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Repetitive Bathing and Skin Poultice with Hydrogen-Rich Water Improve Wrinkles and Blotches Together with Modulation of Skin Oiliness and Moisture

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Abstract: Hydrogen-rich warm water (HW) has not been verified yet for skin anti-aging effects. Daily 10 min HW (dissolved hydrogen: 338–682 μg/mL, 41 °C) bathing and skin poultice with HW-impregnated towels for 11–98 days were demonstrated to improve wrinkle degrees (29 skin-loci) from 3.14 ± 0.52 to 1.52 ± 0.74 ($p < 0.001$) and blotch degrees (23 loci) from 3.48 ± 0.67 to 1.74 ± 0.92 ($p < 0.001$) in five healthy subjects (49–66 years old), by densito-/planimetrically evaluating with an Image-J software, and ranking into six hierarchies (0, 1–5). Meanwhile, skin oiliness was evaluated to increase for the oil-poor skins, but inversely decrease for excessively oily skins, suggesting the HW's function as skin-oiliness modulation, with an appreciably negative correlation in prior oiliness contents versus change after HW application ($r = -0.345$, 23 loci). Skin moisture increased upon HW application, with a negative correlation ($r = -0.090$, 23 loci) in prior moisture contents versus post-HW-application moisture-changing rates, meaning that HW application compensated moisture for water-deficient skins (27.5–40% moisture), but not for wet skins (>41% moisture). Thus, the HW bath together with HW poultice exerted beneficial effects on skin appearances such as wrinkles, blotches and moisture/oiliness, some of which might ensue from enhanced antioxidant ability in blood, as was previously demonstrated for the HW bath.

Keywords: anti-aging; human skin; wrinkle; blotch; oiliness; moisture

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1. Introduction

Wrinkles, blotches, inappropriate moisture levels and excess oiliness in human skin are symptoms typical of skin aging, which are related to the excessive action of reactive oxygen species (ROS) [1–6]. Skin aging is often caused by UV-induced photoaging [7–11]. ROS-induced oxidative stress causes damage to various cellular components, and consequently accelerates cellular senescence; thus, antioxidant-application strategies have been developed as effective solutions for ageing delay, by treating oxidative stress-related symptoms.

Among many antioxidant-application strategies, hydrogen molecules (H_2) are utilized as a novel medical gas with antioxidant properties [2,12,13]. Numerous experimental and clinical studies have demonstrated that hydrogen could protect cells, tissues and organs from oxidative stress-induced injuries by scavenging ROS [14,15]. Hydrogen-enriched electrolyzed water is guaranteed for safety in human body, and hydrogen also has beneficial effects on the human body, including preventive effects on many diseases and radiation-protective effects [16–19]. Several methods of administering hydrogen are represented by inhaling hydrogen gas/mist, drinking hydrogen water (HW) and bathing in HW [13]. The living cells also constantly produce ROS, even under healthy conditions, whereas excess

amounts of ROS are produced by inflammation and stress [19]. It is generally known that skin aging is also caused by stress due to overproduction of ROS [1–6]. Therefore, it has been reported that there are many substances that suppress skin aging [2,3,6–10,14]. Recently, hydrogen water is also one of the methods being used for anti-aging [12].

The newly developed instrument, designated as Lita Life Ver. 2, produces hydrogen-rich water by electrolysis, forming colloidal nanobubbles and stabilizing hydrogen nanobubbles dispersed in water for a long time [13]. Hydrogen-rich water has been suggested to be effective for alleviating oxidative stress [16,17,19]. It has improved the heightening of antioxidant activity, and its nanobubbles are markedly important for permeation into the depth of the living body and organs. By bathing with nanobubble hydrogen-rich water that is prepared by the electrolyzer, the oxygen radical absorption capacity (ORAC)-based antioxidant capacity increased and C-reactive protein (CRP, an index of degree of inflammation) level of human serum as well as inflammatory symptoms decreased [13]. Therefore, the hydrogen-rich bath is also considered to bring about skin-quality improvement. It has been reported that inhibitions by hydrogen-occluding silica microcluster to melanogenesis in human pigment cells and to the melanin-generating key enzyme tyrosinase reaction [12].

For this reason, we aim to investigate the effects of a hydrogen-rich water bath based on the improvement of the cosmetological state of the skin.

2. Subjects and Methods

2.1. Preparation of HW Bath

A hydrogen-rich water (HW) generator, Lita Life Ver.2 (WCJ Co., Ltd., Osaka, Japan), equipped with a lattice-shaped three-blade-type electrode surface-plated with platinum, and which produces hydrogen bubbles via electrolysis for Sakai-city-supplied tap water, was used according to the manufacturer's protocol. The dissolved hydrogen concentrations were evaluated to be 338–682 μg/mL, after 30–60 min electrolysis, with a polarographic diaphragm-type device with hydrogen-selective electrode (DH meter, KM2100DH, Kyoei Electronics Research Institute, Saitama, Japan) [13]. The nano-sized bubbles, which contain gaseous hydrogen, were produced via the electrolysis of warm (41 °C) water of 200 L poured into a bathtub and evaluated with a Nano Sight LM10V-HS (Malvern Co., Ltd., Malvern, UK) system, combined with a sCMOS camera (Hamamatsu Photonics, Hamamatsu, Japan).

2.2. Treatments with HW Bath and HW Poultice

The bath water of 180–200 L was electrolyzed for 60 min, immediately followed by 10 min bathing concurrently with twice continuously compressing of a fresh HW-soaked towel on the face, and finally further washing face. The subjects were two men and three women, aged from 49 to 66 years with no disease nor medical treatment and no intake of hydrogen-related drinking/supplement for the last 60 days. The research was officially approved in 2016 with an approval number of 15C01, under conditions of noninvasive research such as no hemorrhage, painless treatment, and no trace/sign, using a widespread commercially available apparatus, by the Medical Ethics Committee of the Japanese Center for Anti-Aging Medical Sciences, which was officially authenticated by the Hiroshima Prefecture Government of Japan, and all subjects also provided their signed informed consent forms.

2.3. Quantitative Assessments for Degrees of Wrinkles and Blotches

Immediately before and after the HW bath concurrently with the HW poultice compressing the concerned skin parts, the subjects underwent photography of wrinkles (at forehead, eye outer corner, between eyebrows, nasolabial folds, Marionette line and hand) and blotches (at forehead, eye outer corner, cheek, lower head and neck and upper limbs) under the conditions unified for background brightness and illuminating angle. The identification of the concerned area as a blotch was performed where both the subject and the inspector recognized it as a blotch without any pathological symptoms. A digital camera (Compact Digital Camera PowerShot SX730 HS, Canon, Tokyo, Japan) was used to

photograph the wrinkles and blotches on the face and hands. The degrees of wrinkle and blotch on the skin were evaluated in quadruplicate for each affected part as a percentage of the area and density distribution that were objectively recognized by application of the software Image J (NIH, Bethesda, MD, USA) as changes on the surface of the skin, and further expressed in the average value ± SD of the results of each datum, calculated using Microsoft Excel 2013 for Windows as six hierarchies (−, ±, +, ++, +++, ++++, +++++). To investigate the statistically significant difference between before and after HW bath and face poultice, data of six hierarchies of wrinkles and blotches were described as graphics.

The HW application with bath and poultice for the prearranged periods were evaluated for effects on skin oiliness and moisture of the concerned face parts (23 loci, respectively: at eye corner, cheek and hand neck) of five subjects in quadruplicate, by a bioelectricity-impedance-/electroconductivity-based skin-oiliness/moisture meter (HKJ-SK03P, HuntKey Japan, Co., Ltd., Tokyo, Japan), after gently wiping the concerned skin part with purified water-soaking cloth and drying up for 5 min.

2.4. Statistical Analysis

All data were analyzed statistically by Student's *t*-examination of Microsoft Excel (Redmond, WA, USA). Statistical significance was considered when $p < 0.05$.

3. Results

3.1. Effect of Improving Wrinkles on Five Subjects by Bathing and Face Poultice with Hydrogen-Rich Warm Water

Five subjects received bathing in hydrogen-rich warm water (HW) of 338–682 μg/L (another expressed unit: ppb) (41 °C) with 6.86–9.02 × 10^7 nano-bubbles/mL, once a day for 11–98 consecutive days. During the 10 min bathing, the subjects were repeatedly applied with a towel reimpregnated with fresh HW to the relevant skin area every 3 min. For every subject, loci where wrinkles became narrow, shallow, faint or disappeared after HW bathing and face washing were photographed (Figure 1A–H).

In three subjects—a 49-year-old female, 59-year-old male and 57-year-old male—a reduction in small wrinkles around the right-eye outer corner was observed (Figure 1A–C). In one subject—the 49-year-old female—a reduction in wrinkles around the forehead mole was observed (Figure 1D). In one subject—the 59-year-old male—a reduction in wrinkles on the left cheek was observed (Figure 1E). In one subject—a 66-year-old female—a reduction in wrinkles between the eyebrows was observed (Figure 1F). In one subject—a 57-year-old male—an improvement of wrinkles of both nasolabial folds was observed (Figure 1G). In one subject—a 66-year-old female—the second wrinkle from the top among four wrinkles in the total on the outer corners of the right eye almost disappeared, and the other three wrinkles also became shallower and shorter (Figure 1H).

Figure 2A shows changes of degrees of wrinkles for all observed loci of five subjects. Figure 2B shows changes of degrees for each region of five subjects. Figure 2C shows changes for total loci and five subjects. A total of 29 examined wrinkles were improved after the hydrogen-rich warm bath, with a significant decrease in wrinkle degrees of 3.14 ± 0.52 to 1.52 ± 0.74 ($p < 0.001$).

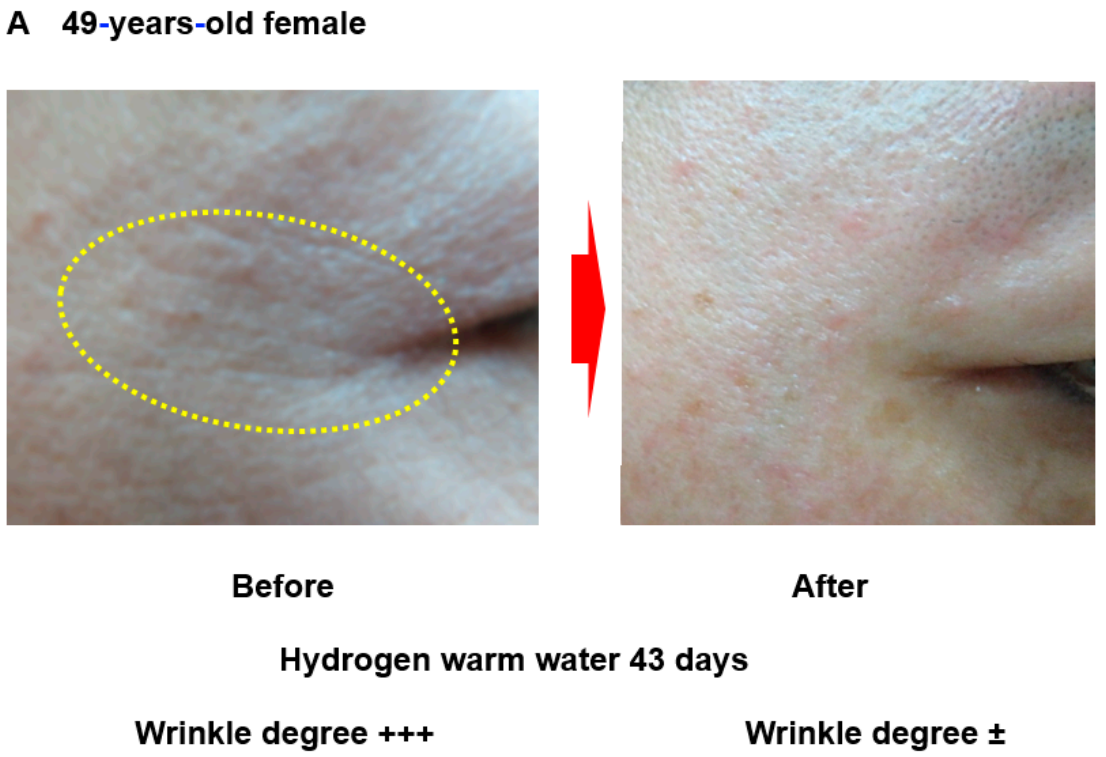
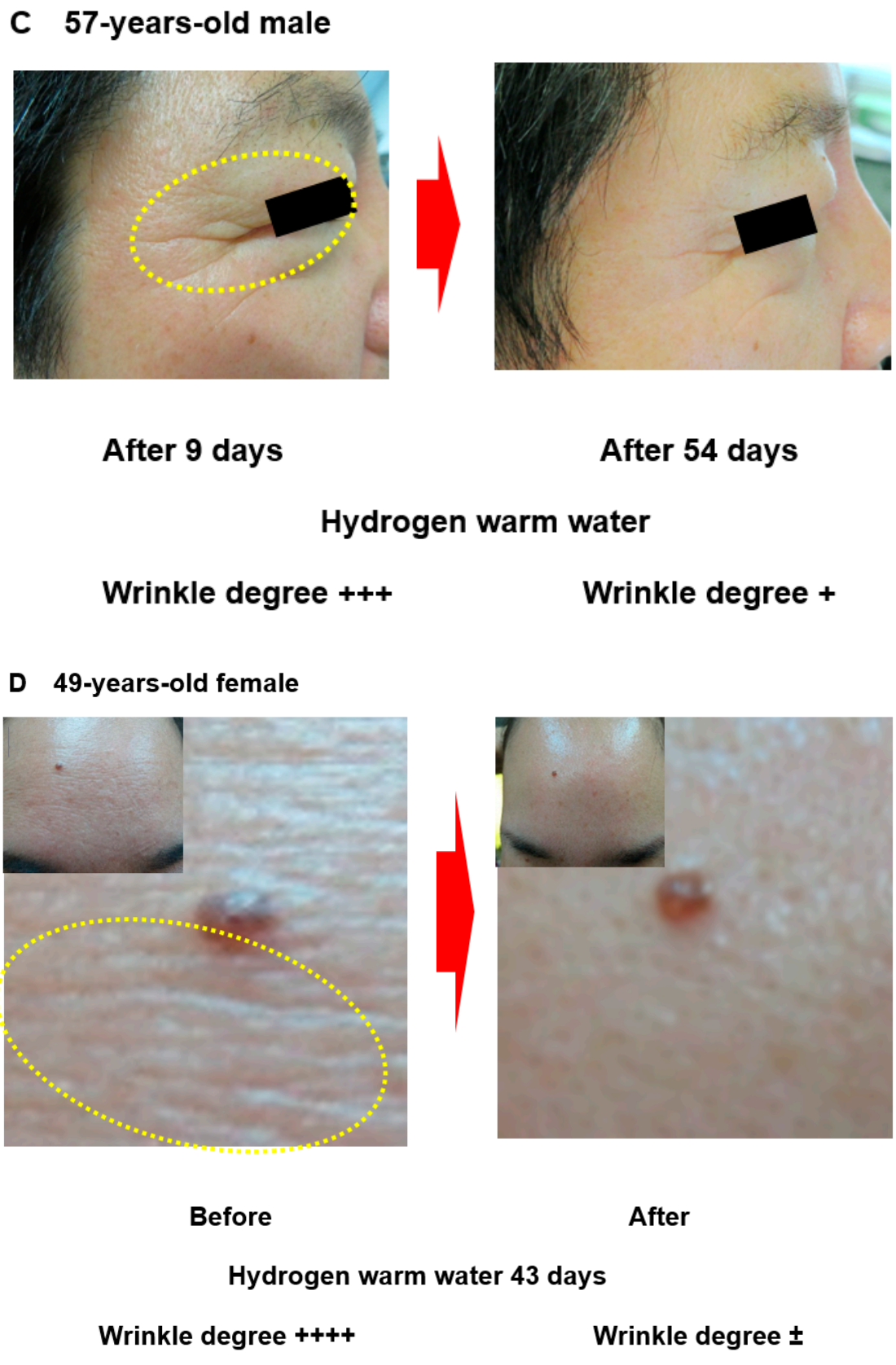


Figure 1. *Cont.*



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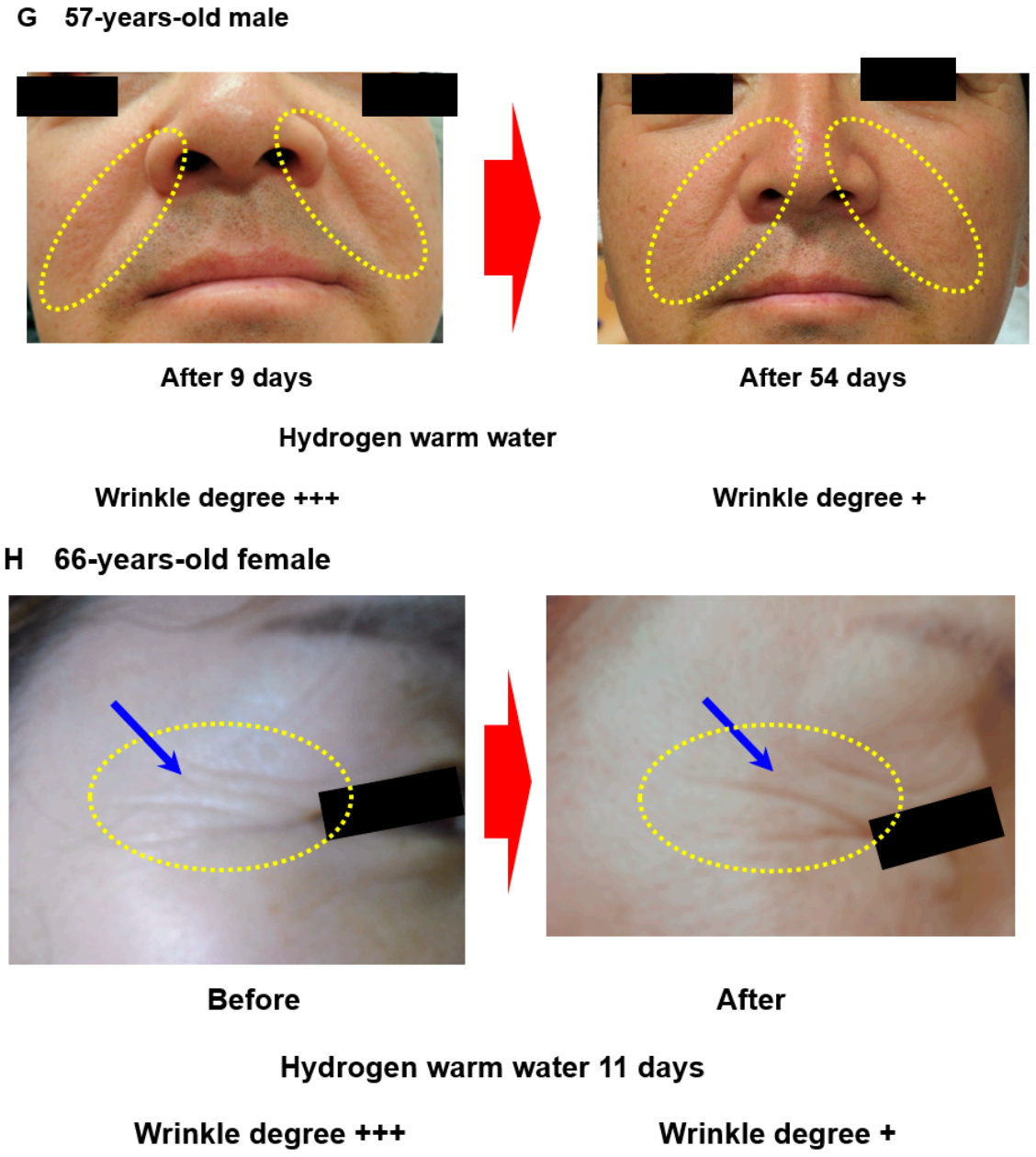
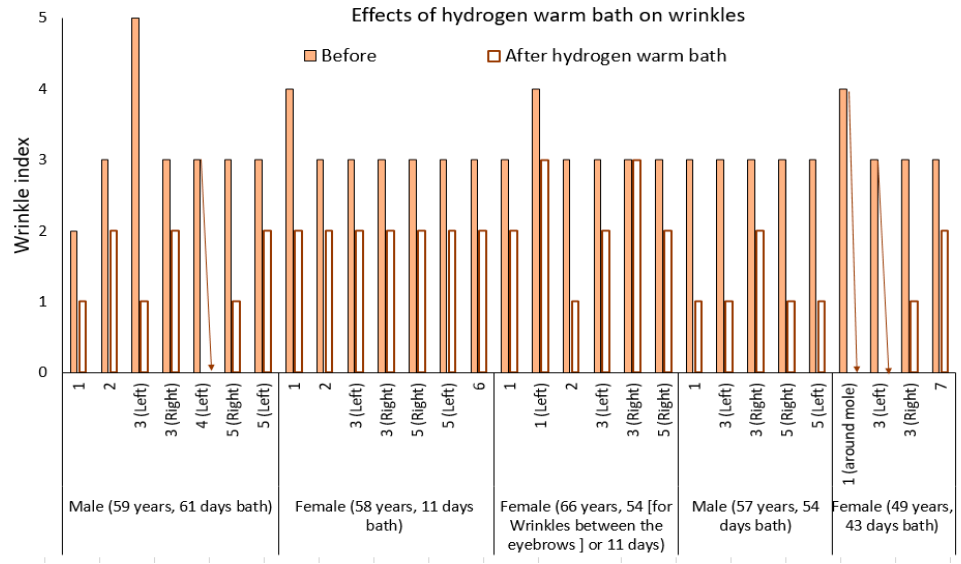


Figure 1. Improving effects on wrinkles of five subjects by 10 min bathing or repetitive three-time 3 min face poultice with hydrogen-rich warm water. Wrinkle degrees were sorted into six hierarchies (−, ±, +, ++, +++, ++++, +++++). (**A**–**C**): Reduction in small wrinkles around right-eye outer corner (indicated by a yellow dotted circle) was observed. (**D**): Reduction in wrinkles around forehead mole (indicated by a circle) was observed. (**E**): Reduction in wrinkles at left cheek (indicated by circles) was observed. (**F**): Reduction in wrinkles between the eyebrows (indicated by circles) was observed. (**G**): Improvement of wrinkles of both nasolabial folds (indicated by circles) was observed. (**H**): Before hydrogen application, among 4 wrinkles on the outer corners of the right eye (indicated by circles), the second wrinkle (indicated by an arrow) from the top was shallow and thin. However, the second wrinkle had almost disappeared, and the other three wrinkles had also become shallower and shorter.



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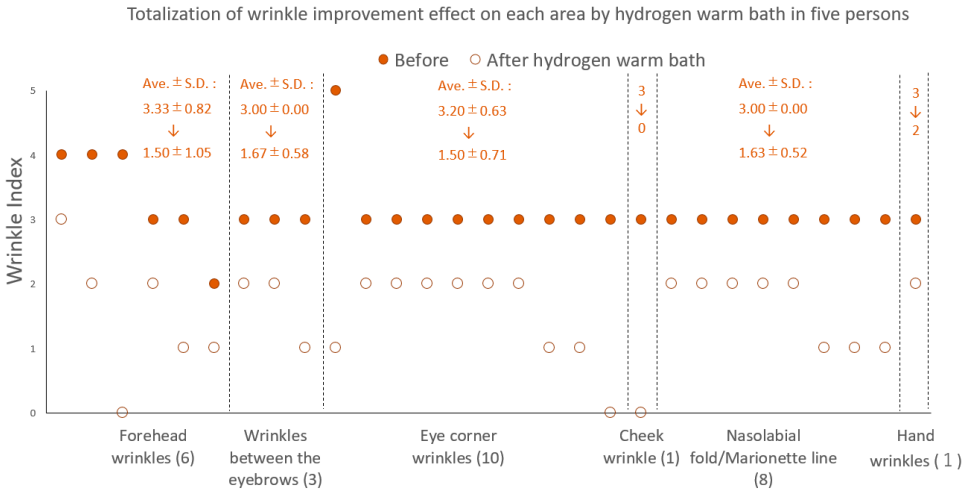

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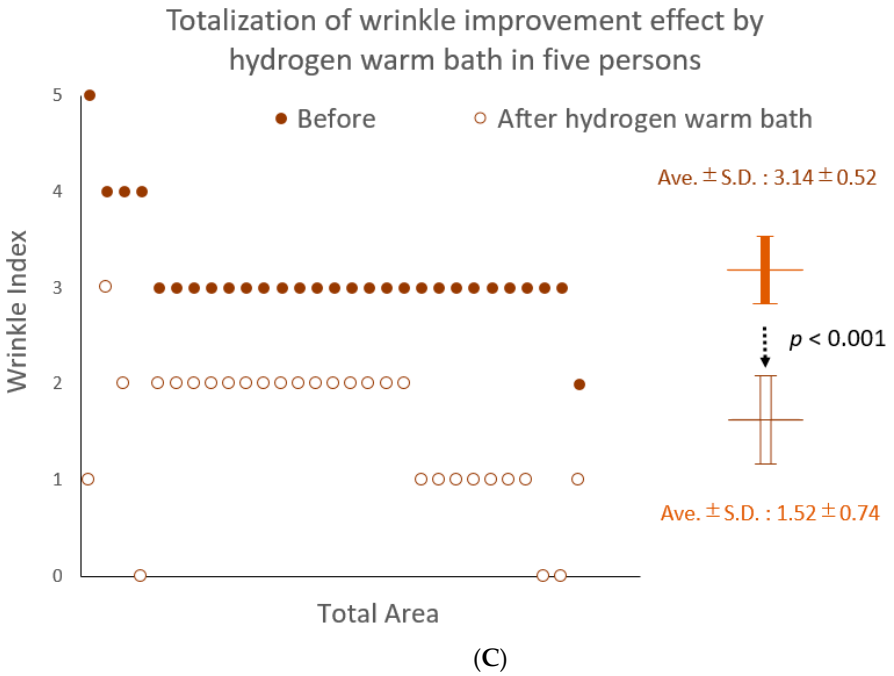


(**C**)

Figure 2. Effects of long-term daily usage of hydrogen warm-water bath and poultice on wrinkles

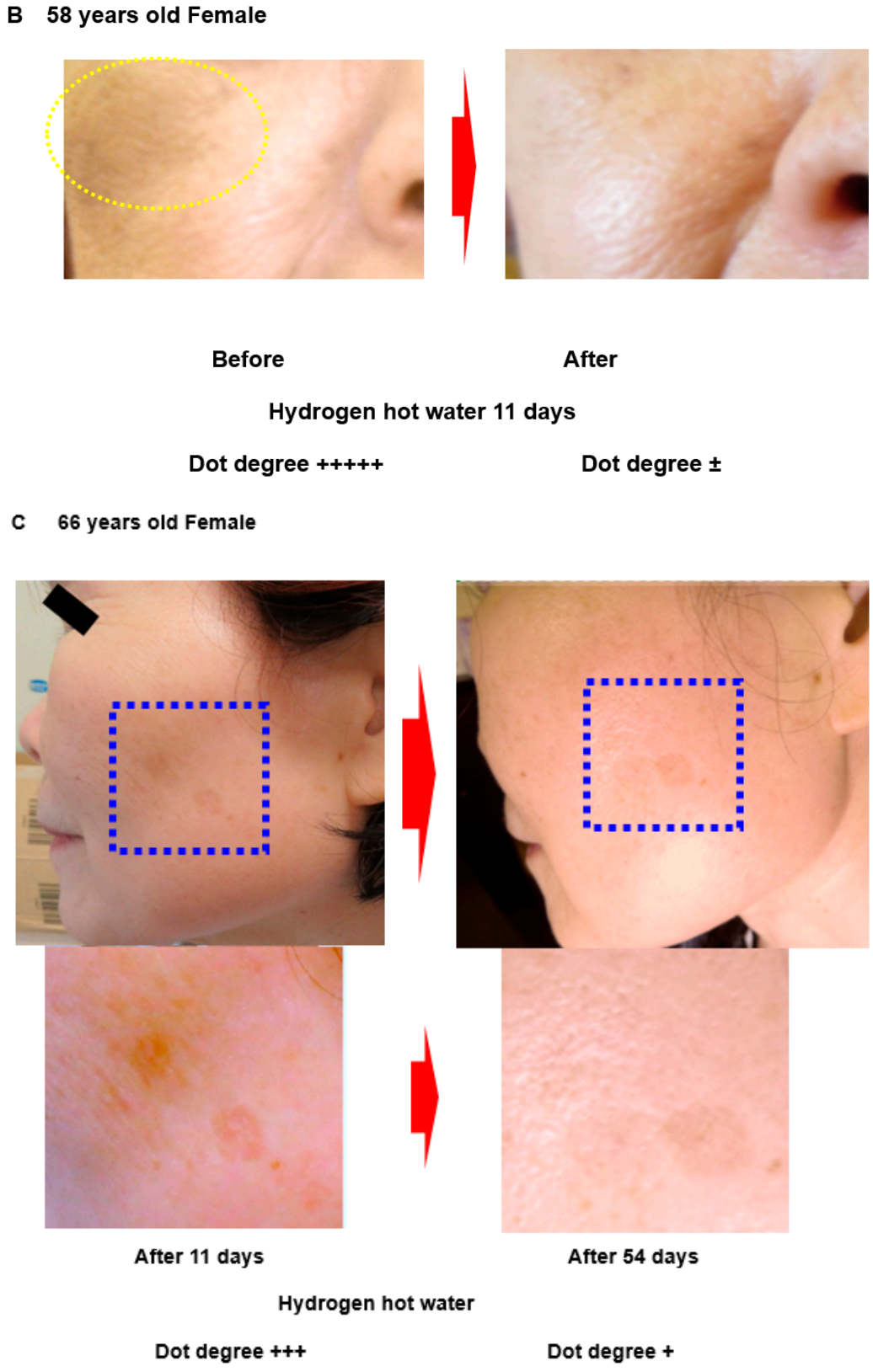
on the skin of face. (**A**): Bar graph indicating change in the degrees within six hierarchies before and after hydrogen warm bath, as divided by five subjects. Vertical axis shows the wrinkle index, while horizontal axis shows the numbering of analyzed areas for each subject, as follows; 1: at forehead, 2: between eyebrows, 3: eye outer corner, 4: cheek, 5: nasolabial folds/Marionette line, 6: hand. (**B**): Dot graph indicating change in degrees with six hierarchies before and after bathing, as divided by each region of all subjects. (**C**): Dot graph indicating change in degrees with six hierarchies before and after bathing for total loci of all subjects (n = 29). For Figure 2B,C, mean values ± SD before and after treatments are described and analyzed by the Student's *t*-test. * $p < 0.05$.

3.2. Effect of Improving Skin Blotches on Five Subjects by Bathing and Face Poultice with Hydrogen-Rich Warm Water

As in Figure 1A–H, five subjects received the bathing and face poultice with hydrogen-rich warm water (HW) for 10 min, for 11–98 consecutive days.

For every subject, several loci were found where the blotches became narrow, faint or disappeared after HW bathing and face poultice (Figure 3A–D).

Figure 3. *Cont.*



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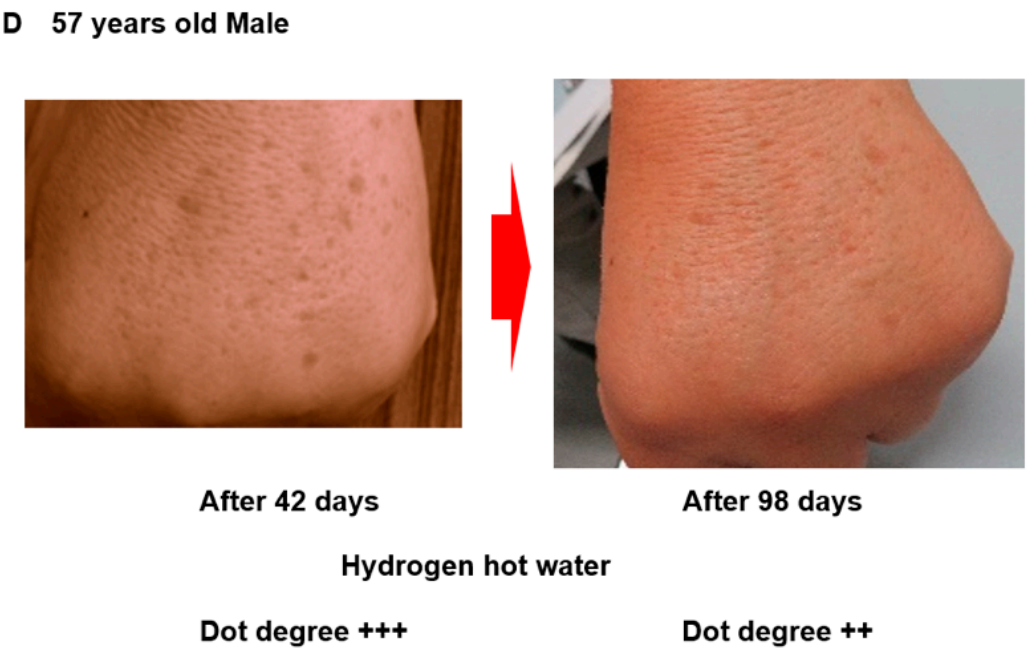
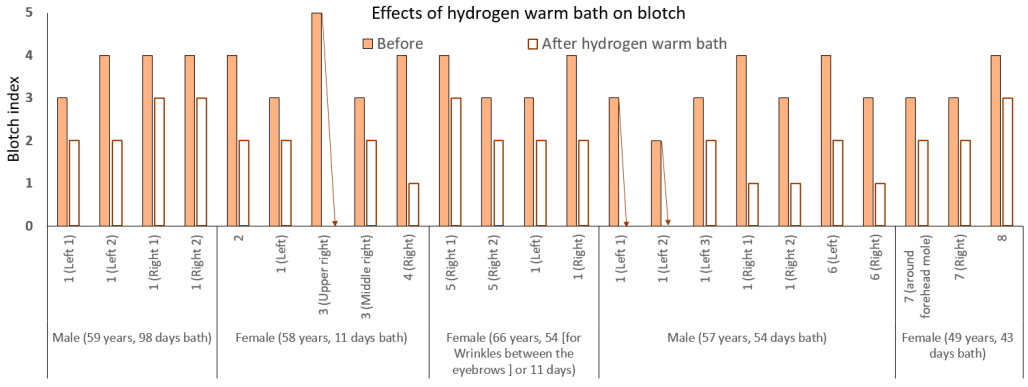


Figure 3. Improvements to blotches in five subjects by bathing and poultice with hydrogen-rich warm water. As in Figure 1A–H, five subjects received the bathing and face poultice with hydrogen-rich warm bath (HW) for 10 min, for 11–98 consecutive days. The face-blotch loci were classified to the degree of area and density of blotches into six hierarchies (−, ±, +, ++, +++, ++++, +++++). (**A**): Reduction in blotches on right cheek (original and magnified) was observed. (**B**): Reduction in a widespread blotch on the right cheek (indicated by a circle) was observed. (**C**): Reduction in blotches on left cheek (indicated by a box and magnified) was observed. (**D**): Reduction in blotches at back of the left hand was observed.

In the 66-year-old female subject, a reduction in blotches on the right cheek was observed (Figure 3A). In the 58-year-old female subject, a reduction in widespread blotches on the right cheek was observed (Figure 3B). In the 66-year-old female subject, a reduction in blotches on the left cheek was observed (Figure 3C). In the, 57-year-old male subject, a reduction in blotches at the back of the left hand was observed (Figure 3D).

Figure 4A shows changes of degrees of blotches for all observed loci of five subjects. Figure 4B shows changes of degrees for each region of five subjects. Figure 4C shows changes for total loci of five subjects. The HW application was found to bring about improvements in all 23 examined blotches, with a significant decrease in blotch degrees of 3.48 ± 0.65 to 1.74 ± 0.90 ($p < 0.001$, Figure 4C).



(**A**)

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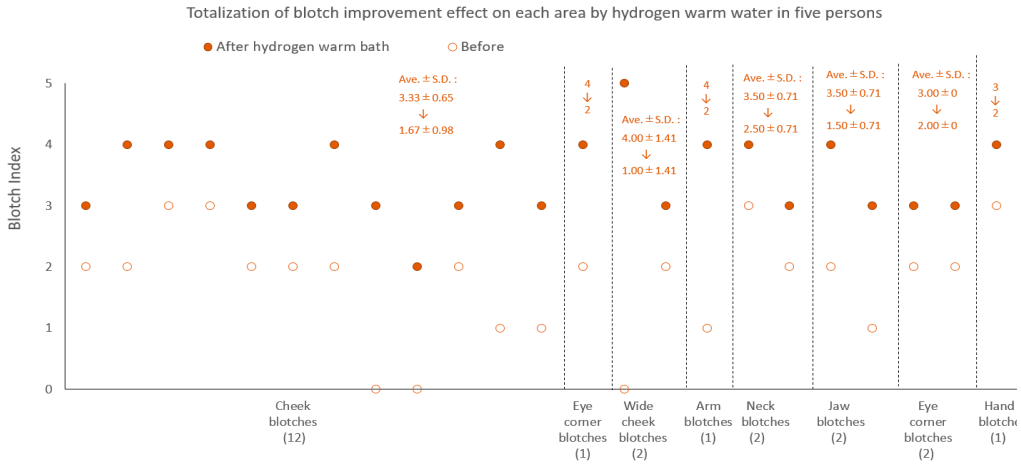

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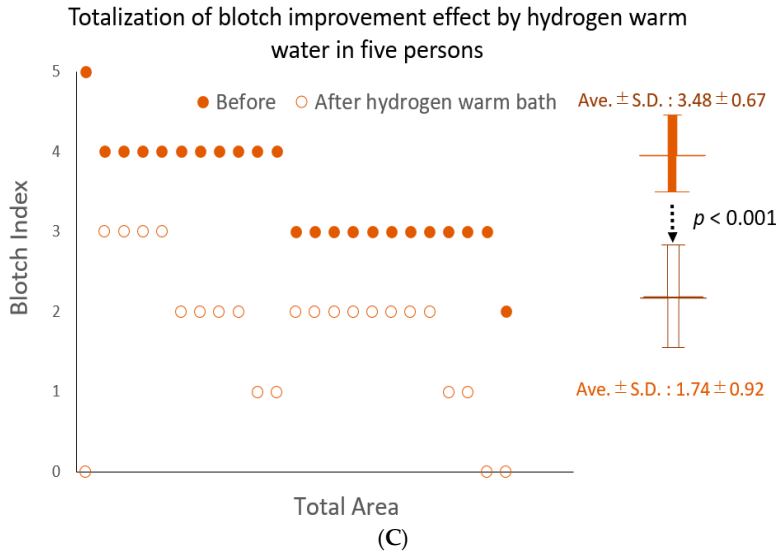
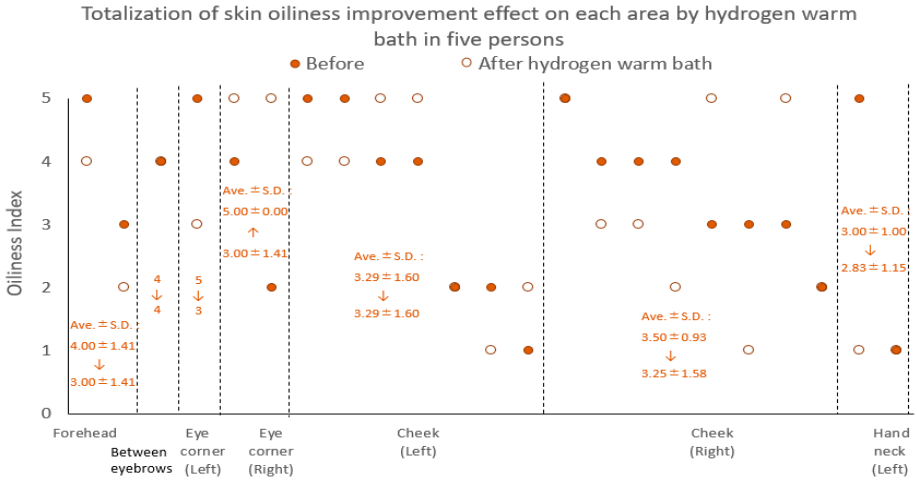


(**C**)

Figure 4. Effects of long-term daily usage of hydrogen rich warm bath and simultaneous affected-part poultice on blotches of the skin of face and hands. (**A**): Bar graph indicating change in the rank with five degrees before and after bathing, as divided by five subjects. Vertical axis shows the blotch index, while horizontal axis shows the numbering of analyzed are-as for each subject, as follows; 1: cheek blotch, 2: blotch of outer corner of the eye, 3: wide-ranged cheek blotch, 4: upper-outside-arm dispersed blotch (Right), 5: neck dispersed blotch, 6: jaw blotch, 7: eye-corner blotch, 8: hand blotch. (**B**): Dot graph indicating change in the rank with five degrees before and after bathing, as divided by each region of all subjects. (**C**): Dot graph indicating change in the rank with five degrees before and after bathing for total loci of all subjects. For Figure 4B,C, the mean values ± SD before and after treatment are described and analyzed by the Student's *t*-test. * $p < 0.05$.

3.3. Improvements to Oiliness in Five Subjects by Bathing and Face Poultice with Hydrogen-Rich Warm Water

Meanwhile, skin-oiliness contents were evaluated as a comparison between oiliness values before and after HW bathing/face poultice. Interestingly, the HW application was found to make the oil-poor skin gain oiliness, but inversely, the oily skin was altered to less-oily or moderate skin, suggesting the HW's function as an oiliness modulation, with an appreciably negative correlation ($r = -0.345$; 23 loci) of prior oiliness values versus the fluctuation for before/after HW application (Figure 5).



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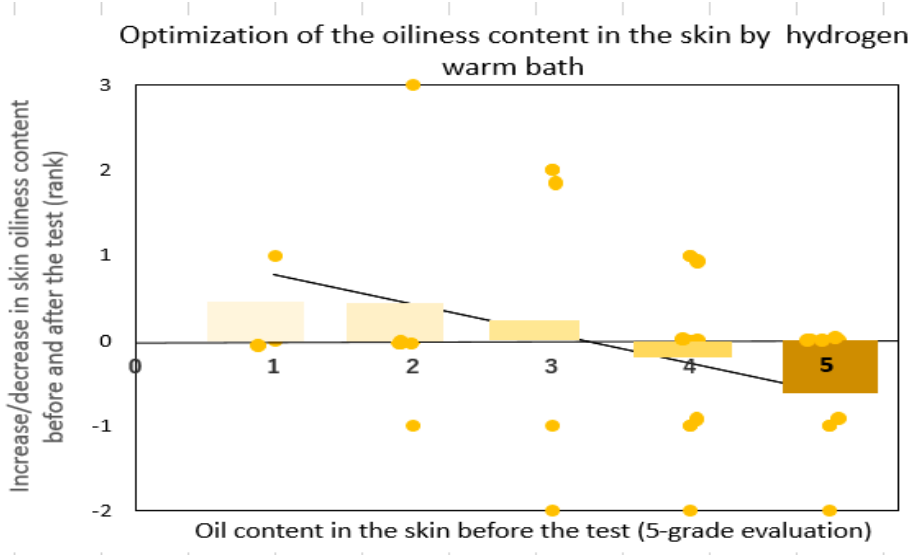
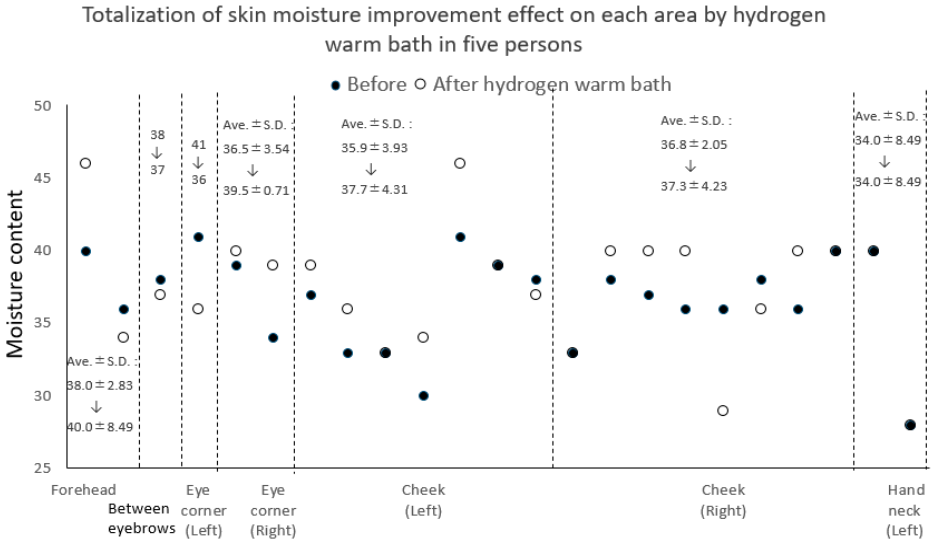


(**B**)

Figure 5. Optimization for the skin-oiliness contents by hydrogen-rich warm-water bath together with the poultice on the concerned skin parts. (**A**): Dot graph indicating change in the rank of the oiliness content with five degrees before and after bathing as divided into seven skin regions of all subjects. Vertical axis shows the oiliness index, while horizontal axis shows the numbering of analyzed areas for each subject, as follows; 1: forehead, 2: between eyebrows, 3: eye corner (left), 4: eye corner (right), 5: cheek (left), 6: cheek (right), 7: hand neck (left). (**B**): Dot graph indicating change in the rank of the oiliness content for 23 skin loci. The horizontal axis shows the degrees (0–5) before hydrogen application, while the longitudinal axis shows the change in the degrees. The negative correlation was observed between the original oiliness content and change in the rank, with a correlation coefficient: −0.345.

3.4. Improvements to Skin-Moisture Content in Five Subjects by Bathing or Face Poultice with Hydrogen-Rich Warm Water

It is suggested that the water content of skin is related to the scavenging action of hydrogen on reactive oxygen species. [7–9] Thus, the effect of hydrogen-rich water treatment on the amount of water content of the face was examined. HW application was found to make dry skins (moistures: 27–35%, 35–45.5%) moist (moisture gains: 2.0%, 0.6%, respectively) concurrently, with scarce changes for the wet skin (moisture: above 40.5%), with a negative correlation ($r = -0.090$; 23 loci) of prior moisture contents versus the fluctuation in before/after HW application (Figure 6).



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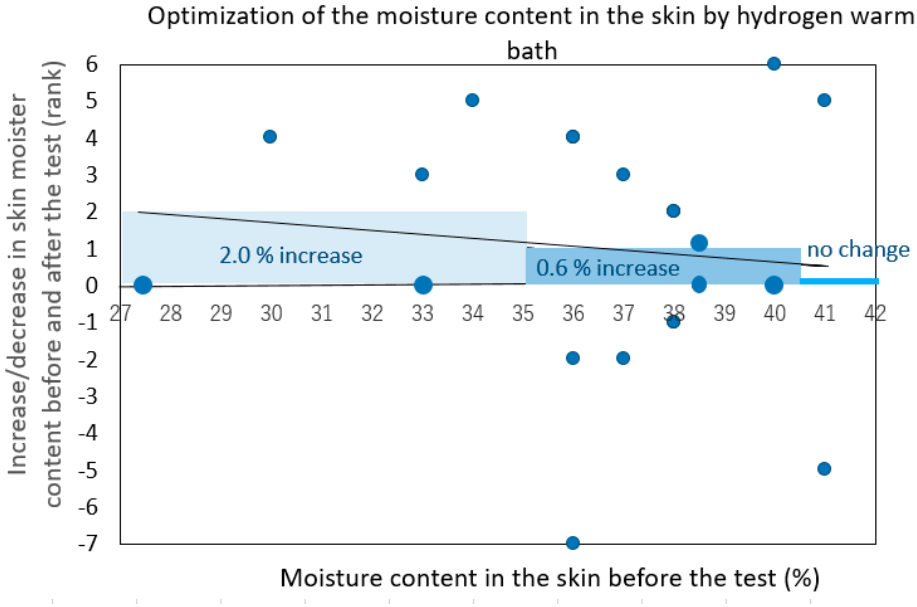

(**B**)

Figure 6. Hydrogen-rich warm-water bath and poultice increased the amount of moisture in dry skin. (**A**): Dot graph indicating change in the rank of the water content with five degrees before and after bathing, as divided by seven skin regions of all subjects. (**B**): Dot graph indicating change in the percentage of the water content for 23 loci of the face and hand. The horizontal axis shows the water content (%) before hydrogen application, while the longitudinal axis shows the change in the water content. The negative correlation was observed between the original water content and change in the water content, with a correlation coefficient: −0.090.

4. Discussion

The examination periods were flexibly set for each subject for as long it was possible to be permitted, in the viewpoint of subjects' individual life styles, for improvements of wrinkles and blotches, although it is difficult to secure sufficiently long-term cooperation among subjects; thus, the limitation of this study is to be terminated in a case study.

Various anti-aging agents were used, such as an inhibitor for skin wrinkles [6–9,14,20–26], an inhibitor for skin blotch [6,9,12,22,24–27], a conditioner for sebum [1,11] and agents with moisturizing activity [7,8,10,15,20,22,25–28]. Most of them have antioxidant effects.

As compared to other diverse antioxidants, the ROS-scavenging effects of hydrogen by itself may be much weaker or moderate. However, because certain levels of ROS are crucial as triggering or relaying signals for some biological processes, excessively strong antioxidants could occasionally have some undesired effects by blocking the ROS-involved cellular-signaling pathway. Hydrogen-dissolved water has been found to be a safe alternative and has few side effects [13]. The effects might be achieved by compression of the HW poultice on concerned skin parts, in addition to hydrogen permeation into skin depth, as previously suggested by elevation of blood antioxidant ability caused by the HW bath, as shown by our previous study [13]. By taking an HW bath and HW skin poultice, hydrogen nanobubbles could rapidly infiltrate into the stratum corneum, reach the epidermis and dermis, and be absorbed into the blood stream. Repeated HW bathing has been clinically demonstrated to repress the inflammatory blood-marker CRP (C-reactive protein) value, which is majorly synthesized in and secreted from the liver, in contrast to no significant CRP value change for normal warm water, suggesting a possibility for the penetration and widespread distribution of hydrogen molecules into the whole liver [13]. In contrast to no effect for a normal tap-water warm bath, the hydrogen-rich warm-water bath was demonstrated previously to promote the ORAC (oxygen radical absorbing capacity)-based antioxidative activity in the blood [13], which might improve skin conditions including wrinkle/blotch prevention and moisture/oiliness modulation, all of which have been numerically and visually verified in the present clinical study. Hydrogen molecules can be distributed in the blood and tissues such as the skin of an organism. Once the hydrogen molecules reach the blood stream, they scavenge ROS and protect the cells and tissues against inflammation-/oxidative-stress-induced damages [13]. Therefore, the hydrogen-rich water bath was used to deliver hydrogen molecules to subjects in the present study. Comparatively, in the present study, our data showed that hydrogen-rich water brought out repetitive effects on human skin.

Repression by HW treatments on wrinkles (Figures 1 and 2) might ensue from combinative effects of HW-bath-induced systemic-circulation-based hydrogen-distribution [13] and HW-poultice-induced local permeation-based hydrogen distribution. Wrinkles in human skin are considered to depend on the ability of collagens to moisturize or retain dermal structural robustness. Collagens are essential components that support the strength and flexibility of the skin structure. Studies have shown that wrinkles in human skin depend on either the deteriorated moisturizing ability of collagen or its reduced expression and the resultant intra-/intermolecular cross-linkage [3,7,8,10,21,22]. In human artificial three-dimensional skin, type I collagen is known to be widely distributed inside the dermis [10,19,23,25]; meanwhile, type IV collagen is localized inside the basement membrane that connects the dermis and the epidermis [22,29]. The "basement membrane" forms a "scaffold" for the growth of keratinocytes that occupy most of the epidermis, and type IV collagen creates a lattice-like structure that becomes the basement membrane.

The ROS-scavenging ability of nanobubbles as abundant as 9.02×10^7/mL (as previously evaluated by NanoSight method [13]) in a hydrogen-rich water bath helps in the lightening and size reduction of the dark dermal spots and stains, improving skin-whitening quality [12]. The present study also revealed that the hydrogen-rich water bath confers anti-aging effects on human skin blotches (Figures 3 and 4). The blotch-repressive effects of the HW bath/poultice might be attributed to both the abilities of hydrogen to reductively bleach the existent blotches and to extracellularly expel the melanin-containing old epidermal keratinocytes and old stratum corneum through cell-proliferation promotion. Hydrogen molecules are assumed to penetrate from the surface to the target pigment-cell layer, through the minimum molecular mass combined with neutral electrostatic property, and alleviate ROS in the target skin part, suppressing skin melanin, as presumed by the fact that nanospheres easily penetrate into the skin [30]. It was presumed that, the skin epidermal layers containing melanin and lipofuscin, which cause blotching, were expelled out of the skin surface, such as dirt through hydrogen-activated keratinocyte proliferation, and/or were reductively bleached by the whitening power of hydrogen [12,31].

Hydrogen application inclined to modify oil-deficient skin into oily skin, and, inversely, excessively oily skin into less-oily or intermediate skin, bringing out the appropriate modulation for skin-oiliness degrees (Figure 5). This finding (oiliness) means that HW application optimizes skin-oiliness degrees, through either hydrogen functions for a possible modulation to the sebum-secreting glands or hydrogen adjusting to adipogenesis by subcutaneous adipocytes, as previously demonstrated using three-dimensional human skin equivalents [32].

Hydrogen-application-induced heightening of skin-moisture contents (Figure 6) is considered to be caused by improvements of skin-moisture-retaining extracellular matrices such as type I/IV collagens, which are demonstrated to be increased by administration of hydrogen-generating silica microparticles to three-dimensional human skin-tissue equivalents [31,32].

Thus, the HW bath in combination with the HW poultice on concerned skin parts exerts diverse beneficial effects on cosmetological aspects of human skin appearances such as wrinkles, blotches and oiliness/moisture. In the future, the issue is whether to promote the expression of stress-related genes such as COX-2, p53, Nfr2, MAPK signaling by hydrogen water, as well as other antioxidants [15,20,21]. Similar effects of the hydrogen-rich water bath could be considered for other tissues and blood/capillaries, helping the systemic improvements of health.

5. Conclusions

Bathing and skin poultice with hydrogen-rich warm water (HW) for 11–98 days improved both wrinkle degrees and blotch degrees in five healthy subjects (49–66 years old). Meanwhile, HW functioned as skin-oiliness modulation, and compensated moisture for the water-deficient skins. Thus, the HW bath together with HW poultice exerted beneficial effects on skin appearances such as wrinkles, blotches and oiliness/moisture, some of which might ensue from enhanced antioxidant ability in blood.

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Institutional Review Board Statement: This research was officially approved in 2016 with an approval number of 15C01, under conditions of noninvasive research such as no hemorrhage, painless treatment, and no trace/sign, using a widespread commercially available apparatus, by the Medical Ethics Committee of the Japanese Center for Anti-Aging Medical Sciences, which was officially authenticated by the Hiroshima Prefecture Government of Japan.

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

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