

## Thoughts and The Progress

### Effect of Electrolyzed Water on Wound Healing

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**Abstract:** Electrolyzed water accelerated the healing of full-thickness cutaneous wounds in rats, but only anode chamber water (acid pH or neutralized) was effective. Hypochlorous acid (HOCl), also produced by electrolysis, was ineffective, suggesting that these types of electrolyzed water enhance wound healing by a mechanism unrelated to the well-known antibacterial action of HOCl. One possibility is that reactive oxygen species, shown to be electron spin resonance spectra present in anode chamber water, might trigger early wound healing through fibroblast migration and proliferation. **Key Word:** Wound healing-Electrolyzed water-Anode water-Cathode water-Hypochlorous acid.

Electrolyzed water, in a variety of forms and made by a variety of different processes, is widely used in Japan as a topical disinfectant (1,2). We describe here our findings that some types of electrolyzed water appear to accelerate the healing of full-thickness cutaneous wounds in rats.

#### Materials and methods

Six different types of water were made and tested. The first, ultrapure water (resistivity  $\cong 18 \text{ M}\Omega \cdot \text{cm}$ ), was produced by a sequence of treatments applied to city tap water: charcoal filtration, reverse osmosis, and ion exchange. One group of experimental animals was treated with this ultrapure, nonelectrolyzed water. Four types of electrolyzed water were made from this ultrapure water. In each case, the water was electrolyzed (voltage gradient 13V and apparent current density  $200 \text{ mA}/\text{cm}^2$ ) while being pumped (500ml/min) through a 3 chamber device (MicroBank Co., Ltd. Korea) (3). One chamber contained a platinum-plated titanium anode. Usually, the middle chamber was filled with a saturated sodium chloride solution made with the

ultrapure water. The third chamber contained the cathode, also made of platinum-coated titanium. Proprietary ion-exchange membranes separated the chambers. The following 3 types of electrolyzed water were made using the MicroBank Co., Ltd. device in this configuration (all measurements made at 25°C). The first, acid pH water, Ac(+), was taken from the anode chamber [pH 2.50–2.63, oxidation-reduction potential (ORP) 1104–1191mV, concentration of residual chlorine (determined by the o-toluidine method) 80 to 100 ppm]. Neutral pH (7.40) anode chamber water, N(+), was made by adding NaOH (1N) to the electrolyzed water taken from the anode chamber [pH 7.4, ORP 749–784 mV, concentration of residual chlorine almost the same as in the Ac(+) solution]. Alkaline pH water, Al(-), was taken from the cathode chamber (pH 10.65–10.85, ORP 212–297 mV, residual chlorine only a few ppm). Replacing the platinum cathode in the MicroBank Co., Ltd. device by one made of carbon and replacing the center-chamber solution by 5 M citrate (organic acid; citric acid) in ultrapure water allowed us to make a fourth type of electrolyzed water. This acidic water [Ac(-)] was taken from the cathode chamber (pH 3.86–3.87, ORP 212–297 mV, no residual chlorine). Finally, hypochlorous acid (HOCl) solution was made by electrolyzing 0.45% NaCl in a single-chamber device (Omuko Co. Ltd., Tokyo, Japan) (Voltage gradient, apparent current density, and pump rate were the same as for the MicroBank Co., Ltd. device.). The pH and ORP of this solution were 7.45 and 780 mV, respectively. When stored in PET bottles, the pH and ORP of all 6 types of water remained stable for at least a month.

Forty-two Wistar rats (8 weeks old) were randomly assigned to 6 experimental groups, housed in individual metabolic cages at 25°C, and fed rodent chow and water ad libitum. Under pentobarbital anesthesia, the back was shaved and two 1.0 cm square, full-thickness cutaneous wounds were made, one behind the other and 1.5 cm apart, on the back of each animal. In each rat, 1 wound (selected randomly) was treated twice a day for 7 days with 1 of the 6 types of water described previously; the other wound was left untreated. The rat was observed carefully until the water was absorbed by the wound to ensure that no spillage occurred. The first treatment was administered immediately after surgery. All wounds were allowed to heal without dressings. For 17 days after surgery, wound areas were measured daily by planimetry, using digital video camera images displayed via a personal computer.

**TABLE 1.** Comparison between the areas of water-treated and nontreated wounds, and comparison between experimental groups

POD	0	1	2	3	4	5	7	9	11	14	17
Water(n=7)		b	b		b	b	b	b	b	b	b
Mean	1.0399	1.3117	1.3082	1.1325	1.0894	0.8760	0.6588	0.3165	0.2280	0.1525	0.0839
SD	0.0777	0.1889	0.1559	0.2135	0.1194	0.1133	0.1203	0.0348	0.0801	0.0159	0.0202
NT											
Mean	1.0931	1.1958	1.1007	1.0242	0.9849	0.8509	0.6558	0.3208	0.2000	0.1685	0.0795
SD	0.0839	0.2697	0.2186	0.2407	0.1832	0.1154	0.1554	0.1189	0.0664	0.0609	0.0416
Ac(+) (n=9)		a,c	a	a	a,c	a,c	a,c	a,c	a,c	a,c	a,c
Mean	1.0347	0.9213	1.0904	0.9001	0.7937	0.6248	0.3709	0.1597	0.0878	0.0225	0.0040
SD	0.1105	0.2005	0.2187	0.1868	0.1832	0.1578	0.1783	0.0868	0.0330	0.0225	0.0083
NT											
Mean	1.0876	1.1913	1.3229	1.2836	1.1969	1.0530	0.7255	0.3509	0.2114	0.1225	0.0430
SD	0.1815	0.2506	0.3939	0.3362	0.3535	0.2524	0.2738	0.1552	0.0967	0.0399	0.0351
N(+) (n=6)		a	a	a	a,c	a,c	a,c	a,c	a,c	a,c	a,c
Mean	1.0457	1.1577	0.9918	0.8878	0.7846	0.6214	0.3754	0.1960	0.1122	0.0230	0.0151
SD	0.1076	0.2757	0.1751	0.1926	0.1775	0.1438	0.1273	0.0957	0.0700	0.0239	0.0371
NT											
Mean	1.0972	1.4260	1.3473	1.3465	1.2990	1.1729	0.8549	0.4755	0.3371	0.1447	0.0811
SD	0.0494	0.2773	0.1889	0.1699	0.1379	0.1614	0.2917	0.1421	0.1781	0.1191	0.0719
Al(-) (n=6)		c			a	a				c	c
Mean	1.0151	0.9611	0.9970	0.8861	0.8052	0.7372	0.4776	0.2692	0.1221	0.0368	0.0305
SD	0.0595	0.1277	0.0876	0.1099	0.1335	0.1400	0.1461	0.1524	0.0280	0.0227	0.0251
NT											
Mean	1.0488	1.0272	1.1619	1.0939	1.0963	0.9781	0.7044	0.3958	0.2025	0.1406	0.0720
SD	0.0643	0.1376	0.3234	0.3316	0.2564	0.2191	0.2431	0.1335	0.0843	0.0510	0.0442
Ac(-) (n=6)			c								
Mean	0.9723	1.1173	0.9656	0.9082	0.8789	0.7429	0.5517	0.2316	0.1471	0.0979	0.0763
SD	0.0642	0.2219	0.3055	0.2881	0.2423	0.2130	0.2021	0.0842	0.0584	0.0482	0.0311
NT											
Mean	0.9374	1.0732	1.0655	1.0394	1.0151	0.8215	0.6636	0.3369	0.1941	0.1110	0.0795
SD	0.0682	0.1278	0.1326	0.1958	0.2027	0.1632	0.1818	0.1109	0.0440	0.0487	0.0318
HOC1 (N=8)											c
Mean	1.1368	1.1986	1.2423	1.1403	0.9823	0.8702	0.6797	0.3585	0.1802	0.0961	0.0509
SD	0.1941	0.2300	0.3154	0.2580	0.2814	0.2474	0.2628	0.1830	0.1005	0.0563	0.0407
NT											
Mean	1.1909	1.1885	1.3253	1.3231	1.2036	1.1455	0.8468	0.3892	0.1901	0.1488	0.0660
SD	0.2448	0.3144	0.3970	0.3555	0.3412	0.2983	0.2766	0.1555	0.1103	0.1155	0.0559

<sup>a</sup>p < 0.05 vs the area of the NT wound (paired Student's t test).

<sup>b</sup>p < 0.05 among the groups (one-way ANOVA).

<sup>c</sup>p < 0.05 versus water (Dunnett).

Values are cm<sup>2</sup>. POD: postoperative day (wound were made on Day 0), Water: nonelectrolyzed pure water, NT: nontreated wounds, Ac(+): acid pH anode water, N(+): neutral pH anode water, Al(-): alkaline pH cathode water, Ac(-): acid pH cathode water, HOC1: hypochloric acid.

Acid and neutralized anode waters were studied by electron spin resonance (ESR; JEOL-JES-RE2X; Nihon Denshi, Tokyo, Japan) with the addition of a spin trapping agent [5,5-dimethyl-1-pyrroline-N-oxide (DMPO, Sigma, St. Louis, MO, U.S.A.)] using a flat cell of 1 mm width (4). This was carried out 24hr after the preparation of the electrolyzed water. The duration of the incubation of the electrolyzed water with DMPO was 2min. The magnetic field was of 335±5mT. Signal strength was compared with the signal derived from Mn<sup>2+</sup> (as a control).

All protocols were approved by the Animal Research Review Committee of Teikyo University Medical School.

For each group, the results are presented as mean wound area ±SD. The comparison between the areas of water-treated and nontreated wounds was performed using a paired Student's t test. The comparisons among the experimental groups were made using a one-way analysis of variance. When statistical significant was detected, Dunnett's test was used to determine which values differed significantly from those obtained using the nonelectrolyzed ultrapure water. Values of p<0.05

were considered significant.

## Results

As shown in Table 1, both types of anode chamber water [acidic and neutral: Ac(+) and N(+), respectively] accelerated wound healing (when compared to the effect of nonelectrolyzed ultrapure water) (p < 0.05). This acceleration of wound healing was evident from as early as postoperative Day (POD) 1 in Ac(+) or POD 2 in N(+). The alkaline water from the cathode chamber [Al(-)] showed a slight, but insignificant, tendency to increase healing (compared with the ultrapure water), but the acidic water from the cathode chamber [Ac(-)] and the HOC1 solution were both ineffective. All the wounds in all animals were free from signs of infection.

As shown in Fig. 1, no significant ESR spectrum was observed for untreated acidic anode chamber water. However, when we added a small amount of ferric salt to the anode water (5) to mimic the in vivo experiment, 4 lines suggestive of hydroxyl radicals were observed in the spectrum. Similar ESR spectra were observed for neutralized anode chamber water (in the absence or presence of ferric salt, respectively).

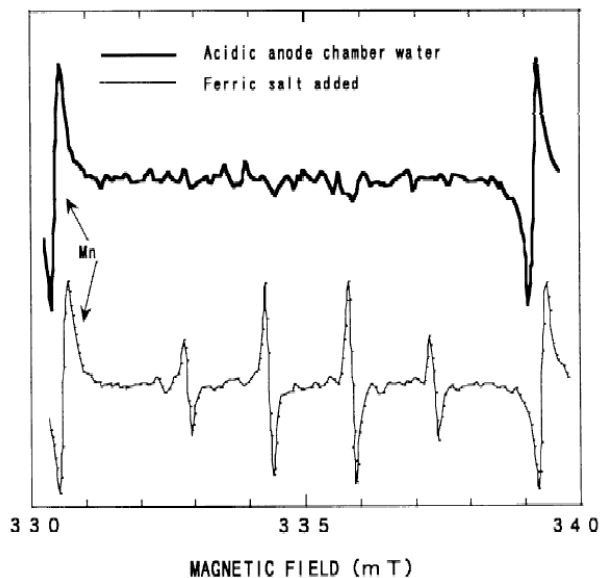


FIG. 1. Shown are electromagnetic spectra of acidic anode chamber water. The upper line is for untreated acidic anode chamber water. There was no significant ESR spectrum. Note that the lower line, the spectrum of the same water with ferric salt added, shows 4 lines suggestive of hydroxyl radicals.

## Discussion

The bactericidal action of various types of anode chamber water in the past has been attributed to their HOCl content (1). Since our HOCl solution was without effect on wound healing, we suggest that the anode chamber waters tested here may have a mode of action unrelated to any antibacterial HOCl produced by the electrolysis. One possibility is that free radicals, generated in the anode chamber waters by contact with the wound (Fig. 1), may be responsible for the very early effect on wound healing seen in this model. This interpretation is supported by the known effects of free radicals on inflammation as well as by what is known of the role of inflammation in the initiation of healing (6) in this model. In our study, differences in pH could not account for the significant difference in the acceleration of wound healing between acidic (pH about 2.5) and neutralized (pH 7.4) anode chamber waters on the one hand, and acidic cathode chamber water (pH about 3.8) on the other. Furthermore, there was no difference in pH or ORP between neutralized anode chamber water (pH 7.4, ORP 749–784 mV) and HOCl (pH 7.45, ORP 780 mV). Thus, ORP presumably did not substantially affect wound healing in our experiment.

Reactive oxygen species were shown to affect DNA synthesis and proliferation in fibroblasts. A low level of these species stimulates DNA synthesis and cell division while a high level inhibits DNA synthesis (the cytotoxicity induced being proportional to the level of reactive oxygen species) (7–9). Moreover, in small amounts, reactive oxygen species were shown to be involved in the mechanism underlying fibroblast chemotaxis into sites of injury or inflammation (10).

To our knowledge, there is no report clearly explaining the direct beneficial effect of HOCl on wound healing. HOCl generates not only chlorine gas,

which is the main cause of the bactericidal effect of HOCl, but also singlet oxygen, which is a reactive oxygen species whose physiological effect is still unknown (11). Kozol et al. demonstrated that sodium hypochlorite had toxic effects on wound modules (e.g., on neutrophils, fibroblasts, and endothelial cells even at dilute concentrations of  $2.5 \times 10^{-2}\%$  to  $2.5 \times 10^{-4}\%$ ) (12). Furthermore, wounds created on mouse ears and treated with 0.25% sodium hypochlorite showed delayed epithelialization and neovascularization (13). This might be the reason that HOCl was without effect in our wound healing model.

In conclusion, acid and neutral anode chamber waters accelerated the healing of full-thickness cutaneous wounds in rats. This effect might be caused by the generation of reactive oxygen species; however, further study is necessary for the elucidation of the mechanism underlying the effects of electrolyzed water on wound healing.

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