM medium containing 10% fetal bovine serum was used as control medium. The ceramic-treated medium was created by treating the control medium with Tadanoumi ceramics® (approximately 1 g for 50 ml medium) for 24 h at room temperature. The medium was used after the filter was sterilized. Human skin cells were grown in the control medium. Full grown cells were trypsinized and used at a concentration of 1.0×10^5 cells/ml. The cells cultured in control or ceramic-treated medium for 48 h were treated with Cell counting kit-8. The number of viable cells is determined by measuring the absorbance at 450 nm of water soluble formazan, which is produced by intracellular dehydrogenase.

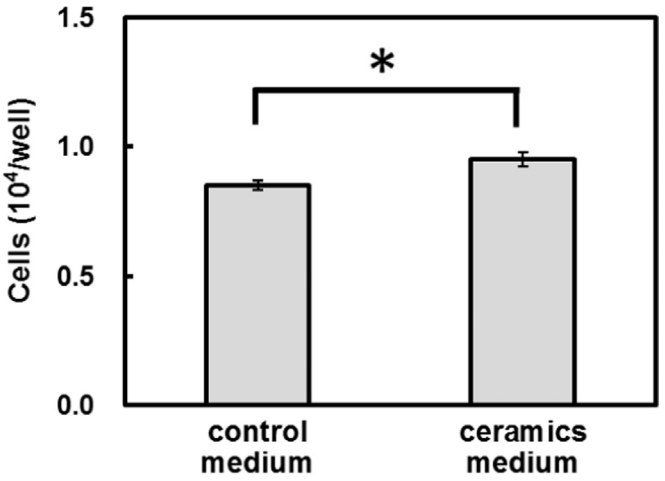

Fig. 1. Proliferation of the human skin cells in the ceramic-treated culture medium (*$P < 0.05$).

3. Results

3.1. Effect of the ceramic-treated water on shelf life of begonia

The Begonia flowers were grown in the tap water or the ceramic-treated tap water, and their flower growing status was compared (Supplemental figure). The Begonia flowers in tap water withered after 7 days, but the Begonia flowers in the ceramic-treated water maintained their original shape after 7 days.

3.2. Influence of the ceramic-treated water on the growth of culture cells

Because the ceramic-treated water has different physical and chemical features, these differences may affect culture cell activity. In this study, we investigated the effect of the ceramic-treated water on the human skin culture cell activity.The results of human skin cell activity assay show that proliferation of the cells culture in the ceramic-treated culture medium was 12% higher than the cells culture in the control medium (Fig. 1), suggesting that the use of ceramic-treated water in the medium promoted the proliferation of cells.

3.3. Effect of ceramics on water permeability of aquaporins

If the nature of water is changed by ceramics, we hypothesize that the modified water molecules can change their permeability through aquaporins. To elucidate how ceramic-treated water affects aquaporin permeability, oocytes expressing human aquaporins (AQP2, AQP3 and AQP5) were transferred directly to the untreated and ceramic-treated tap water. The expansion rates of oocytes expressing different aquaporin in untreated and ceramic-treated tap water samples were recorded, and the water permeability (Pf) was calculated. As shown in Fig. 2, AQP2, AQP3 and AQP5 showed significantly higher permeability in the ceramic-treated tap water than the untreated tap water. The Pf values of the ceramic-treated water through AQP2 increased by 26% compared with the tap water (Pf was 305 μm/s and 386 μm/s for the untreated and ceramic-treated tap water, respectively). Similar to AQP2, the Pf value of ceramics-treated water through AQP3 increased by 26% (Pf was 97 μm/s and 144 μm/s for the untreated and ceramic-treated tap water, respectively), and AQP5 increased by 21% (Pf was 1258 μm/s and 1523 μm/s for the untreated and ceramic-treated tap water, respectively). Statistically, the ceramic-treated water had significantly higher permeability compared to