

Article

Hydrogen-Nano-Bubble-Rich Water in Bucket/Bathtub Improves Intractable Skin Roughness

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Abstract: Determining how to apply hydrogen as a therapeutic/preventive antioxidant for oxidative-stress-related diseases practically in daily life has not been studied. The effects of bathtubs and buckets filled with hydrogen water (41 °C, >10 min bathing) were investigated on six subjects, without a medical prescription, suffering from skin roughness on the foot, hand, finger, or elbow. They were also treated with an electrolyzer composed of a lattice-shaped, microscopically flat, platinum-plated three-layer electrode, except for one subject who was treated with a micro-porous emittance terminal hydrogen-jetting apparatus, resulting in improvements in both cases. For another subject with similar skin roughness on both hands, immersing the right hand in an electrolytically generated hydrogen water bucket showed more marked improvement than immersing the left hand in a bucket with normal water. The nano-bubbles (average, mode, and median sizes of 157 nm, 136 nm, and 94 nm, respectively) increased 3.79 fold to 2.20×10^8 /mL after 30 min electrolysis with 2 L of tap water and were boiling (98 °C, 2 min)-resistant, with heat stability in nano-bubbles as small as 69–101 nm, as evaluated by laser-beam-based Brownian movement trailing Nano-Sight analysis. The marked increase in nano-bubbles caused by electrolysis correlated with an increase in dissolved hydrogen (<15 µg/L to 527 µg/L) but not a decrease in dissolved oxygen (9.45 mg/L to 6.94 mg/L). Thus, the present study proposed the novelty of hydrogen regarding its contribution to health from the perspective that hydrogen-nano-bubble-rich water in a foot bucket, which was additively used together with a conventional bathtub and can be frequently used in daily life, improved diverse types of skin roughness.



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Keywords: hydrogen-molecule-dissolved warm water; electrolyzed hydrogen warm water; nano-bubble; warm water containing hydrogen nano-bubbles; skin roughness; platinum-plated electrolyzer; hydrogen-jetting apparatus; laser-beam-based nano-particle-trailing analysis

1. Introduction

Oxidative stress generated in daily life, such as ultraviolet rays, smoking, air pollutants, physical/mental stress, and excessive exercise, causes reactive oxygen species (ROS) in the body and induces oxidative damage to various body components. Oxidative stress also affects the physiology and maintenance of skin homeostasis and brings about major causes of aging and/or skin diseases, such as pigmentation, wrinkle formation, dryness, and skin cancer [1–4]. Importantly, skin roughness is not only a problem closely associated with aging, cosmetology, and quality of life, but also serves as a sign of disturbed skin homeostasis and modulated skin areas where there are risks of developing and exacerbating skin disease [5,6].

Hydrogen molecules are widely used in semiconductor processing, the petrochemical industry, and fertilizer production. Many new industrial applications are also expected to be found, including uses in the energy field, and various studies are currently underway for their effective use [7–10]. Recently, various research studies on the use of

hydrogen molecules for healthcare have also been vigorously advancing [11–13]. Hydrogen molecules have also recently attracted attention as new antioxidants and are expected to become new gaseous therapeutic and prophylactic agents related to various kinds of disorders [11–13], such as oxidative-stress-induced cellular injuries [4,14–16], wound repair [14,17], blood fluidity [18–20], ischemia-reperfusion injuries [21,22], inflammation [23,24], lipid metabolism [25,26], type 2 diabetes [27], neurodegenerative diseases [28], and cancer [29].

Various methods of supplying hydrogen molecules to the human body have been proposed, such as the ingestion of dietary supplements or hydrogen-rich water (HRW), the inhalation of hydrogen gas, the infusion of hydrogen-rich saline, hydrogen production using Mg, and topical administration via a bath, and it is important to use different methods depending on the target organs [30]. Regarding the effects of hydrogen molecules on the skin, bathing has attracted attention as a simple and effective method. In particular, HRW containing nano-sized hydrogen molecules has been expected to be more efficient owing to the stability of the hydrogen molecule in the solution and its permeability into the organs [31,32]. More recently, a home-use electrolysis-type hydrogen molecule generator has been developed, which can produce HRW-containing nano-bubbles and make it possible to routinely carry out hydrogen molecule intake at home within one's usual lifestyle for treatment [26,32–34]. It is expected to function in the prevention of oxidative-stress-related modulations and/or diseases [11–29]. The combination of a home-use electrolysis-type hydrogen molecule generator with bathing is expected to provide a more efficient approach for the skin. In fact, to date, we have demonstrated that immersion in hydrogen-containing water can improve inflammation levels in serum [32], skin blotch [26], wrinkle [26,34], and heat-retention effects [33]. However, its effects on diverse types of skin roughness are not yet well understood.

In the present study, the effect of warm hydrogen water containing hydrogen molecules and nano-bubbles in a bathtub and a foot-immersing bucket (foot bucket), generated by a household electrolytic device, on skin roughness was investigated to clarify the possibility of this new treatment and prevention methods for oxidative-stress-related skin roughness.

2. Materials and Methods

2.1. Electrolysis Apparatus Supplying the Hydrogen-Molecule-Dissolved Warm Water

An electrolysis apparatus with a three-blade, lattice-shaped, and microscopically non-bumpy platinum-gilded electrode (the electrolysis type) was used as a generator of hydrogen-molecule-dissolved warm water (hydrogen-dissolved warm water), referred to as “an electrolyzer”, and was supplied under the product name “LitaLife Ver.2” by WCJ Co. Ltd., Osaka, Japan. The hydrogen-generating electrolysis apparatus is composed of two parts: one is an electric-power-supplying apparatus and the other is an electrode where hydrogen molecules are generated concurrently with oxygen molecules. The high performance of the electrode is achieved by a three-blade, lattice-shaped, and microscopical non-bumpy platinum-gilded electrode [34]. The electrolyzer prepared hydrogen water for a bathtub and foot-bucket using 180–200 L and 2–50 L of tap water (Osaka City Water-Works Bureau) at 41 °C, respectively. The subjects used a hydrogen-rich bathtub/bucket according to the manufacturer's operating instructions and the examination protocols to electrolyze or bubble-jet the water for 10 min under a ventilation fan. The bathing procedures and the hardware of the hydrogen-generating apparatus minimized the gaseous chlorine. The hydrogen water obtained showed diverse physicochemical properties (Table 1). The dissolved hydrogen-molecule concentration (DH) was measured by a diaphragm polarographic electrode-type hydrogen-molecule concentration meter (KM2100DH, Kyoei-Electron-Lab, Saitama, Japan) [32]. The dissolved oxygen molecule concentrations were measured by a polarographic dissolved oxygen-molecule meter (DO-5519E, Lutron, PA, USA). Most of the subjects suffering from skin roughness were examined using an electrolyzer in the present study.

Table 1. Physicochemical properties of hydrogen-dissolved warm water that was electrolytically generated at 41 °C in a foot bucket filled with 2-L tap water, together with the numbers of the relevant figures.

Average particle size (mean; average value of the measured diameters of all the particles)	157 nm	Figure 4B(c)
Mode size (the most frequent size)	136 nm	Figure 4B(c)
Median diameter (diameter of the nanoparticle at which the cumulative frequency in particle size order becomes 50% of all the particles)	93.8 nm	Figure 4B(c)
Maximum value of the dissolved hydrogen concentration	526 µg/L	Figure 4D
Average value of the dissolved oxygen concentration	6.81 mg/L	Figure 4E

2.2. Hydrogen-Jetting Apparatus Supplying the Hydrogen-Dissolved Warm Water

As the reference versus the above-mentioned electrolyzer, one subject was examined using the apparatus of hydrogen-jetting from an emittance terminal of a jetting nozzle (spouting pressure: 0.55 kg/cm³) through a microporous silica filter for 20 min per 100 mL of warm water from a built-in hydrogen-filled-tank, which is called “a jetting hydrogen supplier” [35]. The hydrogen water that was prepared by a jetting hydrogen supplier showed diverse physicochemical properties: a dissolved hydrogen (DH) value of 739 ± 106 µg/L (theoretical maximum, 1600 µg/L; pre-jetting normal water, below 15 µg/L of the detection limit), an oxidation-reduction potential (ORP) of -419 ± 57.7 mV (pre-jetting normal water, +95 to +195 mV), and pH 6.6–7.4 (ibid., pH 6.8–7.1). These parameters were measured with a diaphragm polarographic electrode-type DH meter (DH-35A, DKK-TOA Co. Ltd., Tokyo, Japan), a pH/DO meter (D-55, Horiba Inc., Kyoto, Japan), and an ORP meter (RM-20P, DKK-TOA Co. Ltd., Tokyo, Japan), respectively.

2.3. Alleviation of Skin Roughness by Hydrogen Water Bucket/Bathtub

Seven volunteers with rough skin on their heels, hands, or elbows were bathed for the whole body, below the neck, or without/with the immersion of the diseased skin locus, which was immersed in a bathtub or a bucket (the following is otherwise called “hydrogen bathtub” or “hydrogen bucket” as the shortened form of “hydrogen-dissolved warm water”) filled with warm water containing hydrogen generated by an electrolyzer. The environmental conditions were standardized so that it could be considered as a daily-life experiment in response to diverse differences for personal subjects, such as the degrees of skin roughness, the size/shape of the bathtub in subjects’ homes, and tap-water-hardness quality, although the electrolytic time, bathing time, and the immersing time of the diseased skin locus were unified to 30 min, 10 min, and 10 min, respectively. Changes in the degrees of rough skin were observed in 6 subjects who could be followed up throughout the procedure. After subjecting the respective rough skin portions (such as the hand palms, fingers, elbows, and heels) of each of the 6 subjects to hydrogen bathing for periods of days, as shown in Table 2, the changes in the rough skin portion were examined. All the subjects did not receive any medical prescription, neither before nor during the examination period, attributing any skin roughness improvement to the hydrogen and the relevant endogenous cure. For some subjects, a bathtub for the whole body, below the neck, or a bucket for the diseased skin locus was used together, after being poured with the hydrogen bath. It was confirmed that no subjects received medical skin treatment or ointment prescription other than hydrogen bathing, as ensured by the protocol that was given previously to the subject and the report that was periodically proposed by the subject, both of which described no usage of prescriptions/treatments except hydrogen. The effects of hydrogen molecules on the rough skin area were shown as the result for each rough skin portion. As the reference versus the electrolyzer, one subject underwent the hydrogen bathtub/bucket filled with an above-mentioned jetting hydrogen supplier. The skin locus and skin roughness degree were significantly different from one another for all patients of the definite person number, and therefore the control group could not be set, but the characteristics that would influence the

states of skin roughness of the subjects were standardized, together with the organization of the correlation of the clinical examination for improvement of skin roughness and the conditions for hydrogen treatment (Table 2).

Table 2. Information of subjects that suffered from skin roughness and were treated with hydrogen-dissolved warm water.

Subject The Relevant Figure	Skin Locus	Age	Gender	Days for Hydrogen Treatment	Practice of Treatments: Electrolytically Generated Hydrogen-Dissolved Warm Water, Unless Noted	Treatment/ Observation Period	Other Simultaneous Prescription
A Figure 1A	Heel	66	Man	162	Hydrogen bathtub, Hydrogen foot bucket	2/11-7/23	No
B Figure 1B	Heel	42	Woman	150	Hydrogen bathtub, Hydrogen foot bucket	2/1-7/1	No
C Figure 1C	Heel	46	Woman	90	Hydrogen bathtub	9/5-12/5	No
D Figure 2A,B	Hand	38	Woman	152	Hydrogen bathtub, Hydrogen foot bucket	7/9-1/31	No
E Figure 2C	Finger	49	Woman	11	Hydrogen bathtub	6/20-7/1	No
F Figure 3	Elbow	51	Man	195	Hydrogen bathtub, Hydrogen foot bucket	7/24-2/15	No

Six subjects without simultaneous prescription were chosen out of thirteen candidate patients, primarily from a viewpoint of diverse skin loci and symptoms being intractable even for long periods or by other remedies. Periods for hydrogen-dissolved warm water treatment were set within the period of the previous history of an intractable state for each subject to minimize or deny the contribution of a possible natural cure. As characteristics of the concerned daily-life practical study, there were diverse appropriate electrolytic conditions for the generation of hydrogen-dissolved warm water, which were adjusted to actual situations of each subject, such as differences in (1) water hardness of the pre-electrolytic tap water, (2) the size of foot bucket/bathtub, and (3) the number of times each subject bathed with those outside of their family without changing water, which is a part of Japanese customs.

The previous history of skin roughness and the characteristics of skin roughness treatment were as follows:

Subject A: The skin roughness, such as cracks, dryness, and keratinization in the back side of the feet, especially heels, did not improve with the normal warm water bathtub over the last year, but appreciably improved within half a year due to the hydrogen treatment.

Subject B: The skin roughness in the heels did not improve with the normal warm water bathtub over the last year, but appreciably improved by the hydrogen treatment within 5 months.

Subject C: The hydrogen-jetting bath was used in contrast to other subjects for electrolysis. The skin roughness was not improved by skin-peeling with fruit acid/glycolate and pumice exfoliation, over the past 20 years, but appreciably improved due to hydrogen treatment within 3 months.

Subject D: The skin roughness was pathognomonic of frequent and spot-scattered occurrence had not been prevented over the last 6 months, but appreciably improved on the right hand due to the hydrogen treatment after less than 6 months. As the reference, the left hand was treated with normal warm water, resulting in scarce relief.

Subject E: The skin roughness was characteristic of frequent and widespread skin peeling from as shallow as the stratum corneum to deeper layers of the epidermis that did not resolve over the last month. Each finger in both hands was appreciably improved after less than 2 weeks by hydrogen treatment.

Subject F: Recurrence of intractable skin roughness was observed with severe itching, reddish swelling, and peeling of the skin on the left elbow, which had not alleviated over the last year, but appreciably improved after less than 7 months of hydrogen treatment. Supporters were used only when the pain was felt.

2.4. Analysis for the Correlation of Nanoparticle-Sizes Versus Frequencies in Hydrogen Water by Nano-Sight Method

Electrolysis was carried out using tap water from the Osaka City Waterworks Bureau. The detection and characterization for nanoparticles in the water of hydrogen generated by LitaLife Ver.2 were analyzed by a nanoparticle analyzer Nano-Sight (LM10 type manufactured by Malvern Instruments Ltd. (Malvern Worcestershire, UK); 405 nm laser module, high-sensitivity CCD camera, nano-particle tracking analysis software), which analyzes the trajectory by examining the halation due to laser-beam for Brownian movement of nanoparticles/bubbles and evaluates the size versus frequency distribution of nano-particles [34]. The particle size was calibrated three times by polystyrene latex beads of approximately 100 nm (Thermoscientific Co. Ltd., Tokyo, Japan) as estimated by the transmission electron microscope and was evaluated using the Nano-Sight method to be 104.1 ± 0.4 nm for the median and 109.3 ± 1.0 nm for the average. The length-indicator scales on the microphotographs were evaluated based on the diameter of the Nano-Sight minimum halation being attributed to the standard particle of the average electron-scopical diameters.

2.5. Physico-Chemical Properties of Hydrogen Water That Is Prepared by the Electrolyzer

The dissolved hydrogen molecule concentration was measured by a diaphragm polarographic electrode-type hydrogen-molecule concentration meter (KM2100DH). The dissolved-oxygen-molecule concentrations were measured using a polarographic dissolved oxygen-molecule meter (DO-5519E).

2.6. Compliance with the Code of Ethics and Review by the Ethics Committee

All clinical studies were conducted following the guidelines of the Ministry of Health, Labor, and Welfare in Japan. The study protocol was officially approved as a non-invasive examination, as there was no hemorrhage, no pain, and no remaining symptoms, together with all the subjects' informed consents, by the Medical Ethics Committee of the Japanese Center for Anti-Aging Medical Sciences (Approval No. 08S02; authenticated 2011 by the Hiroshima Prefectural Government) on 15 October 2018.

2.7. Anonymization and Double-Blind Processing in the Clinical Analysis

In this study, all personal information of the subjects was double-anonymized and strictly conducted as follows: The subjects were not informed of details of the treatment ahead of time. A series of clinical photographs were taken and delivered as a batch with the randomized codes from the photographer to the individuals evaluating. The correlation of detailed treatment menu versus changes in skin roughness was estimated independently by three researchers, finally resulting in the summarized evaluation.

3. Results

3.1. Alleviation of Rough Skin in the Heel by Hydrogen-Dissolved Warm Water Bucket/Bathtub

Rough skin on the heel was examined for signs of alleviation due to treatment consisting of a combination of the hydrogen water foot bucket and hydrogen water bathtub use under specified conditions (Table 2). Subject A was soaked in 47 L foot bucket for 10 min daily. Warm water (41 °C) was enriched with hydrogen by 30 min electrolysis and was also poured into the hydrogen water bathtub (the electrolysis-type hydrogen generator (the electrolyzer)). As shown in Figure 1A and Table 2, the skin roughness of the right foot of Subject A was alleviated visually, with slightly more markedly improvement compared to the left foot after the 27th day, and the softness for the heel skin was further confirmed when touched. The subject's right heel was gradually alleviated in terms of chaps and cracks in

the skin, and finally, the feeling of dryness became less noticeable. Subject A received no dermatological or medical/cosmetic care except for the combination of a hydrogen water foot bucket and hydrogen water bathtub use.

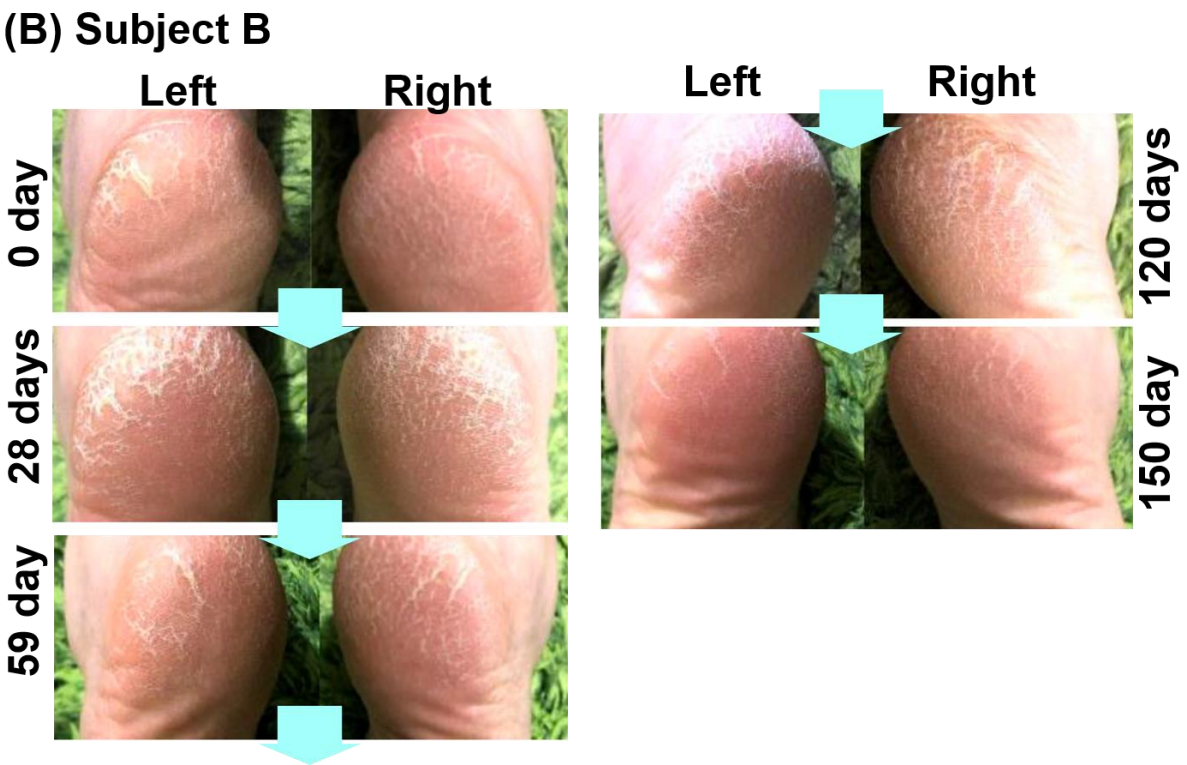
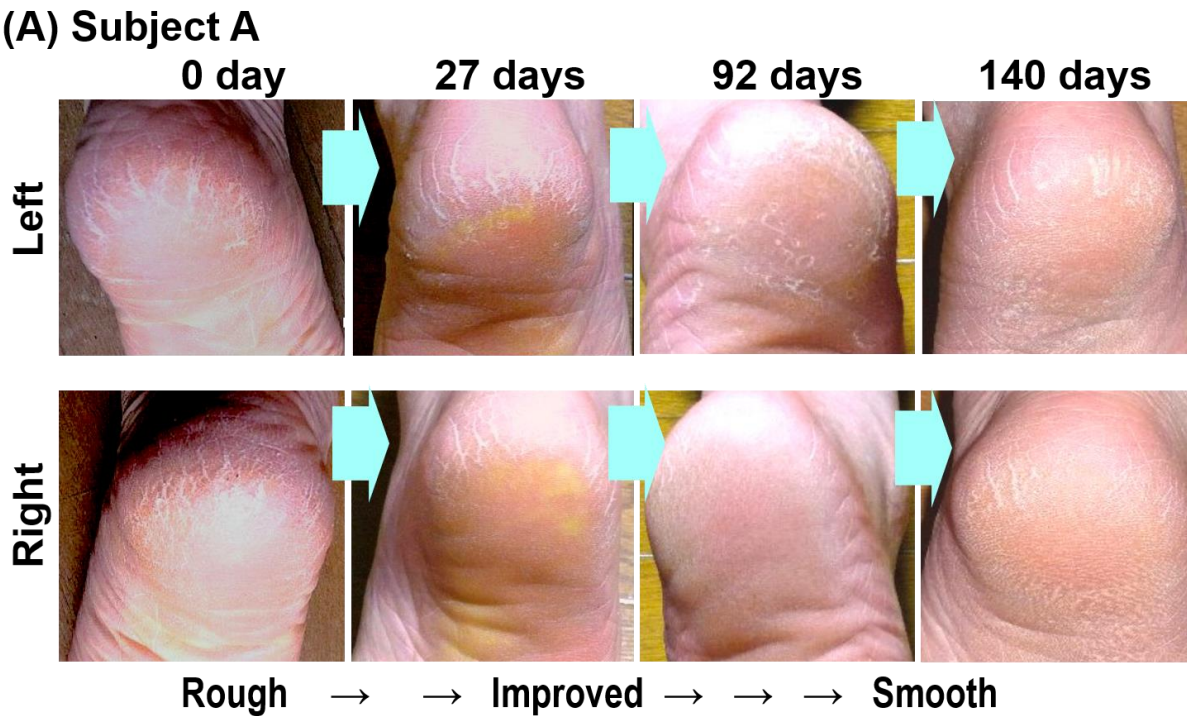


Figure 1. Cont.

(C) Subject C

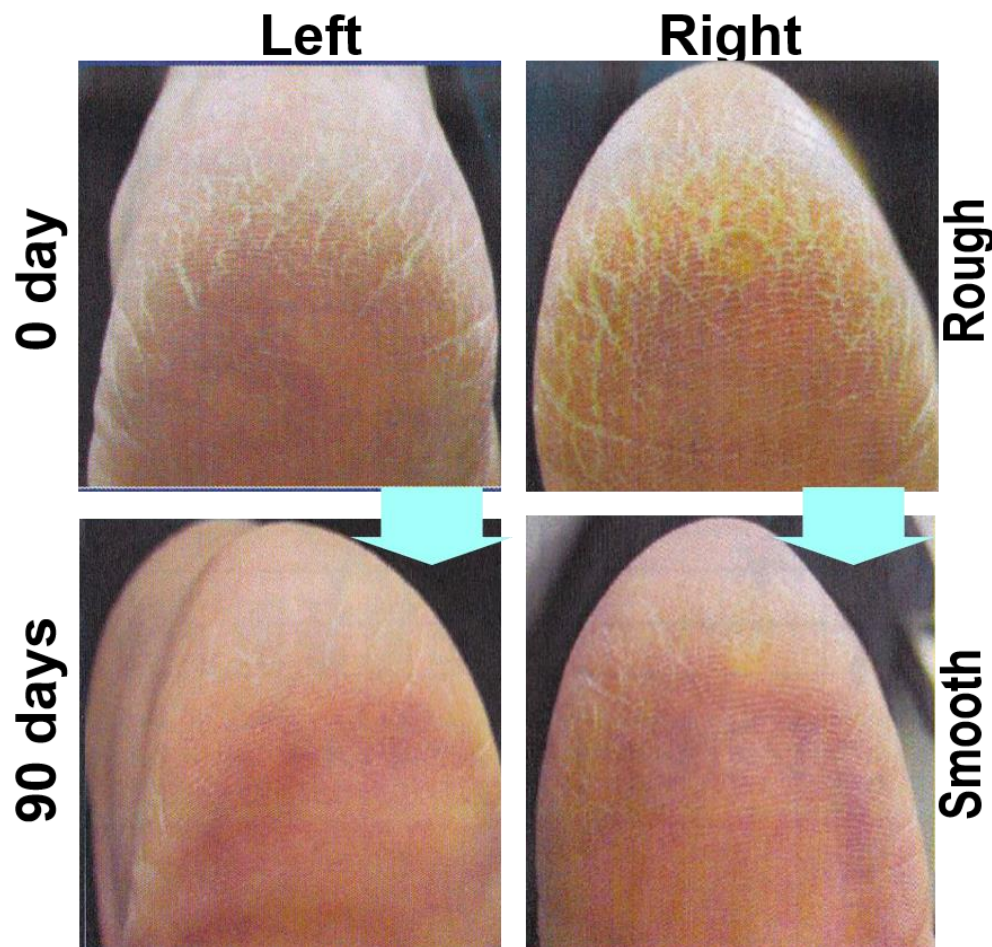


Figure 1. Alleviation of the rough skin in the heel by hydrogen-dissolved warm water bucket/bathtub. The information on subjects and hydrogen-dissolved warm water treatments was shown in Table 2.

In Subject B, who had rough skin on the heels, both heels were soaked up to the shin for 30 min every day, with 60 min of electrolysis for hydrogen generation in a foot bucket filled with 50 L of warm water (41 °C), and then Subject B used the hydrogen water bathtub similarly prepared by the electrolyzer for 150 days. After the start of the test (on the 28th and 59th days), the rough skin temporarily worsened, but the combined use of the hydrogen water foot bucket and the hydrogen water bathtub alleviated the skin roughness of the heel (the 150th day) (Figure 1B). Meanwhile, Subject B did not apply skin medications or use cosmetic creams as for other subjects.

3.2. Difference in Hydrogen-Supplying Methods between Electrolytic Generation and Hydrogen-Gas Jetting

To examine which of the improvement of skin roughness was achieved via a hydrogen water bathtub with hydrogen-jetting from micro-porous gas-emitting terminals of a hydrogen cylinder, compared with electrolysis-type (Figure 1A,B), the effects on the rough skin of both the left and right heels of Subject C was investigated by a hydrogen water bathtub (dissolved hydrogen molecule concentration: 721 µg/L; redox potential: minus 360 mV, 41 °C, pH 7.43; bathing for 5 min every day for 90 days consecutively). The results showed that rough skin on the heels had appreciably alleviated to similar degrees between the left and right heels (Figure 1C). Thus, bathing in hydrogen-dissolved warm water prepared either by electrolysis or microporous bubbling was demonstrated to alleviate skin

roughness. Skin cracks in Subject C disappeared in both the left and right heels, together with the restored feeling of moisturized skin.

(A) A 66-year-old man (Subject A) with skin roughness in right and left heels was bathed for 10 min every day in a foot bucket and, in a time-staggered mode, a bathtub, both of which were filled with 47 L and 180 L, respectively, of hydrogen-dissolved warm water (41 °C) that had been generated via electrolysis over 30 min every day for 162 days.

(B) A 42-year-old woman (Subject B) with rough skin on right and left heels soaked up to below the shin for 30 min every day in a bucket filled with 50 L of hydrogen-dissolved warm water (41 °C), which was generated by electrolysis over 60 min, in addition to a separate bath up to the neck in a 180 L hydrogen-water-filled bathtub, generated in a similar method, for 150 days.

(C) Immediately before the start, a 46-year-old woman (Subject C), whose skin roughness degree was slightly more marked on the right heel than on the left heel, was subjected to a hydrogen bath (dissolved hydrogen concentration: 721 ppb, oxidation-reduction potential: minus 360 mV, 41 °C) (pH 7.43, bathing for 5 min) every day for 90 days. The hydrogen bath was a microporous emission terminal-jetting hydrogen water type.

3.3. The Cases of Examination for Alleviation Degrees of Rough-Skin in the Hands with Hydrogen-Bathing for one Hand and Normal Water-Bathing for Another Hand as the Reference

Subject D, who suffered from skin roughness on both hands, soaked only their right hand in a hydrogen water bucket twice a day for 152 days and bathed for 10 to 20 min while only immersing the right hand with the left hand placed on their head so as to not immerse it (Table 2). For the reference examination, it was intended for the left hand with skin roughness to be immersed in normal warm water, similar to the right hand, except with non-hydrogen-containing water. Immediately after soaking in the hydrogen bath, the skin roughness of the right hand improved (Figure 2A,B) and gave a considerable skin-moisturizing feeling without any medical skin treatments. A subjective comfortable feeling was obtained when touching the skin. In contrast, the left hand receiving no hydrogen showed little improvement the skin roughness.

(A) Subject D-Right

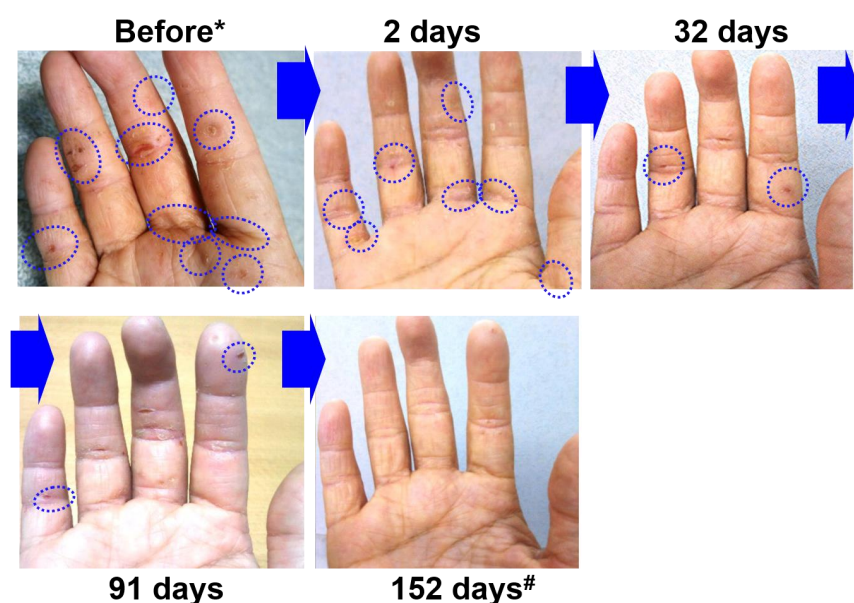


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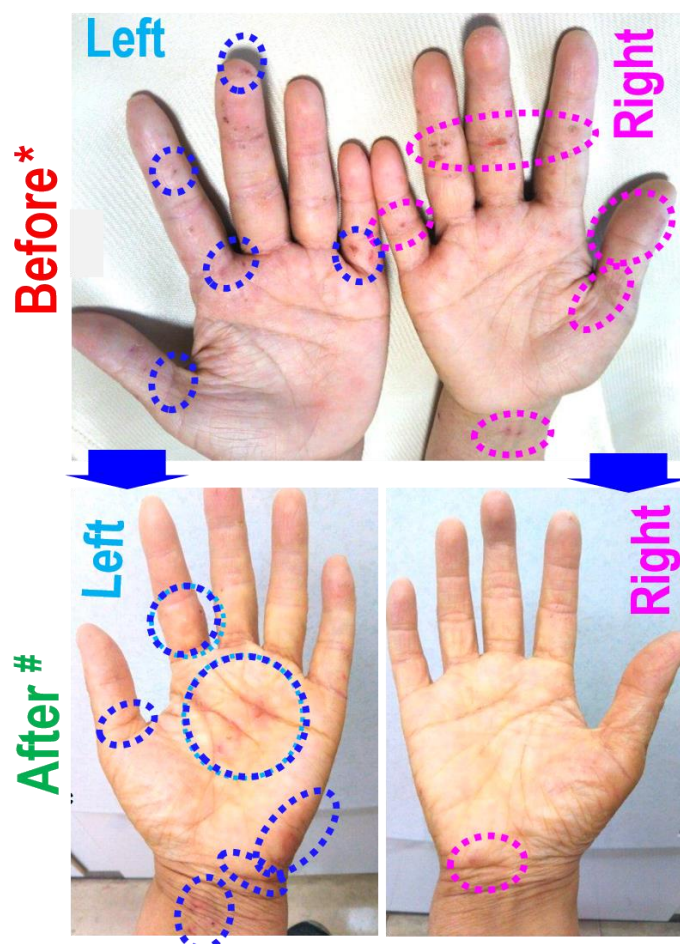
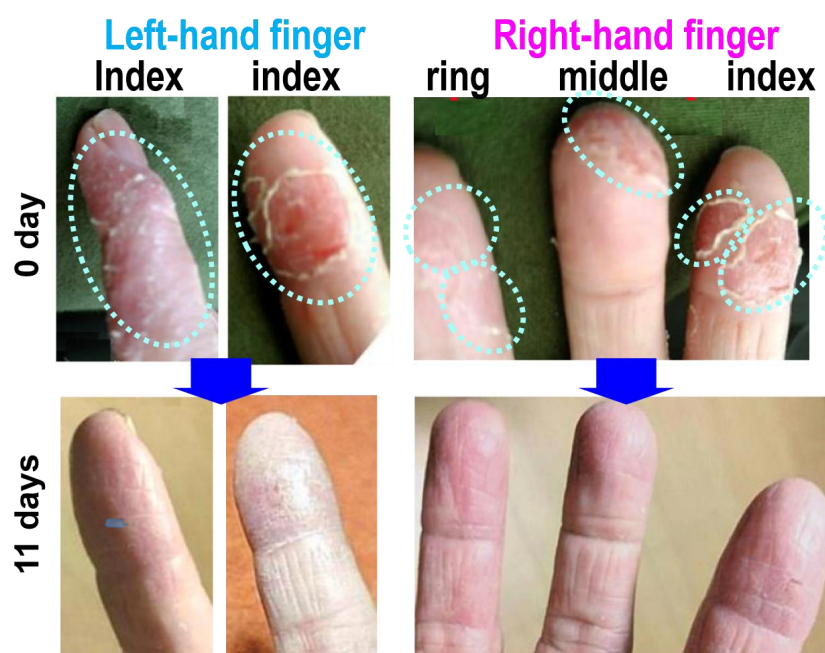
(B) Subject D**(C) Subject E**

Figure 2. A case of alleviation of the rough skin in the hands by the hydrogen-dissolved warm water bath.

(A) A 38-year-old woman (Subject D) with rough skin on both hands also soaked each hand in a hydrogen-dissolved warm water bucket twice a day for 152 days for 10–20 min and also took a hydrogen bath, separately, without immersing either hand for 10 min once every day while only her right hand was immersed in the hydrogen bucket. The rough-skin locus is indicated by a blue dashed-line ellipsoid. *, 23 days before the start of hydrogen treatment. #, 31 days after the end of hydrogen treatment.

(B) For 152 days, Subject D immersed only their right hand in a hydrogen water bucket, similar to the immersing of the left hand in the normal warm water bucket. The rough-skin locus is indicated by a blue or pink dashed-line ellipsoid. *, #: *ibid.* in Figure 2A.

(C) A woman with skin roughness and peeling (Subject E) in diverse hand-skin sites, such as the index finger of the left and right hands, the middle finger of the left hand, and the ring finger was immersed for 10 min once every day in a hydrogen water bath for 11 days. The light blue dashed-line ellipsoids indicate the areas of skin roughness like atopic dermatitis.

3.4. Effects of Hydrogen Bath on Atopic Dermatitis-like Skin Roughness

The effects of the hydrogen water bathtub on atopic-dermatitis-like skin roughness (Subject E) with the simultaneous immersion of both hands were also examined (Table 2). By continuing the hydrogen bath for 11 days, skin symptoms, like atopic dermatitis, on various areas of skin on the hand and fingers, such as the index finger of both hands, the middle, and the ring fingers of the left hand, were alleviated (Figure 2C). Thus, the hydrogen water bathtub was also shown to be effective for repairing skin that has peeled from the stratum corneum and deeper layers of the epidermis.

A 51-year-old man (Subject F), who had severe itching, reddish swelling, and peeling of the skin on the left elbow, used a foot bucket for 20 min once a day for 180 days. The light blue dashed-line ellipsoids indicate the skin roughness. \$, 11 days before the start of hydrogen treatment.

3.5. Alleviation of Rough Skin on the Elbow by Taking a Hydrogen Foot Bucket and a Hydrogen Bathtub Together

Subject F had severe itching and peeling of the skin on the left elbow, which scarcely showed atopy-like symptoms (Table 2). The skin roughness on the elbow was alleviated using a hydrogen water bathtub by immersing of both the elbows and separately using a hydrogen foot bucket for 10 and 20 min once a day for 180 days. Initially, itching of the affected skin area of the elbow skin was relieved, with improvement in the redness (Figure 3) and alleviation of excess skin tension. Even in the winter during the final stage of the examination period, when skin is prone to dryness and roughness, the rough skin on the elbows was moisturized with the removal of scabs, became smooth to the touch, and reduced itchiness. Subject F could sleep soundly without skin irritation or stinging during the treatment period. Even when the affected skin area was itchy, Subject F used neither medications, medical treatments, nor cosmetics, but applied an analgesic upon feeling pain several times.

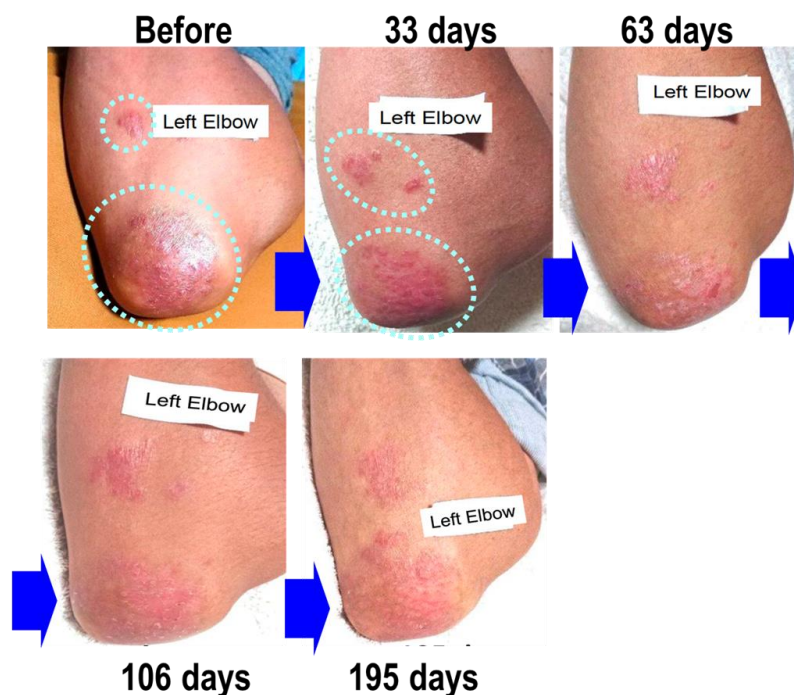
Subject F

Figure 3. Alleviation of the rough skin in the elbow by combined utilization of a foot bucket/bathtub filled with hydrogen-dissolved warm water.

3.6. Quantification of Nano-Particles and the Size-Frequency Distribution in Hydrogen Water Generated by the Electrolytic Hydrogen Generator

The presence of nanoparticles was investigated for hydrogen-dissolved warm water produced by the electrolyzer. A laser-beam-halation-trail-based nanoparticle analyzer, Nano-Sight, was used to examine the size-frequency distribution of nanoparticles before and after electrolysis for hydrogen molecule production as described in the Materials and Methods section (Figure 4A). Few nanoparticles were present in the aspirator-adopted tap water that was degassed for 10min before electrolysis (upper panel), whereas the presence of abundant nanoparticles was confirmed after hydrogen molecules were generated by electrolysis (lower panel), which were suspended for a long term, as evidenced by the Brownian-like movement, i.e., in a state of neither floating nor sedimenting.

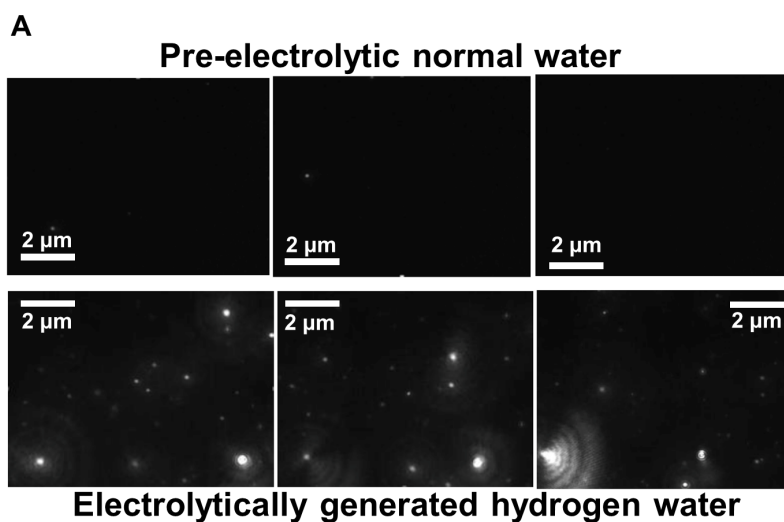


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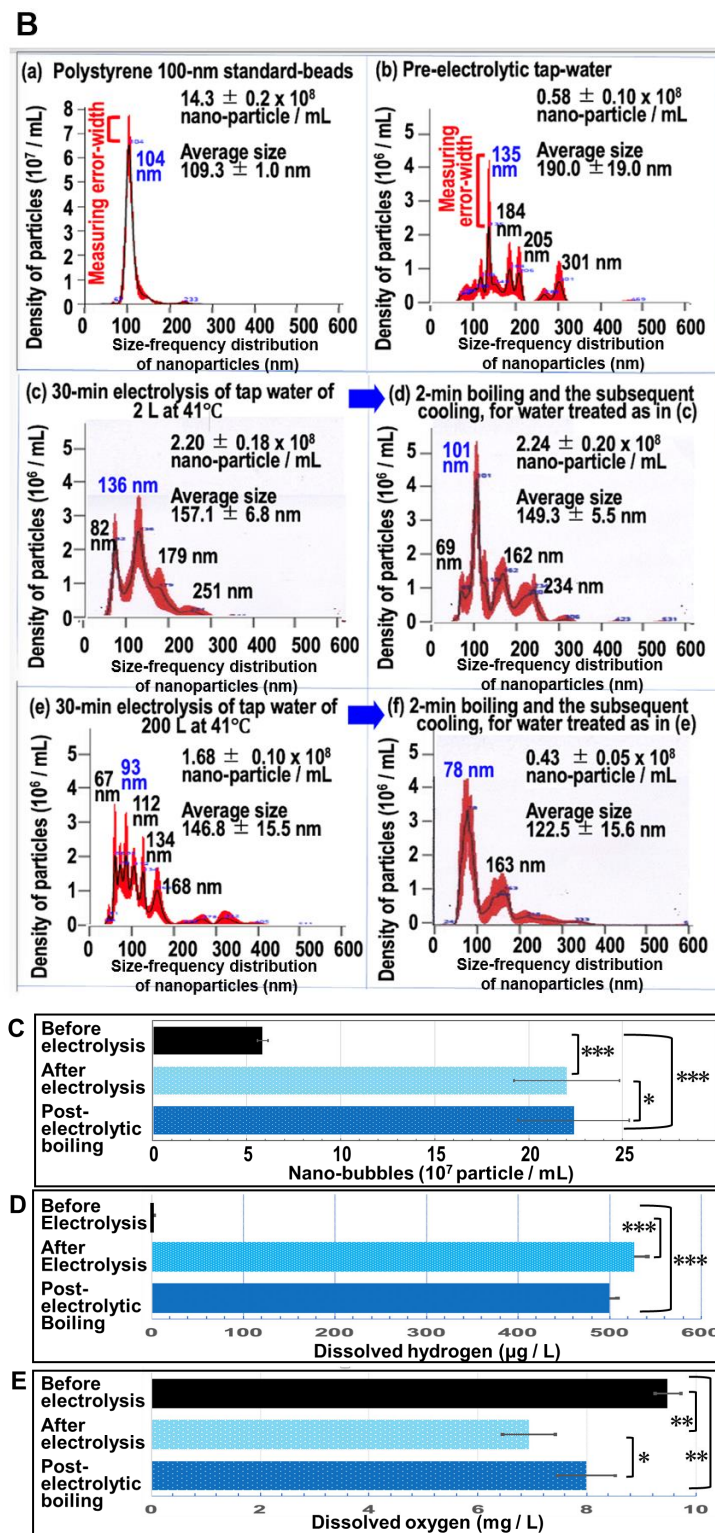


Figure 4. Physico-chemical properties of electrolyzer-generated hydrogen-dissolved warm water. Some major values of measurement are shown in Table 1.

(A) Using a laser-beam-based nanoparticle analyzer, the size-frequency distribution of nanoparticles before and after hydrogen generation by electrolysis was microscopically examined. Although few nanoparticles were present in tap water before electrolysis (upper panels), the presence of nanoparticles was confirmed by the halation due to the laser-

beam causing Brownian movement after hydrogen molecule generation by electrolysis (lower panels).

(B) The size-frequency distribution of nanoparticles generated by the electrolyzer was evaluated five times (except (a) three times) using the laser-beam-based particle-locus-chasing Nano-Sight method and is expressed as the line-histogram in the average. (a) Polystyrene latex standard beads that were 100 nm in diameter. (b) Osaka-City-supplied tap water before electrolysis. (c) A total of 2 L of tap water was electrolyzed at 41 °C using the electrolyzer for 30 min to generate hydrogen molecules. (d) The treatment in (c) and the subsequent boiling for 2 min at 98 °C, followed by natural cooling. (e) A total 200 L of tap water was electrolyzed at 41 °C using the electrolyzer for 30 min to generate hydrogen molecules. (f) The treatment in (e) and the subsequent boiling for 2 min at 98 °C, followed by natural cooling. The length value in a unit of nm expresses the average diameter of each peak. Specifically, the blue value represents the mode. The red area expresses the measuring error range for the Nano-Sight analysis.

(C–E) The density of nanoparticles (C) and concentrations of dissolved hydrogen (D)/oxygen molecules (E) were evaluated five times in the pre-electrolytic tap water, hydrogen-dissolved warm water generated at 41 °C from 2 L of tap water via the electrolyzer, and the subsequent boiling at 98 °C for 2 min, followed by natural cooling. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

3.7. Size-Frequency Distribution of Nanoparticles Generated by the Electrolytic Hydrogen Molecule Generator

The correlation between nano-particle size and frequency of the electrolyzer-generated hydrogen water was investigated and expressed as line histograms (Figure 4B). The polystyrene latex standard beads with a diameter of approximately 100 nm were evaluated as 109.3 ± 1.0 nm for the average, 102.1 ± 1.0 nm for the mode (the most frequent value), and 104.1 ± 0.4 nm for the median (the value with a cumulative frequency of 50% in the order of particle size) using the Nano-Sight analyzer (Figure 4B(a),C), indicating the precision within 10% of measuring error ranges. Based on the standard particle, the length scales on the microphotographs were evaluated. The pre-electrolytic tap water was shown for the size-frequency distribution of multiple peaks without a single peak being significantly higher (Figure 4B(b)), whereas the 2 L of hydrogen water used in the case of the foot-foot bucket that was prepared by 30 min electrolysis showed two major higher peaks, and the number of nano-particles created by the electrolyzer increased from 0.58×10^8 /mL to 2.20×10^8 /mL (Figure 4B(c)). For the 200 L of tap water at 41 °C, in the case of the bathtub, the nano-particles were increased to 1.68×10^8 nano-particle/mL (Figure 4B(e),C). Thus, the postelectrolytic increase in nano-particles was shown to be more marked for 30 min application of the electrolyzer in a smaller volume of tap water than for 200L used in the bathtub. The average, mode, and median of the nano-particles of hydrogen water electrolytically generated for the 2 L of tap water over 30 min are summarized in Table 1. It was confirmed that the maximum dissolved hydrogen-molecule concentration reached 527 µg/L (another unit formula: ppb) with 30 min of electrolysis on 2 L of tap water.

3.8. Resistance of the Electrolytically Generated Hydrogen Water against Transient Boiling

The hydrogen water was boiled at 98 °C for 2 min, and naturally cooled to 25 °C. The post-boiling nano-particles were markedly retained for the 2 L of hydrogen water but scarcely retained for the 200 L of hydrogen water, as shown by the histograms of nano-particle size–density distribution (Figure 4B(d,f)). The results showed that the nano-particles that survived post-boiling were mostly occupied by nano-particles as small as 78- to 101 nm rather than nano-particles of sizes larger than 134- to 136 nm (Figure 4B(b)), suggesting that the resistance against bubble disappearance due to transient boiling was attributed to a smaller size of the nano-particles.

3.9. Correlation of the Number of Nano-Particles and the Dissolved Hydrogen/Oxygen Concentrations

The nanoparticle densities were almost positively correlated with the dissolved hydrogen molecule concentrations, which were applied to the 0-/30 min electrolysis and the subsequent transient boiling/cooling (Figure 4B(c–f),C). In contrast, the nano-particle densities were scarcely correlated with the dissolved oxygen molecule concentrations (Figure 4C,E). Thus, the electrolysis-caused increase in nano-particle was indicated to be mostly occupied by the dissolved hydrogen molecules as dilute as 498–527 $\mu\text{g/L}$ but scarcely by the dissolved oxygen molecules, even for concentrations as dense as 6.8–9.3 mg/L .

4. Discussion

The causes of rough skin are considered to be diverse disorders such as the disturbance of metabolism, the lowering of renewing replacement between old skin cells and new skin cells, skin dryness, and the reduction/deregulation of skin immunity [4,36]. It is generally believed not only that these phenomena tend to increase with aging but also that ROS, such as hydroxyl radicals, which are the main causes of aging, are involved. Very interestingly, in recent years, much attention has been paid to hydrogen molecules as new antioxidants, but it has been clarified that hydrogen molecules specifically react with hydroxyl radicals to eliminate them [11,12,31]. Moreover, it has also been demonstrated that typical ROS-scavenging endogenous substances, like ascorbic acid, are more markedly retained against spontaneous auto-oxidation to dehydroascorbate or diverse reductones by coexistent hydrogen-nano-bubbles than when existing alone [37]. In the present study, it was shown that roughness in human skin tended to be alleviated by hydrogen-dissolved warm water containing hydrogen molecules of nano-sizes (Figure 4A,B), higher density, and heat-resistance (Figure 4B(d,f),C,D), in contrast to scarce effects for normal warm water (Figure 2B,C), as suggested for some skin cases [14,38–41].

By using an electrolyzer, which can be applied not only to bathing the whole body and below the neck in the bathtub but also to foot baths/buckets as shown in Figure 5A, it is possible to combine a whole-body bathing bathtub and a foot/hand-immersing bucket in the routine [32,33]. The cuticle, nail bed, and lower nail that are in contact with the nails are not easily exposed to the outside air, and the stratum corneum is thin or poor, as shown in Figure 5B. Therefore, in the foot bucket, it is considered that hydrogen molecules easily penetrate the body depth through the cuticle, nail bed, and lower nail, as they have the smallest molecular mass of any element on the earth and the non-charged state for the lower-dispersed hydrogen molecule. At this time, as the skin-roughness-caused impairment of the skin barrier is more serious than in healthy skin, hydrogen molecules were penetrated more markedly through the disorganized stratum corneum into skin depth surrounding the capillary vessels. However, this study showed that bathing in warm water containing hydrogen nano-bubbles generated by an electrolyzer may be effective in alleviating rough skin.

(A) As a common household item that can be used for the consistent intake of hydrogen into the body, a 2–50 L foot/hand-immersing bucket can be used more frequently/easily adopted into a new routine, in combination with hydrogen water bathtub, than a bathtub that requires 170–200 L of tap water and a hydrogen production time requiring 30–60 min of electrolysis.

(B) The skin adjacent to the nails ((1) cuticle, (2) nail bed, (3) lower nail skin) is hardly exposed to the outside air, and so the stratum corneum is thin and scanty. It is estimated that hydrogen nano-bubbles easily penetrate the epidermis or depth of skin from the gap between the nail and toes. It is presumed that hydrogen molecules easily penetrate the skin from (1) the skin in contact with the nail, (2) the arch of the foot sole, (3) the pores of the shin, and (4) the space between the toes. It also might be considered to be effective as a substituted means for elderly, sick, or frail people who have some difficulties with bathing, and/or as an auxiliary means for hydrogen water bathtubs. In terms of electric safety, the electric shock can be avoided as follows: The electric current interruption apparatus is built in two loci, such as the electric input gate and the electrolyzer inside for the insulation, to

avoid elevation beyond the safety standard value. The gap in the cover of the electrolytic terminal is designed to be below 2 mm distant to prevent the entry of objects.

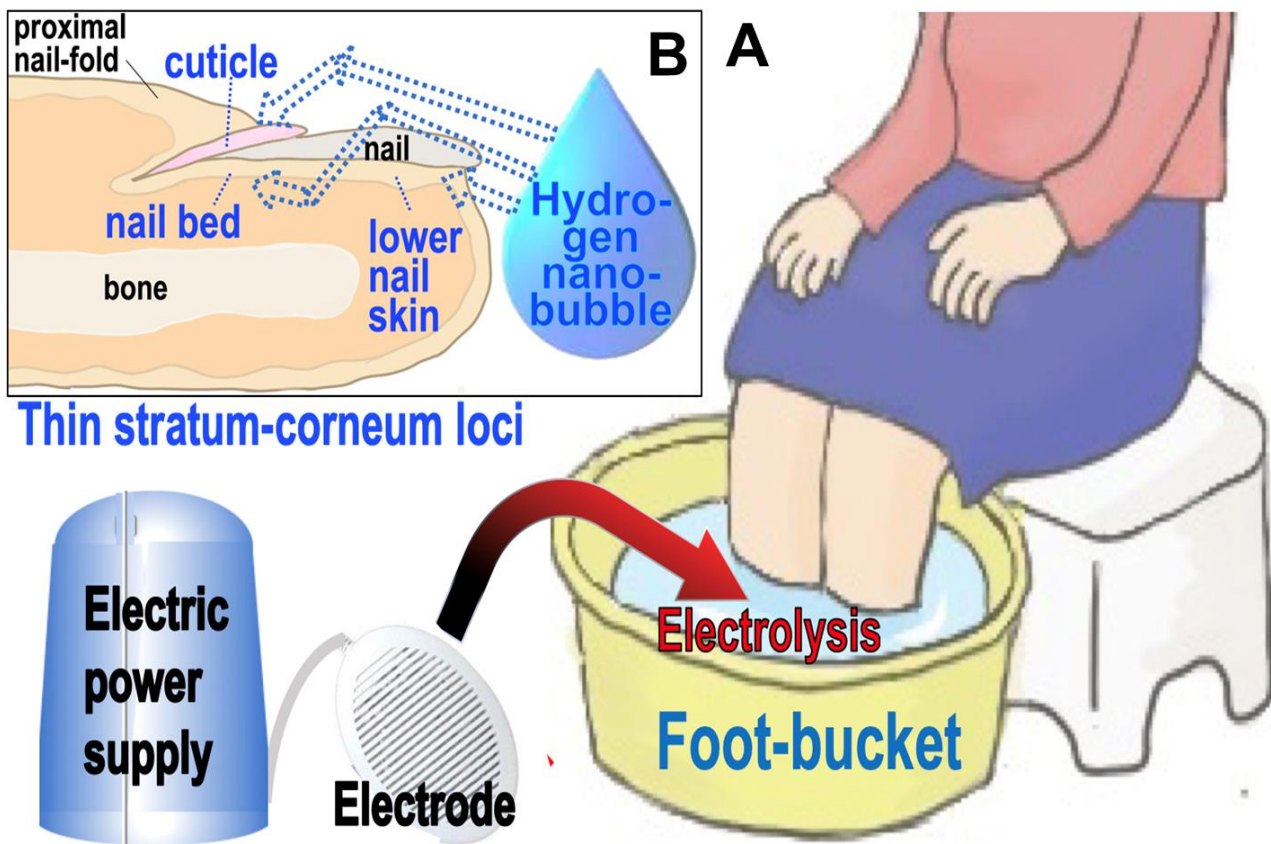


Figure 5. Hypothetical mechanism of permeation of hydrogen molecules in warm water into adjacent skin by a foot bucket.

The mechanism whereby a bathtub and a frequently-used foot bucket that is filled with hydrogen-nano-bubble-rich water can improve the diverse type of skin roughness is suggested to be attributed to both the abilities of hydrogen molecule (1) to permeate to the depth of the epidermis via the stratum corneum [42,43] and (2) to efficiently diminish the reactive oxygen species in the skin roughness locus through its reducing power [14]. For example, (1) the barrier of the skin surface is suggested to have to a molecular mass below 500 but not above 1000 [42], although the order is known as follows: methylene blue dye (319.85) > ascorbic acid (176.12) > water (18.015) >> hydrogen (2.01588). Hydrogen nano-bubbles, but not ascorbic acid, were demonstrated to permeate a water-impermeable polyethylene-film bag that was filled with the redox dye methylene blue and water and changed the blue dye colorless [43]. Thus, hydrogen nano-bubbles might efficiently permeate into the skin surface, especially at skin roughness loci that are at least partly damaged in the stratum corneum. (2) As the tap water at an ORP of $+266.8 \pm 15.4$ mV underwent electrolysis for longer terms, ORP was evaluated to be diminished from -430.6 ± 24.8 mV for 10 min to -468.0 ± 4.2 mV for 30 min ($N = 5$, respectively), indicating the increase in its reducing power [14,15]. And, it has been demonstrated that the hydrogen-rich bath made an appreciable increase in the antioxidant ability of the participants' serum, in contrast to the scarce increase in the serum antioxidant ability by the control bathing using tap water [32]. In addition, every case of intractable skin roughness, which was dealt with in the present research, was demonstrated not to be or scarcely healed for more days or by other therapies, such as chemical peeling and ointments, being applicable to the control experiment for the same subject. As a possible mechanism, the skin roughness improvements

of hydrogen nano-bubbles might not ensue from the direct effect of hydrogen contained in the bathtub/bucket. However, hydrogen is well-known to be so inert as to react with other non-physiological substances under normal temperature and pressure. However, hydrogen might affect the redox of physiological substances, such as some enzyme-related cofactors towards the reduced state, most of which might function in the human body toward a benefit state. Thus, it might be assumed as the mechanism that hydrogen-nano-bubble-rich water in a daily bathtub and frequently used foot bucket could permeate into a skin roughness locus, and repress the reactive oxygen species at least partly responsible for the deterioration of the skin.

Although the present examination was the first trial for the improvement of intractable skin roughness, the present research could achieve six subjects of diverse skin loci without regimens other than hydrogen. In the cases of skin roughness that the present research handled, there was the intractable skin roughness that could not heal by itself, nor was improved yet by other regimens, for example, fruit acid/glycolic acid peeling and pumice exfoliation, even for several years, indicating that the hydrogen-nano-bubble method might be worth being utilized because of the proof of improvement even for longer days through the gentle action of hydrogen nano-bubbles. As new aspects, which the present work will bring to the health-caring fields, it can be exemplified to potentially improve some types of intractable skin roughness by daily utilization via a bathtub/bucket from a viewpoint of the hydrogen-based repression of oxidative stress. Secondly, the advantage of the hydrogen nano-bubble technique can be found for scarce incidences of such side-effects that can be caused by some ointments, whereas the limitations include the decreased improvement in the wide range of symptoms that go beyond the oxidative-stress-induced level. Thirdly, as a part of the unique method that is proposed in the present investigation, it was emphasized that the hydrogen-rich water bucket, which can be utilized daily, should be combined with a bathtub being utilized once a day.

5. Conclusions

The combination of the bathtub and the foot bucket filled with warm water containing the dissolved hydrogen molecules with nano-bubbles generated by an electrolyzer was demonstrated, under prescription-free conditions, to mitigate the intractable skin roughness that could not be improved for a longer period nor other past regimens.

Author Contributions: N.M. wrote the initial draft. Y.S., Y.T. and N.M. completed the final draft. The clinical and most basic examinations were executed by N.M., who proposed the resultant data. Y.T. examined the diverse physicochemical parameters of hydrogen-dissolved warm water. The illustration drawing for the bucket application and mechanism was conducted by N.M. The scientific validity of all clinical and basic data in this study was carefully examined by Y.S. and N.M. Y.S., Y.T. and N.M. read the final manuscript for publication. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted by the Declaration of Helsinki and approved by the Medical Ethics Committee of the Incorporated Non-profit Organization the Japanese Center for Anti-Aging Medical Sciences (Approval No. 08S02; authenticated 2011 by Hiroshima Prefectural Government) on 15 October 2018.

Informed Consent Statement: The documents for informed consent have been obtained from all the patients enrolled in the present clinic study.

Data Availability Statement: All scientific data included in this study are available upon reasonable request by officially contacting the corresponding author.

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