# Antioxidative effect of water treated by granular ceramics

Dept. of Applied Physiology, Faculty of Medicine, University of Miyazaki, Japan

## Abstract:

It has been reported that treatment of water by granular kaolinite ceramics modified the physical and biological properties of water (Ref.1-4). It was shown that treated-water was positively charged without any absorption-deposition phenomena to substances contained in water. The positive charge of the treated-water was indirectly affirmed by thermally stimulated depolarized current measurements and Nuclear Magnetic Resonance spectrometry analysis. In the present study, we investigated an antioxidative effect of treated-water employing fibroblast cell culture system and Vitamin C preservation test..

After incubation for 24 hours, the survival rate of the cells cultured in tMEM was significantly high compared to that of the cells cultured in MEM. Dissolved Vitamin C loses its reducing activity in time course. However, the reducing activity of Vitamin C in tMEM was significantly higher than that of Vitamin C in MEM, after prolonged incubation of the solution, suggesting the preventive effect of the treated-water against auto-oxidation of Vitamin C. Although the exact mechanism involved in the cell resistance against oxidative stress is not clear yet, the results indicated anti-oxidative property of the water.

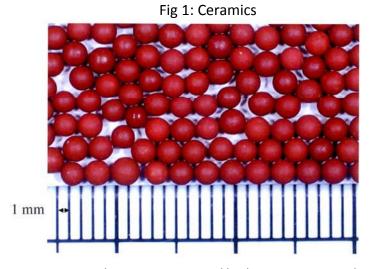


Fig. 1: High-tempreture sintered kaolinite ceramic granules

#### **Methods**

- a) Preparation of ceramics-treated water Five hundred ml of Milli-Q water (Milli-Q SP UF system) was mixed with 10 g of high-temperature sintered kaolinite ceramic granules in plastic bottle. Then vigorously shake the bottle 20 times. Milli-Q water was used as untreated control water.
- b) Preparation of treated-culture medium (tMEM) MEM powder was dissolved by

treated-water prepared as mentioned above. After sterilized by autoclave, the medium was used as ceramics-treated medium (tMEM). MEM by Milli-Q water was assigned as control medium (MEM).

- c) Cell culture and oxidative stress by hydrogen peroxide (H2O2) Human fibroblast cell line, TIG3-20 (10,000/well) was cultured in MEM with 10% fetal calf serum at 37 in a humidified atmosphere containing 5% CO2 for 24 hrs. Then culture media were changed into MEM or tMEM, with or without H2O2 (50  $\mu$  M). After cultured for 24 hours, living cells were counted by MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide) method.
- d) Vitamin C preservation test Vitamin C (0, 500, 1000 $\mu$ M) was dissolved in MEM or tMEM with or without H2O2 (50 $\mu$ M). The reducing activity of Vitamin C was estimated utilizing MTT as a substrate at 0 hr and after 24 hrs incubation at 37 in a humidified atmosphere containing 5% CO2.

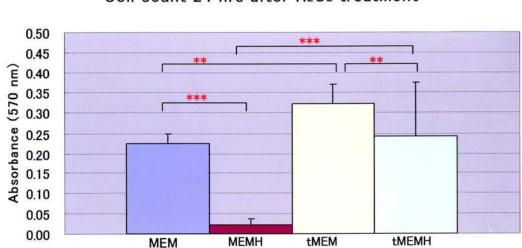


Fig. 2

Cell count 24 hrs after H2O2 treatment

### Fig.2 Cell count after 24 hours

After 24 hrs, the number of the cells cultured in tMEM was significantly higher than that of The cells in MEM. Twenty four hours after the treatment with H2O2, most of the cells in MEMwere dead, while considerable number of the cells in tMEM was survived. MEM; cultured in MEM without H2O2, MEMH; cultured in MEM with H2O2 (50  $\mu$ M), tMEM; cultured in tMEM without H2O2, tMEMH; cultured in tMEM with H2O2(50 $\mu$ M).Each column represents mean  $\pm$ SD (n = 16). \*\*;p < 0.01,\*\*\*;p < 0.001 (ANOVA, Bonferroni).

Fig. 3a

Vitamin C activity at 0h in MEM

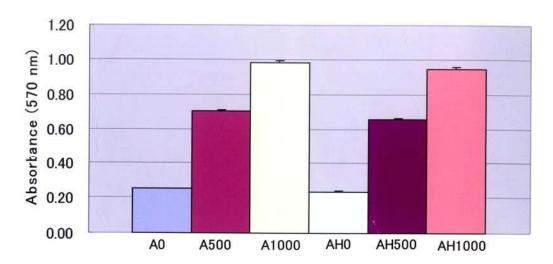


Fig. 3c
Vitamin C activity after 24 hrs in MEM

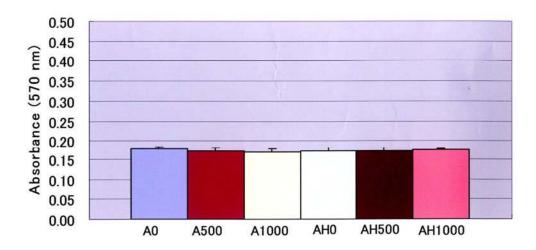


Fig. 3 Preservation of Vitamin C activity in tMEM

- a. Vitamin C activity in MEM at 0 h
- b. Vitamin C activity in tMEM at 0 h
- c. Remaining Vitamin C activity in MEM after 24 hrs
- d. Remaining Vitamin C activity in tMEM after 24 hrs

MTT reducing activity of Vitamin C in MEM was completely disappeared after 24 hrs incubation, while the activity was considerably preserved in tMEM even after 24 hrs. A0; control, A500; Vitamin C 500  $\mu$ M , A1000; Vitamin C 1000  $\mu$ M, AH0; control, AH500; Vitamin C 500  $\mu$ M with 50  $\mu$ M H2O2, AH1000; Vitamin C 1000  $\mu$ M with 50  $\mu$ M H2O2. Each column represents mean ±SD (n = 8).

Fig.3b

Vitamin C activity at 0h in tMEM

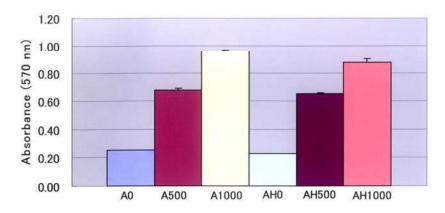
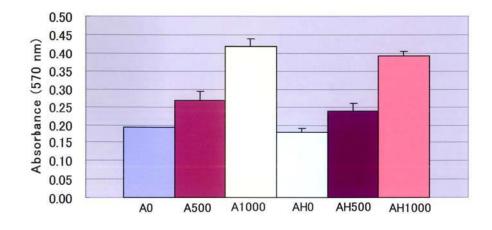


Fig.3d

Vitamin C activity after 24 hrs in tMEM



### **Conclusion:**

It was strongly suggested that the water treated with high-temperature sintered kaolinite ceramic granules possessed anti-oxidative property.

#### References:

- 1. The structure of water induced by specific ceramics treatment and effects of treated water in view of some biological aspects. K. Sato, M. Ago, K. Ishikawa, T. Sato and K. Okajima Environ. Control Biol., 43 (3) pp211-221(2005)
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