

# Electrochemically reduced water, a type of functional water, has reactive oxygen species scavenging activity in HT1080

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**Introduction:** There are many functional waters that give us benefits such as disease prevention and cure. Of these, electrochemically reduced water (ERW) exhibits an alkaline pH, contains richly dissolved hydrogen (H<sub>2</sub>), and a small amount of platinum nanoparticle. As a result of such characteristics, ERW has reactive oxygen species (ROS)-scavenging activity (1). Also, recent studies demonstrated that H<sub>2</sub>-dissolved water exhibits ROS-scavenging activity (2). Thus, the anti-oxidative capacity of ERW is postulated to be dependent on the presence of H<sub>2</sub> levels; however, there is no report verifying the role of dissolved H<sub>2</sub> in ERW. In this report, we clarify whether the responsive factor for anti-oxidative activity in ERW is dissolved H<sub>2</sub>.

**Methods:** We used human fibrosarcoma cell line HT1080. Change in the water quality with electrolysis intensity was evaluated by pH, electrical conductivity, oxidation-reduction potential, dissolved oxygen, and dissolved hydrogen. Intracellular ROS scavenging activity of ERW and H<sub>2</sub>-dissolved water was tested by using 3'-O-Acetyl-6'-O-pentafluorobenzenesulfonyl-2',7'-difluorofluorescein (BES-H<sub>2</sub>O<sub>2</sub>), oxygen radical absorbance capacity (ORAC) assay, and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Transition of ERW compositional factor with the degree of electrolysis intensity was detected by ICP-MS. Observation of platinum electrode was used by scanning electron microscope (SEM).

**Results and Discussion:** We confirmed that ERW possessed electrolysis intensity-dependent intracellular ROS-scavenging activity and ERW exerted significantly superior ROS-scavenging activity in HT1080 cells than the equivalent level of H<sub>2</sub>-dissolved water (Fig.1). ERW retained its ROS-scavenging activity after removal of dissolved H<sub>2</sub>, but lost its activity when autoclaved (Fig.2). ORAC assay and the DPPH assay could not detect radical-scavenging activity in both ERW and H<sub>2</sub>-dissolved water. We searched for Pt from 62 elements that increased in amount in an electrolysis-dependent manner by ICP-MS analysis and grasped that the surface structure appeared to be a sheet of aggregated nanoparticles by using SEM (3). These results indicate that ERW contains electrolysis-dependent H<sub>2</sub> and an additional anti-oxidative factor predicted to be platinum nanoparticles.

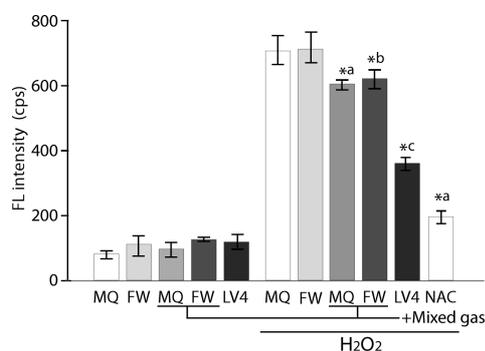


Fig. 1

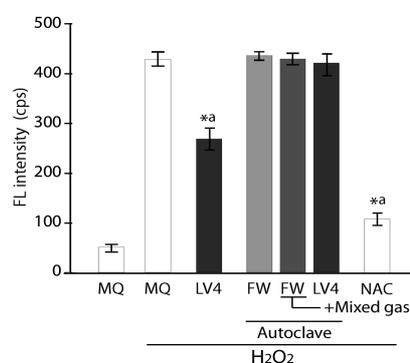


Fig. 2

## References:

1. Sanetaka Shirahata et al., Trends in Food Science & Technology. 2012
2. Ohsawa et al., Nature medicine. 2007
3. Uscatequi AV et al., Electrochim Acta. 2013