Applied Cell Biology

ISSN : 2320-1991 Online
ISSN : 2320-1983 Print
Volume 13 Issue 1
DOI: https://dx.doi.org/10.53043/2320-1991.acb13002

Research Article
Appl Cell Biol, 12(1), 2025 [8-13]
Published: February 07, 2024

The Benefits of Hydrogen-Enriched Water - Studies on Cultured Kidney Epithelial Cells

Peter C. Dartsch*

Dartsch Scientific GmbH, Institute for Cell Biological Test Systems, Oberer Anger 1, D - 86911 Dießen am Ammersee, Germany

***Corresponding author:** Peter C. Dartsch, Dartsch Scientific GmbH, Institute for Cell Biological Test Systems, Oberer Anger 1, D - 86911 Dießen am Ammersee, Germany

ABSTRACT

Background: Molecular hydrogen has not yet been widely used and accepted in conventional medicine. However, recent findings indicate that hydrogen has a variety of pharmacological effects which have a positive impact on various diseases. Among them are kidney diseases. In the present study, cell biological test methods were used to investigate whether hydrogen-enriched water possesses beneficial effects at the cellular level in direct comparison to the same tap water without treatment.

Experimental: Hydrogen-enriched water was produced by two different devices from misterwater GmbH, Germany, from initial local tap water in accordance with the operating instructions. Investigations of the resulting hydrogen-enriched water were carried out immediately after hydrogen generation. The same tap water without treatment with the hydrogen-producing devices was taken as control. For the experiments, a well established kidney epithelial cell line (MDCK; Madin-Darby Canine Kidney Cells) was used. At least three independent experiments for each test parameter were conducted. Statistical analysis of the data was done using the two-tailed Wilcoxon-Mann-Whitney rank-sum test.

Results: The use of the freshly prepared hydrogen-enriched water resulted in a statistically significant stimulation of the cell metabolism of kidney epithelial cells ($p \leq 0.01$) compared to the initial tap water. The most significant stimulation of 21.4 ± 7.4% was achieved at a volume fraction of 20 vol% (mean value ± standard deviation). Even higher concentrations of hydrogen-enriched water showed a significant increase by about 16%. In comparison to the initial tap water, cell regeneration was stimulated by the hydrogen-enriched water by 31.5 ± 4.7% (20 vol%) and 28.9 ± 4.5% (40 vol%). This improvement in cell regeneration was statistically significant in all experiments ($p \leq 0.01$). In the experiments with exogenously induced oxidative stress by reactive oxygen species, the difference in cell viability was improved for hydrogen-enriched water vs. initial tap water at all hydrogen peroxide concentrations tested and was significant for 2 and 5 mM hydrogen peroxide ($p \leq 0.01$). This protective effect of hydrogen-enriched water was also clearly visible morphologically.

Conclusions: The regular drinking of freshly prepared hydrogen-enriched water produced by the devices of mister water GmbH are able for improving cell metabolism of kidney cells, stimulating the regeneration process in the case of cell damage and giving more cell protection in the case of oxidative stress. Therefore, hydrogen-enriched water might also possess a beneficial impact on human well-being and the attitude to life.

Keywords

Kidney epithelial cells
MDCK
Hydrogen-enriched water
Cell viability
Cell metabolism
Regeneration
Oxidative stress
Reactive oxygen species
Cell culture

Research Article

INTRODUCTION

Molecular hydrogen has not yet been widely used and accepted in conventional medicine. However, recent findings indicate that hydrogen has a variety of pharmacological effects such as antioxidant, anti-inflammatory or anti-apoptotic properties [1]. Meanwhile, an increasing number of studies have been conducted on the use of molecular hydrogen for various diseases. Molecular hydrogen has several advantages: It penetrates rapidly into tissues and cells and does not interfere with metabolic redox reactions or reactive oxygen species (ROS), which play an important role in cellular signaling in the body [2]. Therefore, the use of molecular hydrogen should have no adverse effects, as demonstrated in a study of mutagenicity, genotoxicity, and subchronic oral toxicity up to a daily intake of 20 ml of hydrogen-enriched water per kilogram of body weight [3].

There are several methods of ingesting hydrogen, such as inhaling hydrogen gas or drinking hydrogen-enriched water. Water has the advantage that it is very easy to prepare and use and hydrogen can be dissolved to a concentration of 1.6 ppm under normal atmospheric pressure at room temperature. Contrary to initial expectations, the drinking of hydrogen-enriched water was found to have similar effects to inhaling hydrogen gas [2].

The main function of the kidneys is to remove toxins and metabolic waste products from the blood and to regulate the body's salt and water balance. This is done by excreting urine. The kidney tubules are involved in filtering the blood to remove waste products and are lined on the inside with a layer of epithelial cells. Oxidative stress can cause permanent damage to the kidneys and kidney epithelial cells, so the use of hydrogen offers both a preventive and therapeutic option [1,4-6].

In the present study, cell biological test methods were used to investigate whether hydrogen-enriched water has beneficial effects at the cellular level in direct comparison to the same initial local tap water without treatment. Due to the positive effects described by users, the investigations in this study were carried out exclusively with cultured kidney epithelial cells.

MATERIALS AND METHODS

Preparation and use of hydrogen-enriched water

Two different devices from misterwater GmbH, D-85540 Haar OT Salmdorf, Germany, were provided for the duration of the tests and used with local tap water in accordance with the operating instructions. Investigations of the resulting hydrogen-enriched water were carried out immediately afterwards, as the generated and dissolved hydrogen is very short-lived. By sealing the cell culture dishes, precautions were also taken to allow the hydrogen to act on the cell cultures as long as possible. As a control, the same initial tap water without treatment with the hydrogen devices was used. To avoid osmotically induced cell alterations and cell volume regulations, the initial water and the hydrogen-enriched water were added to the culture medium or reaction mixture up to a maximum volume fraction of 40 vol%.

Kidney epithelial cells

The parent strain of Madin-Darby Canine Kidney (MDCK) cells was used for the studies [7]. This is a mammalian kidney epithelial cell line that was isolated from the kidney tubule of an adult female dog in 1958 [8] and is now used in biomedical research for a variety of cell biological studies such as protein transport, cell polarity and cell contacts. The cell line is also used to study the transport of drugs into and through cells [9]. The importance of this cell line is documented by more than 100,000 publications in the scientific literature.

The cells were purchased in cryopreserved state from Sigma-Aldrich/ECACC (Deisenhofen, Germany) and routinely cultured after thawing in Dulbecco's modified Eagle's medium with 10% growth mixture and 0.5% gentamycin in a gassed incubator at 37°C in an atmosphere of 5% CO_2 and 95% air at more than 90% humidity. Cells were routinely subcultured twice a week and taken for the studies after some passages. Experiments were carried out over a period of almost 6 months.

Basal cell metabolism

For the experiments, cells from 80-90% confluent mass cultures were seeded at a density of 50,000 cells/well in 96-well culture plates (200 µl culture medium/well) and incubated for 48 hours until complete attachment and spreading of the cells as well as a normal cell metabolism was achieved. A reaction mixture consisting of phosphate buffer saline with calcium and magnesium and 5 mM glucose as the energy source, the appropriate test concentrations of the freshly prepared hydrogen-enriched water or initial local tap water and the water-soluble tetrazolium dye WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetra-zolio]-1,3-benzene disulfonate; Sigma-Aldrich, Deisenhofen, Germany) was added to the cells. The cleavage of the dye and its resulting color change is directly related to the mitochondrial energy metabolism and cell vitality [10-12]. The color change was recorded as a differential measurement at $\Delta OD = 450 - 690$ nm with the Elisareader (BioTek Elx 808 with software Gen 5 version 3.00) at definite time points up to a maximum of 120 min and was analyzed using Microsoft Excel. Four independent test series ($n = 4$) with duplicate wells were performed.

Cell regeneration

In this model, the granulation phase, which is characterized by the occurrence of migration and proliferation of cells for closing a defect after injury [13-16], was simulated. Cells were seeded at a density of 100,000 cells/ml into the individual compartments of a silicone 4 well-culture insert (ibidi, Gräfelfing, Germany). The single compartments of the inserts are separated by a 500 µm thick silicone bar with an outer silicone frame of 700 µm. Due to the special adhesion area, a silicone insert adheres firmly to the bottom of a culture dish and forms a distinct cell-free area (= artificial wound), which the cells can colonize by migration and proliferation.

Upon reaching confluency within 48 hours after cell seeding, the silicone inserts were removed with tweezers to achieve a sharp edge of the cell-free area between the compartments. 20 vol% and 40 vol% of hydrogen-enriched water or initial local tap water were added to the cells in separated dishes. Kidney epithelial cells were allowed to migrate and proliferate for 12 hours. Finally, cell cultures were fixed with 100% methanol, stained with Giemsa's azur eosin methylene blue solution (Merck, Darmstadt, Germany) and air-dried. The colonized area was examined under the microscope at 6 different points and calculated by a specialized software with artificial intelligence from KML Vision, Graz, Austria (IKOSA AI software). Three independent experimental series (n = 3) were performed.

Cell viability after exogenously induced oxidative stress

Our established hydrogen peroxide-induced oxidative stress (HP-IOS) test system was used for these studies. From a 3% hydrogen peroxide solution (= 880 mM), 10-fold concentrated hydrogen peroxide stock solutions of the later cell culture test concentrations were prepared by further dilution with phosphate buffered saline. Kidney epithelial cells were seeded at a density of 100,000 cells/well in 24-well plates and were allowed to attach, spread and stabilize their metabolism for 48 hours until a confluency of about 90% was achieved.

Then, cells were incubated with various concentrations of hydrogen peroxide (0 to 2 mM) and a volume fraction of 20 vol% of either hydrogen-enriched water or initial local tap water as control for another 24 hours. Finally, the activity of the surviving cells was measured by a redox color reaction after the addition of XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide; Xenometrix, Allschwil, Switzerland) with an Elisareader (BioTek ELx 808 with software Gen 5 version 3.00) as a differential measurement at ΔOD = 450 – 690 nm at definite time points up to 120 min. Three independent test series (n = 3) were performed.

STATISTICAL ANALYSIS

Statistical analysis was done using the parameter-free two-tailed Wilcoxon-Mann-Whitney rank-sum test.

RESULTS AND DISCUSSION

In four independent experiments, the use of the freshly prepared hydrogen-enriched water resulted in a statistically significant stimulation of the cell metabolism of kidney epithelial cells ($p \leq 0.01$) compared to the initial tap water in the reaction mixture (Figure 1). The most significant stimulation of 21.4 ± 7.4% was achieved at a volume fraction of 20 vol% (mean value ± standard deviation). Even higher concentrations of hydrogen-enriched water showed a significant increase of 15.6 ± 6.3% at a volume fraction of 30 vol% and 16.7 ± 5.4% at a volume fraction of 40 vol% (mean values ± standard deviations).

The microscopic examination of the cell cultures for the examination of the regeneration process showed a clear promotion of the colonization of the cell-free space by exposure to the freshly prepared hydrogen-enriched water compared to the initial tap water (Figure 2). Both tested volume fractions of hydrogen-enriched water (20 vol% and 40 vol%) stimulated the regeneration process by 8.4 ± 6.5% (20 vol%) and 14.3 ± 3.3% (40 vol%) compared to basic cell culture medium (not depicted). This means that even the dilution of nutrients in the culture medium by the addition of hydrogen-enriched water was more effective than the culture medium itself. In addition, the initial tap water caused a reduction in cell regeneration in all experiments, so that the hydrogen-enriched water

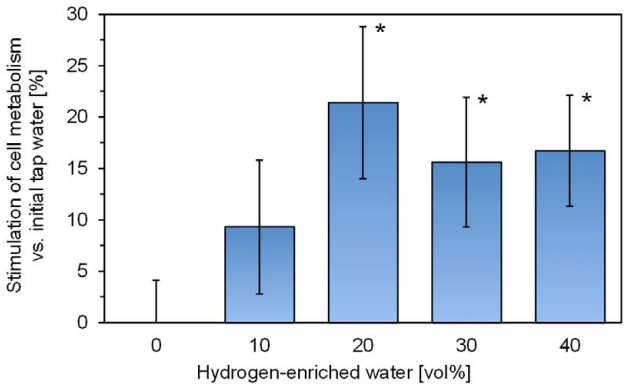

Figure 1: Graphical presentation of the relative stimulation of the basal cell metabolism of kidney epithelial cells by hydrogen-enriched water compared to the initial local tap water at different volume fractions in the reaction mixture. Data show mean ± standard deviation of four independent experiments. (*) indicates statistical significance at $p \leq 0.01$ (two-tailed Wilcoxon-Mann-Whitney rank-sum test).

Research Article

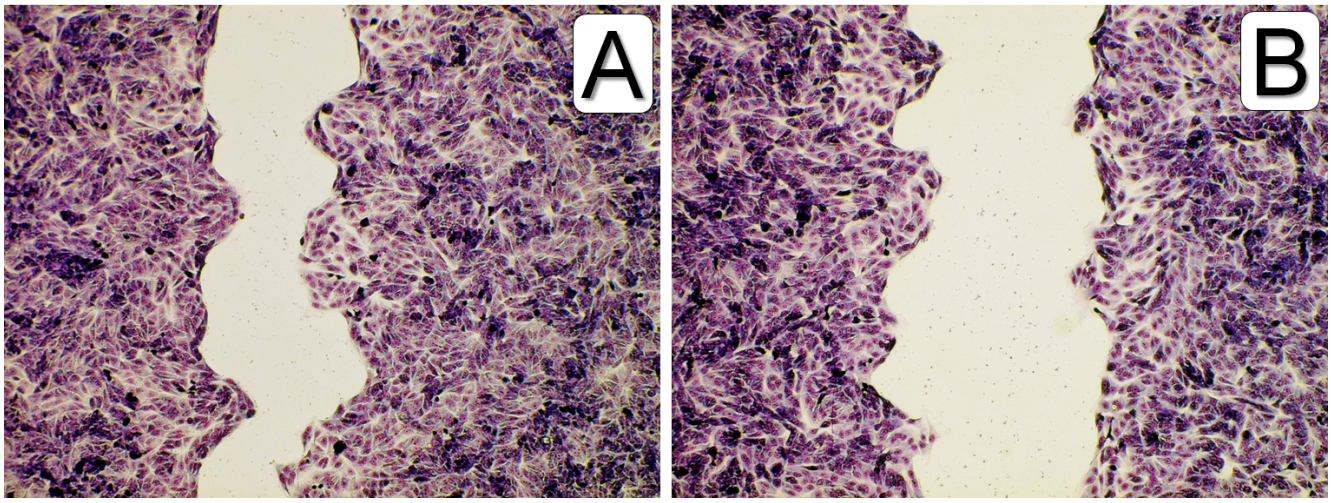

Figure 2: Microscopic visualization of the beneficial effect of hydrogen-enriched water on cell regeneration of cultured kidney epithelial cells within 12 hours. (A) Relatively decreased cell-free space and thus better cell regeneration by using 40 vol% of hydrogen-enriched water in the culture medium. (B) Relatively increased cell-free space due a reduced cell regeneration by using 40 vol% initial local tap water in the culture medium. Fixed and stained samples examined with an Olympus IX-50 inverted microscope equipped with a 10x planachromate lens and an Olympus E-10 digital camera at 4 megapixel resolution using brightfield illumination.

always performed 31.5 ± 4.7% (20 vol%) and 28.9 ± 4.5% (40 vol%) better in direct comparison. This improvement in cell regeneration by hydrogen-enriched water compared to initial local tap water was statistically significant in all experiments ($p \leq 0.01$).

When examining the effect of exogenously induced oxidative stress on the resulting cell viability, we found that the viability decreased with increasing concentrations of hydrogen peroxide compared with cells cultured without the addition of reactive oxygen species. However, cell viability with hydrogen-enriched water was always better when compared to initial tap water (Figure 3). The difference was between 12% and 17% for an improved cell viability for hydrogen-enriched water vs. initial tap water at a concentration of 0.25 to 2 mM hydrogen peroxide and even 34.0 ± 5.2% for 5 mM hydrogen peroxide (mean value ± standard deviation). The difference was statistically significant ($p \leq 0.01$) for 2 and 5 mM hydrogen peroxide. This protective effect of hydrogen-enriched water was also clearly visible morphologically (Figure 4).

As shown here with cultured kidney epithelial cells, the freshly produced hydrogen-enriched water from misterwater GmbH proved its beneficial effects in comparison to the initial local tap water. In direct comparison to the untreated initial tap water, the hydrogen-enriched water was able to improve the metabolism and regeneration of cultivated kidney epithelial cells significantly as well as the inactivation of endogenously formed radicals at the cellular level. This is in accordance with

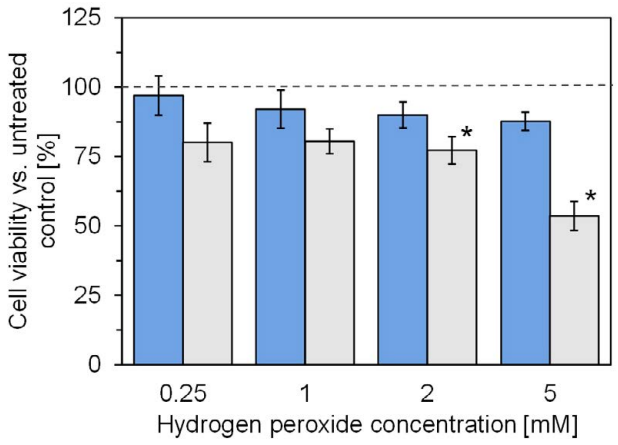

Figure 3: Graphical presentation of the results of the cell-protective effect of hydrogen-enriched water (blue bars) compared to the initial local tap water (grey bars) compared with pure culture medium without the addition of exogenous reactive oxygen species. The culture medium is set as 100% viability. The hydrogen-enriched water always has a better protective effect at all hydrogen peroxide concentrations. Data represent mean ± standard deviation of three independent experiments.

findings of other scientists which have shown that molecular hydrogen or hydrogen-enriched water has serious medical applications and health benefits for kidney diseases [1,5,6,17-20]. Thus, regular drinking of freshly prepared hydrogen-

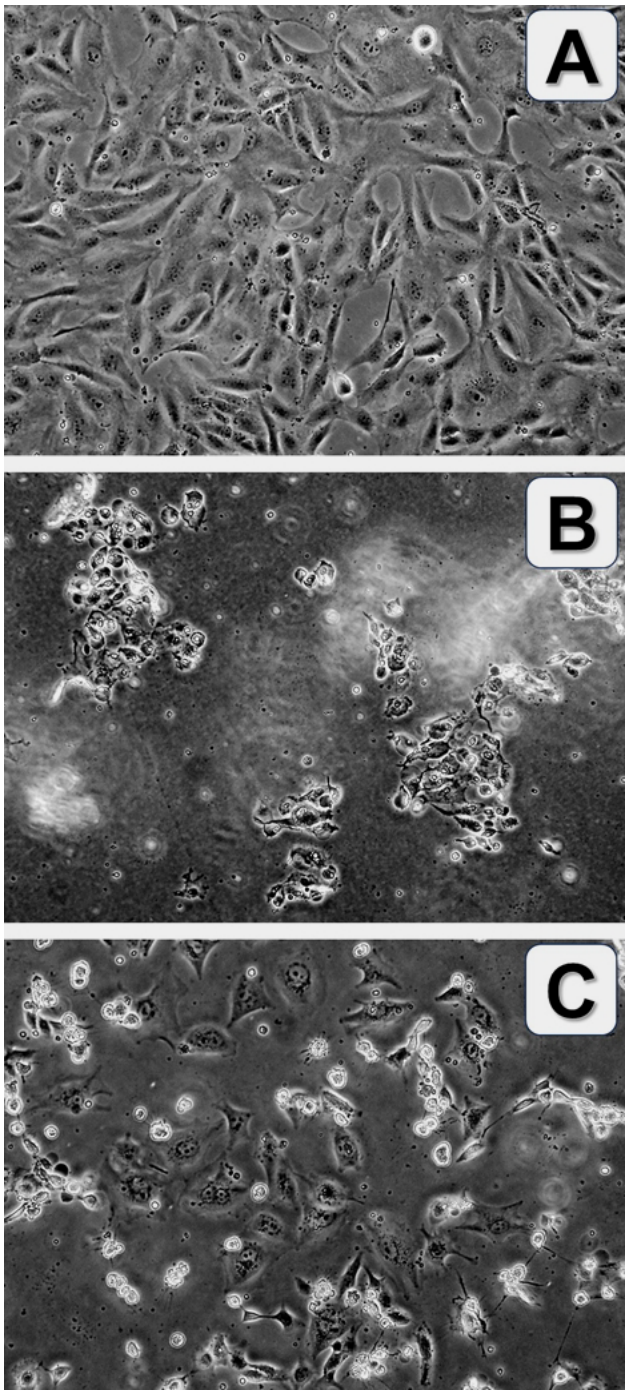

Figure 4: Microscopic visualization of the beneficial effect of hydrogen-enriched water on kidney cell viability after oxidative stress within 24 hours. (A) Control culture without oxidative stress. (B) Rounded, detached and floating cells with decreased viability after treatment with 2 mM hydrogen peroxide with a volume fraction of 20 vol% initial local tap water in the culture medium. (C) Smaller quantity of rounded and detached cells with still adherent cells and a better viability after treatment with 2 mM hydrogen peroxide with a volume fraction of 20 vol% hydrogen-enriched water in the culture medium. Olympus IX-50 inverted microscope equipped with a 10x planachromate lens and an Olympus E-10 digital camera at 4 megapixel resolution using phase-contrast illumination.

enriched water produced by the devices of misterwater GmbH are able for improving cell metabolism of kidney cells, stimulating the regeneration process in the case of cell damage and giving more cell protection in the case of oxidative stress. Therefore, hydrogen-enriched water might also possess a beneficial impact on human well-being and the attitude to life.

REFERENCES

1. Wang B, Li Z, Mao L, Zhao M, Yang B, et al. (2022) Hydrogen: A novel treatment strategy in kidney disease. Kidney Disease 8: 126-136.
2. Ohta S (2011) Recent progress toward hydrogen medicine: Potential of molecular hydrogen for preventive and therapeutic applications. Current Pharmaceutical Design 17: 2241-2252.
3. Saitoh Y, Harata Y, Mizuhashi F, Nakajima M, Miwa N (2010) Biological safety of neutral-pH hydrogen-enriched electrolyzed water upon mutagenicity, genotoxicity and subchronic oral toxicity. Toxicology and Industrial Health 26: 203-216.
4. Small DM, Coombes JS, Bennett N, Johnson DW, Gobe GC (2012) Oxidative stress, anti-oxidant therapies and chronic kidney disease. Nephrology 17: 311-321.
5. Guo SX, Fang Q, You CG, Jin YY, Wang XG, et al (2015) Effects of hydrogen-rich saline on early acute kidney injury inseverely burned rats by suppressing oxidative stress induced apoptosis and inflammation. Journal of Translational Medicine 13: 1-15.
6. Ohsawa I, Ishikawa M, Takahashi K, et al (2007) Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. Nature Medicine 13: 688-694.
7. Dukes JD, Whitley P, Chalmers AD (2011) The MDCK variety pack: Choosing the right strain. BMC Cell Biology 12: 1-4.
8. Gaush CR, Hard WL, Smith TF (1966) Characterization of an established line of canine kidney cells (MDCK). Proceedings of the Society for Experimental Biology and Medicine 122: 931-935.
9. Volpe DA (2011) Drug-permeability and transporter assays in Caco-2 and MDCK cell lines. Future Medicinal Chemistry 3: 2063-2077.
10. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods 65: 55-63.
11. Stockert JC, Horobin RW, Colombo LL, Blázquez-Castro A (2018) Tetrazolium salts and formazan products in Cell Biology: Viability assessment, fluorescence imaging, and labeling perspectives. Acta Histochemica 120: 159-167.
12. Xie L, Dai Z, Pang C, Lin D, Zheng M (2018) Cellular glucose metabolism is essential for the reduction of cell-impermeable water-soluble tetrazolium (WST) dyes. International Journal of Biological Sciences 14: 1535.
13. Hosgoo G (2006) Stages of wound healing and their clinical relevance. Veterinary Clinics: Small Animal Practice 36: 667-685.

14. Mendonça RJD, Coutinho-Netto J (2009) Cellular aspects of wound healing. Anais Brasileiros de Dermatologia 84: 257-262.

15. Mercandetti M, Cohen AJ (2017) Wound healing and repair. Emedicine 14: 12-20.

16. Wallace HA, Basehore BM, Zito PM (2019) Wound Healing Phases. In: StatPearls. StatPearls Publishing, Treasure Island (FL).

17. Jafta N, Magagula S, Lebelo K, Nkokha D, Mochane MJ (2021) The production and role of hydrogen-rich water in medical applications. Applied Water Science 1: Fundamentals and Applications, 273-298.

18. Bradauskienė DDV, Šilas G, Ežerskienė S, Moščenkova E (2024) Hydrogen-enriched water: Benefits for the body, prevention and reduction of symptoms of various diseases. 11. International European Congress on Advanced Studies in Basic Sciences.

19. Cheng J, Shi M, Sun X, Lu H (2024) Therapeutic effect of hydrogen and its mechanisms in kidney disease treatment. Medical Gas Research 14: 48-53.

20. Hirano S-i, Ichikawa Y, Sato B, Takefuji Y, Satoh F (2023) Clinical use and treatment mechanism of molecular hydrogen in the treatment of various kidney diseases including diabetic kidney disease. Biomedicines 11: 2817.

Citation: Dartsch PC (2025) The Benefits of Hydrogen-Enriched Water - Studies on Cultured Kidney Epithelial Cells. Appl Cell Biol, 13(1), [1-13]