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Advanced research on the health benefit of reduced water☆

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In Japan, research on functional water, especially on reduced water, is developing at a rapid pace. Reduced water such as electrochemically reduced water and natural reduced water can scavenge reactive oxygen species in cultured cells. Reduced waters are expected to have preventive and positive effects on oxidative stress-related diseases such as diabetes, cancer, arteriosclerosis, neurodegenerative diseases, and side effects of hemodialysis. It has been suggested that the active agents in reduced water are hydrogen (atoms and molecules), mineral nanoparticles, and mineral nanoparticle hydrides.

Introduction: electrochemically reduced water is beneficial for health

In the field of food science and technology, water is an important ingredient influencing taste, rheology and preservation of foods. Research on functional foods is currently popular; however, it is not yet well known that drinking water also has physiological functions, and that there are some health-beneficial effects of water (Shirahata, 2002, 2004). In the past decade, the decrease in the quality of tap water because of pollution of the global environment over time has become a major social problem. Air pollution affects water in soils, rivers, and farm products by acid rain. Chemicals in polluted water are considered to generate oxidative stress in the placenta of pregnant women, and this can cause various types of diseases in newborns (Obolenskaya *et al.*, 2010).

The human body is approximately 60–80% water. The function of water in the body is mainly classified as follows. (1) The water molecule itself: flowing water affects cellular function and both development and functions of organs (Hirokawa, Tanaka, Okada, & Takeda, 2006; Hove *et al.*, 2003), and hydration and Brownian movement of water are fundamentally important for protein function (Iwaki, Iwane, Shimokawa, Cooke, & Yanagida, 2009); (2) atoms and molecules derived from water molecules, such as protons (H^+), hydrogen atoms (active hydrogen [H]), hydrogen anions (H^-), hydrogen molecules (H_2), oxygen molecules (O_2), and reactive oxygen species (ROS); and (3) molecules dissolved in water, such as mineral ions, mineral nanoparticles, organic and inorganic compounds, and gases.

Functional water is activated water exhibiting specific functions. There are many activation methods such as electrolysis, treatment with a magnetic field, light irradiation, ultrasonication, bubbling with gases, strong water flow and collision, and treatment with some types of minerals or rocks. Functional water is defined by The Functional Water Association of Japan as water in which both treatment and function have been scientifically demonstrated or reproducible useful functions have been demonstrated among artificially treated waters. Among functional waters, electrolyzed water has been mostly investigated. Electrochemically reduced water (ERW) is produced near a cathode and electrochemically oxidized water (EOW) is produced near an anode. Potable ERW is a health-beneficial water as discussed here. EOW is also termed electrolyzed acidic water and is functional water exhibiting a sterilizing action, mainly due to hypochlorous acid, chlorine gas, and ozone (Bari, Sabina, Isobe, Uemura, & Isshiki, 2003) (Fig. 1A).

Potable ERW (pH 8–10) is popular as a health-beneficial water in Japan. ERW is also termed alkaline electrolyzed water, alkali-ionic water, alkaline cathodic water, and alkaline ionized water, based on its physicochemical and physiological aspects. ERW exhibits an alkaline pH, is hydrogen molecule-rich, and has a negative oxidation–reduction potential (ORP) and reactive oxygen species (ROS)-scavenging activity (Shirahata *et al.*, 2007). Studies on the functions of ERW were initiated in Japan in 1931, and its application to agriculture was first

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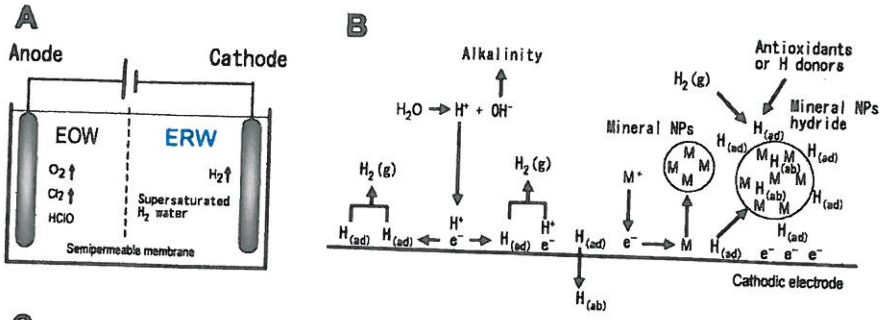

C

	Control >10000	ERW	ERW >10000	ERW <10000
[Pt] (ppb)	N.D.	0.19	12.04	2.16

N.D., not detectable

D

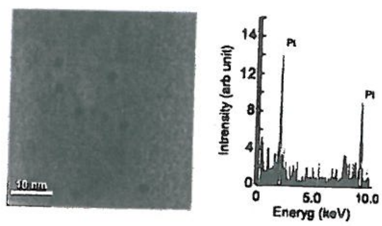

Fig. 1. Electrochemical preparation of reduced water, mineral nanoparticles and mineral hydrides. A. Principle of preparation of electrolyzed water. Electrochemically reduced water nearby a cathode is hydrogen molecule-rich water. Electrolyzed oxidized water nearby an anode contains oxygen gas, chloride gas, and hypochlorous acid if the original water contains Cl^- ions. B. Chemical reactions on the surface of a platinum electrode. Proton dissociated from water is reduced to adsorbed H atoms (H_{ad}) on the surface of the platinum plate. H_{ad} is changed to H_2. H_{ad} is absorbed into Pt metal to produce absorbed H (H_{ab}). Mineral ions in the original water are reduced to metal atoms and then self-organized to mineral nanoparticles. Mineral nanoparticles protected by organic protectors are stable and dispersed in water for a long time. Mineral nanoparticles adsorb or absorb H atoms in the presence of H_2- or H-donors such as organic antioxidants. C. ERW contains Pt nanoparticles. ERW was prepared from 2 mM NaOH solution by electrolysis at a direct current of 100 V for 1 h, as reported by Ye *et al.* (2008). The ERW was ultra-filtrated with a cut size of 10,000. The Pt content of fractions of more than 10,000 Da and less than 10,000 Da was determined with ICP-MS. The control is 2 mM NaOH solution before electrolysis. D. The left panel shows a photograph (×5000) of the fraction of ERW (>10,000 Da) determined by a transmission electron microscope. The right panel shows energy depressive X-ray spectrometer analysis of the left panel, indicating that the nanoparticles are composed of Pt.

attempted in 1954. In 1960, it was applied to medical care as a health-beneficial water, and in 1966, the Ministry of Health, Labour and Welfare of Japan admitted that ERW was effective for chronic diarrhea, indigestion, abnormal gastrointestinal fermentation, antacid, and hyperacidity, and it authorized an ERW-producing device for home-use (see the homepage of the Association of Alkaline Ionized Water Apparatus: http://www.3aaa.gr.jp/english/alkali/kl.html). In 1994, to mainly promote electrolyzed water use in society, the Functional Water Foundation was established with the support of the Ministry of Health, Labour and Welfare of Japan. Hayakawa (1999) reported that rats administrated alkali-ionic water for 8 weeks exhibited a significantly lower amount of total short chain fatty acids in the appendix than that in control rats; however, alkali-ionic water did not affect the flora of intestinal bacteria. Rats administrated ERW of pH 10 had a more negative ORP in the intestine than that in control rats. A double-blind placebo-controlled study on the effects of alkali-ionized water was performed using subjects who had abdominal symptoms such as pyrosis, dysphoria, abdominal distension, chronic diarrhea, and constipation from January 1996 to January 1999. The placebo control water was purified water obtained from tap water using an activated charcoal filter, which was then electrolyzed to obtain alkali-ionized water. The number of patients was 84 in the alkali-ionized water group and 79 in the purified water group. The patients drank at least 0.5 L of alkali-ionized water of pH 9.5 or purified water per day for 2 weeks. The results showed that alkali-ionized water significantly improved the abdominal complaints. In particular, chronic diarrhea patients who drank alkali-ionized water showed a significantly higher improvement efficacy of 94.1% compared with those who drank purified water (64.7%) (Tashiro, Kitahora, Fujiyama, & Banba, 2000). When the Drugs, Cosmetics and Medical Instruments Act of Japan was revised in 2005, a device for preparation of ERW was re-authorized based on considerable scientific evidence as a home managed medical device. The purpose of use was recognized to generate potable alkaline electrolyzed water for the improvement of gastrointestinal symptoms. The Japanese Society for Functional Water was established in 2001, and active studies on various functional waters including ERW have been performed to date.

Mechanism of action of reduced water containing hydrogen and mineral nanoparticles as a newly recognized ROS scavenger

Clinical data suggested that ERW improved oxygen stress-related diseases (Hayashi & Kawamura, 2002). The authors reported that ERW scavenged ROS and inhibited ROS-induced DNA damage *in vitro* (Shirahata *et al.*, 1997). Electrolysis of water produces a strong reducing circumstance in the vicinity of a cathode, because most voltage is applied in the very narrow water layer nearby the cathode, forming very high electric field. Platinum-coated titanium electrodes are often used for electrolysis of water in the commercial ERW-producing apparatus. On the cathodic platinum plate, hydrogen atoms (active hydrogen) and hydrogen molecules are generated. Mineral nanoparticles and mineral nanoparticle hydrides are also formed as shown in Fig. 1B. Actually we found that ERW prepared from NaOH solution contained a small amount of Pt nanoparticles (Fig. 2C and D). Synthesized Pt nanoparticles scavenged $O_2^{\cdot-}$, ·OH, and H_2O_2 (Hamasaki *et al.*, 2008; Kajita *et al.*, 2007) (Fig. 2). Synthesized Pt nanoparticles also activated hydrogen molecules to hydrogen atoms by their catalysis action. Natural reduced waters (NRWs) such as Hita Tenryosui water in Japan and Nordenau water in Germany also exhibited ROS-scavenging activities (Li *et al.*, 2002). We propose an active hydrogen mineral nanoparticles hypothesis of reduced water to explain the mechanism of action of both ERW and NRW (Fig. 3). See supplementary information for detailed discussion.

Anti-diabetic effect of reduced water

According to a national health and nutrition survey in 2007, 22.1 million people, amounting for one sixth of the entire population, are patients with diabetes or people with suspected diabetes in Japan. Diabetes mellitus is mainly classified into two types: type 1 insulin dependent diabetes mellitus and type 2 non-insulin dependent diabetes mellitus. Type 1 diabetes mellitus is caused by insulin deficiency due to the oxidative damage of pancreatic β cells attacked by immune cells. Type 2 diabetes mellitus is also strongly associated with the oxidative damage of myotube and adipocyte cells due to stress, hyperphagia, and lack of exercise. ERW, Hita Tenryosui water and Nordenau water have been shown to scavenge intracellular ROS in a hamster pancreatic β cell line HIT-T15 cells, and remarkably accelerate the secretion of insulin. The oxidative damage induced by alloxan, a type 1 diabetes inducer, is suppressed by ERW and NRW in cells and in alloxan-induced type 1 diabetes model mice (Li *et al.*, 2002, 2005, 2010, 2011).

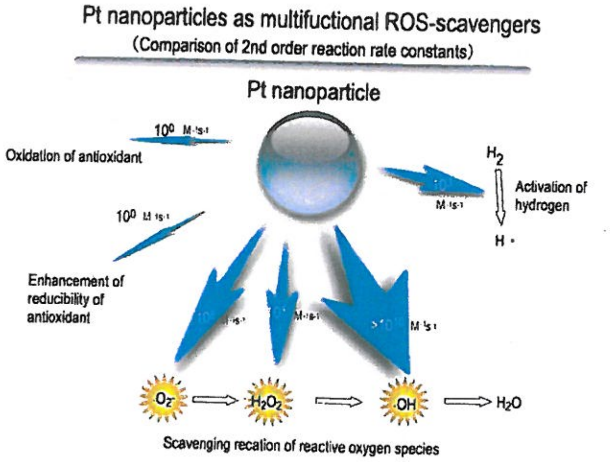

Fig. 2. Multi-functional ROS-scavenging activity of Pt nanoparticles. The 2nd order reaction rates of synthesized Pt nanoparticles of 2–3 nm were determined as reported by Hamasaki *et al.* (2008). Pt nanoparticles exhibit superoxide anion radical-scavenging activity as well as SOD enzyme activity. They also exhibit catalase-like activity. The hydroxyl radical-scavenging activity of Pt nanoparticles is as strong as that of ascorbic acid, one of the strongest scavengers. Pt nanoparticles activate hydrogen molecules to active hydrogen and stimulate the reducibility of antioxidants. The autoxidation activity of Pt nanoparticles on antioxidants is weak. The numbers show *Ks* values of second reaction rate constants.

ERW, Hita Tenryosui water and Nordenau water scavenge ROS in rat L6 myotube cells and enhance sugar uptake (Oda *et al.*, 1999). Nordenau water and Hita Tenryosui water promote the phosphorylation of the insulin receptor *via* suppression of the activity of tyrosine protein phosphatase, which is a redox-sensitive protein, and activate PI3 kinase and Akt, as well as promote the translocation of the sugar transport carrier GLUT4 to the cell membrane to promote sugar uptake (Shirahata *et al.*, 2001, 2007). These waters also alleviate sugar tolerance damage in type 2 diabetes model mice (Gadek & Shirahata, 2002; Osada *et al.*, 2010). ERW derived from tap water improves the symptoms of diabetes model mice (Jin *et al.*, 2006; Kim, Jung, Uhm, Leem, & Kim, 2007; Kim & Kim, 2006).

It has been reported that in 45% of 411 type 2 diabetes patients (mean age, 71.5 years) who drank 2 L of Nordenau water per day, blood glucose and HbA1c levels were significantly decreased after drinking this water for 6 days. These levels were further decreased after long term drinking. Additionally, blood cholesterol, low density lipoprotein (LDL), and creatinine levels were significantly decreased and high density lipoprotein levels were significantly increased. Drinking this water for a longer period resulted in an increase in the percentage of patients who improved (Gadek, Hamasaki, & Shirahata, 2009; Gadek, Li, & Shirahata, 2006). In an open clinical test performed at the First Central Hospital in Jilin Cangchun City in China, 65 patients with diabetes and 50 patients with hyperlipidemia drank 2 L of Hita Tenryosui water per day for 2 months. This resulted in a significant decrease in blood sugar levels in 89% of patients with diabetes. Additionally, blood triglyceride and total cholesterol levels in 92% of patients with hyperlipidemia were significantly decreased (Osada *et al.*, 2010). Furthermore, a double-blind random clinical trial for 29 patients with type 2 diabetes was performed at the Fukuoka Tokusyukai Hospital, Fukuoka city, Japan. Urinary 8-OH dG (an internal oxidation marker) levels of patients who drank 1 L of Hita Tenryosui water per day for 6 months were significantly decreased (Matsubayashi, Hisamoto, Murao, & Hara, 2008). In addition, in a double-blind clinical trial with 100 subjects performed at Hiroshima University from November 2008 to September

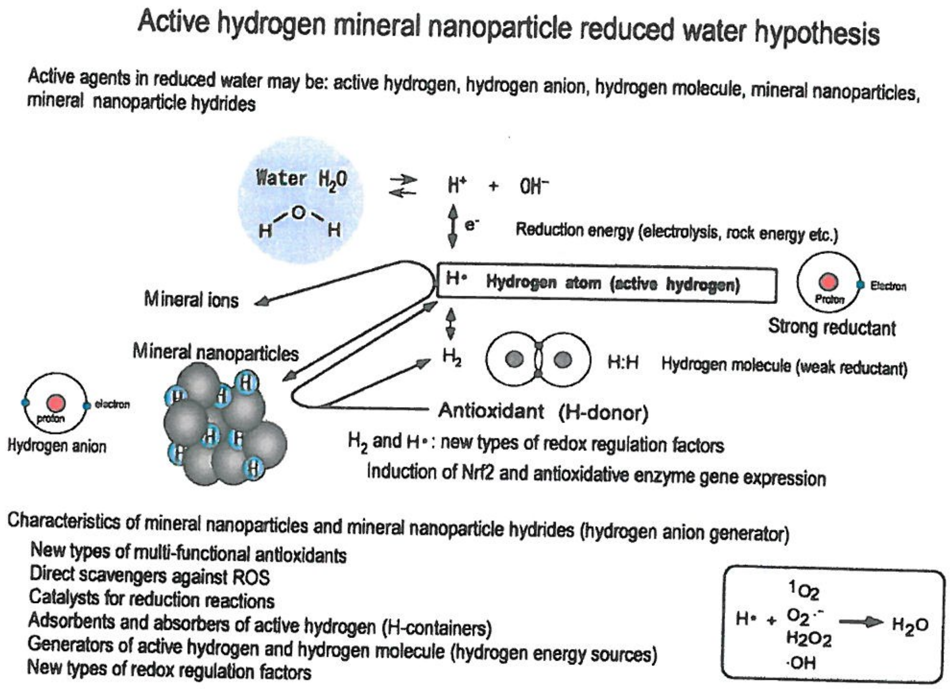

Fig. 3. Active hydrogen mineral nanoparticle reduced water hypothesis. As a common mechanism in both ERW and NRW, water is reduced by electric energy, rock energy and other energy to produce active hydrogen (H atom) and mineral nanoparticles. H atoms produce hydrogen molecules, which are weak reductants, but can function as H-donors. Mineral nanoparticles sustain reduction energy, because they gradually dissociate to mineral ions, releasing electrons. Mineral nanoparticles directly scavenge $O_2^{\cdot-}$, $\cdot OH$ and H_2O_2 by catalysis mechanisms. Mineral nanoparticles stimulate H atom release from many organic substances such as antioxidants and methanol to enhance reducibility. Mineral nanoparticle hydrides can release hydrogen anions, which can function as a reductant.

2009, when 2 L of Hita Tenryosui water was drunk a day, it was found to have anti-metabolic syndrome effects such as a significant decrease in starved blood sugar levels, blood pressure, total cholesterol, LDL cholesterol, GOT, γ-GTP, and triglyceride levels, arteriosclerosis index, and uric acid levels, and a significant increase in leptin levels, as well as improvement of constipation (Higashikawa, Kuriya, Noda, & Sugiyama, 2009). In the clinical trial, it was suggested that drinking 2 L of natural mineral water per day itself was beneficial for health.

Other physiological functions of reduced water

Elongation effect on the lifespan of nematodes

A recent theory on aging suggests that there are adequate ROS levels in living organisms to elongate lifespan, and both insufficient and excess ROS levels shorten the lifespan (Brewer, 2009). We have reported that ERW significantly extends the lifespan of nematodes (*Caenorhabditis elegans*) by scavenging ROS in nematodes (Yan *et al.*, 2010). The active agent responsible for the lifespan extension in ERW is suggested to be Pt nanoparticles of ppb levels, but not hydrogen molecules (Yan *et al.*, 2011). It has also been reported that Pt nanoparticles of an optimum concentration extend the lifespan of nematodes by scavenging ROS (Kim *et al.*, 2008; Kim, Shirasawa, & Miyamoto, 2010).

Anti-cancer effects

ERW causes telomere shortening in cancer cells (Shirahata *et al.*, 1999). It suppresses tumor angiogenesis by scavenging intracellular ROS and suppressing the gene expression and secretion of vascular endothelial growth factor (Ye *et al.*, 2008). ERW suppresses the growth of cancer cells and microorganisms (Hamasaki *et al.*, 2005; Komatsu *et al.*, 2001) and induces apoptosis together with glutathione in human leukemia HL60 cells (Tsai, Hsu, Chen, Ho, & Lu, 2009). ERW induces differentiation of K562 cells to megakaryocytes (Komatsu *et al.*, 2003), and when supplemented with Pt nanoparticles, it strongly suppresses the two step transformation of NIH3T3 cells by a carcinogen (Nishikawa *et al.*, 2005).

Anti-arteriosclerosis effects

ERW suppresses the Cu^{2+}-catalyzed oxidation of human LDL and suppresses triglyceride levels in mice fed high fat foods (Abe *et al.*, 2010). Hydrogen-supplemented water also suppresses arteriosclerosis (see the supplemental information).

Anti-neurodegenerative effects

ERW suppresses neural cell death by oxidative stress (Kashiwagi *et al.*, 2005). Hydrogen-supplemented water also exhibits various anti-neurodegenerative disease effects (see the supplemental information).

Application of ERW to electrolyzed water hemodialysis

Recently, the application of ERW to hemodialysis has been intensively investigated to establish a new dialysis method using ERW (Huang, Yang, Lee, & Chien, 2003; Huang *et al.*, 2006, 2010; Nakayama *et al.*, 2007, 2009, 2010; Zhu *et al.*, 2011).

Suppressive effect of the side effects of anti-cancer drugs

Hydrogen-supplemented water suppresses the side effects of anti-cancer drugs (see the supplemental information).

Aquaporin penetration of water

Kitagawa, Liu, and Ding (2011) recently reported that extractable organic solvents and freeze-labile special components in Hita Tenryosui water promote aquaporin activity in penetration of water into cells. Such components moving via aquaporin are suggested to activate cellular immune responses, which allow prevention and/or treatment of some chronic diseases.

Other effects

Naito *et al.* (2002) reported that chronic administration with electrolyzed alkaline water inhibits aspirin-induced gastric mucosal injury in rats. Hydrogen-enriched electrolyzed water has been demonstrated to be safe in mutagenicity, genotoxicity and subchronic oral toxicity (Saitoh, Harata, Mizuhashi, Nakajima, & Miwa, 2010). ERW exhibits hepatoprotective effects against CCl_4-induced liver damage in mice (Tsai *et al.*, 2009). Ionized alkaline water improves the symptoms of metabolic acidosis in experimental animals (Abo-Enein, Gheith, Barakat, Nour, & Sharaf, 2009). ERW also exhibits an anti-hangover effect (Park *et al.*, 2009). Silica hydride found in Funza water in Pakistan suppressed carbon tetrachloride-induced hepatotoxicity in mice (Tsu *et al.*, 2010).

Recently, many papers have been published on the suppressive effects of hydrogen molecules contained in ERW on oxidative stress-related diseases (see the supplementary information). The major functions of reduced water are summarized in Fig. 4.

Conclusions and perspective

Accumulating evidence has shown that reduced waters are health beneficial and they suppress oxidative stress-related diseases such as diabetes, cancer, arteriosclerosis, neurodegenerative diseases, and the side effects of hemodialysis. The mechanisms of action of reduced water for scavenging ROS are considered to be complicated. ERW contains hydrogen molecules and mineral nanoparticles. Hydrogen molecules and active hydrogen may be new redox regulation factors that can induce the gene expression of antioxidative enzymes. Hydrogen molecules may be converted to active hydrogen by catalyst action of metal nanoparticles to exhibit more potent reducibility. Mineral nanoparticles themselves are new types of multi-functional antioxidants. Mineral hydride nanoparticles, which are H-donors as well as organic antioxidants like ascorbic acid, are also candidates of active agents in reduced waters. NRW may have one or some of the active agents described above. Further investigation on activation methods of water by electricity, magnetic fields or light are likely to contribute to the development of energy-rich waters, which will be beneficial for human health. Reduced water may suppress harmful effects of environmental pollution on the embryo in pregnant women by purifying amniotic fluid and blood. Reduced water might also contribute to the food industry by improving the taste, texture and preservation of foods. In industries, the usage of ERW is

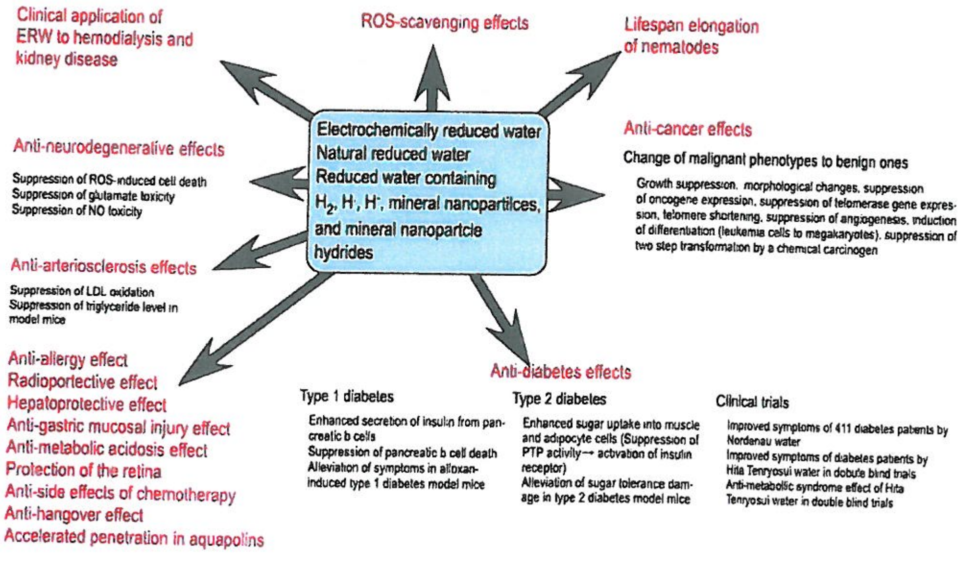

Fig. 4. Variety of functions of reduced water.

expected as washing water to prevent the rust of semiconductors. In the field of environmental remediation, reduced water will prevent the rotting of river and lake water, because the proliferation of bacteria or organisms causing the rotting will be suppressed in a reduced circumstance. Further research on water itself may ultimately reveal the secret of the origin of life.

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Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.tifs.2011.10.009.

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