IN VITRO ASSESSMENT OF A HYPOCHLORITE FREE PURE HYPOCHLOROUS ACID-BASED WOUND SOLUTION COMPARED TO OTHER TYPES OF CLEANSERS

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BACKGROUND

Wound irrigation is a critical procedure for cleansing, hydrating, and debriding tissue, thereby supporting the healing of both acute and chronic wounds. In clinical settings, wound solutions — particularly topical antimicrobial preserved solutions — can be diluted or rendered less effective by wound exudate. This presents a well-recognized challenge in wound management, with important implications for treatment outcomes. This loss of efficacy is most pronounced in heavily exudative wounds, where germ removal activity may be significantly compromised. This study aims to evaluate the physicochemical properties (pH and ORP), *in vitro* biocompatibility, and germ elimination efficacy of four commercially available wound solutions at varying dilution levels.

METHOD

Test Solutions: A pure HOCl solution* (0.033% HOCl, pH=4.28), NaOCl solution (0.125%, pH=9.79, this is essentially quarter strength, or q.s, Dakin's solution), HOCl/NaOCl solution** (0.01%, this solution is pH-neutral and contains 0.005% HOCl and 0.005% NaOCl;), and a PHMB solution*** (0.1%, pH-neutral).

***pH* measurement:** The pH values of the test materials and their dilutions in PBS at 0, 5, 10, and 15 minutes were measured by using a pH meter (FP20, Mettler Toledo, Schwerzenbach, Switzerland).

Oxidation-reduction potential (ORP) measurement: ORP values of the test materials were measured as the open-circuit potential (OCP) of a Pt electrode vs. an Ag/AgCl reference electrode using an electrochemical potentiostat (Digi-Ivy, Austin, Texas, USA).

Antibacterial efficacy test: The antibacterial activity of each solution was assessed by exposing *P. aeruginosa* ATCC 15442 (10^6 CFU/mL) to the solutions for 10 minutes. Log reduction values were calculated relative to the untreated negative control.

Cytotoxicity test: Cell viability of HaCaT keratinocytes, HDFa fibroblasts, and human mesenchymal stem cells (MSCs) after treatment with the test materials and their dilutions for 5, 10, and 15 minutes was determined using the CyQUANT cell proliferation assay kit (Molecular Probes, Invitrogen, UK). Cell counts were converted to percentages relative to the DMEM group at the same time.

*:Vashe® wound solution: Hypochlorous acid solution; **: Granudacyn wound solution: HOCl/NaOCl solution; ***: Prontosan wound irrigation: PHMB solution

RESULTS

Table 1. pH values of the test solutions (diluted in PBS which is pH=7.48)

	HOCl solution				NaOCl solution				HOCl/NaOCl solution				PHMB solution			
Time (mins)	0	5	10	15	0	5	10	15	0	5	10	15	0	5	10	15
50%	6.96	6.96	6.98	6.96	9.53	9.52	9.51	9.52	7.35	7.43	7.46	7.33	7.50	7.44	7.43	7.47
75%	6.61	6.63	6.60	6.63	9.67	9.68	9.69	9.69	7.32	7.35	7.35	7.32	7.50	7.46	7.49	7.48
100%	4.28	4.29	4.34	4.37	9.79	9.78	9.76	9.77	6.89	6.92	6.91	6.93	7.09	7.07	7.03	6.99

Table 2. The maximum ORP (in mV) of the tested solutions and their dilutions in PBS

	% in PBS	ORP Max (mV)	ORP Time (S)
HOCl solution	0	488	0
	50	1102	833
	75	1114	850
	100	1307	2
NaOCl solution	0	488	0
	50	939	883
	75	936	876
	100	944	1172
HOCl/NaOCl solution	0	491	0
	50	1077	1170
	75	1092	1166
	100	1108	1194
PHMB solution	0	499	0
	50	502	626
	75	505	224
	100	508	0

Table 3. Antibacterial data for the test solutions against *P. aeruginosa* for a contact time of 10 minutes.

Sample	100%	75%	50%
HOCl solution	NG (>6.08)	NG (>6.08)	NG (>6.08)
NaOCl solution	NG (>6.08)	NG (>6.08)	NG (>6.08)
HOCl/NaOCl solution	G (0.34)	G (0.18)	G (0.00)
PHMB solution	NG (>6.08)	NG (>6.08)	NG (>6.08)

The log reduction values, in brackets, and growth results, G = Growth and NG = No Growth.

#: 100% original HOCl solution is ~0.033% HOCl solution; ##: 100% original NaOCl solution is ~0.125% NaOCl solution; ###: 100% original HOCl/NaOCl solution is 0.005% HOCl and 0.005% NaOCl solution; ####: 100% original PHMB solution is ~0.1% PHMB solution.

RESULTS

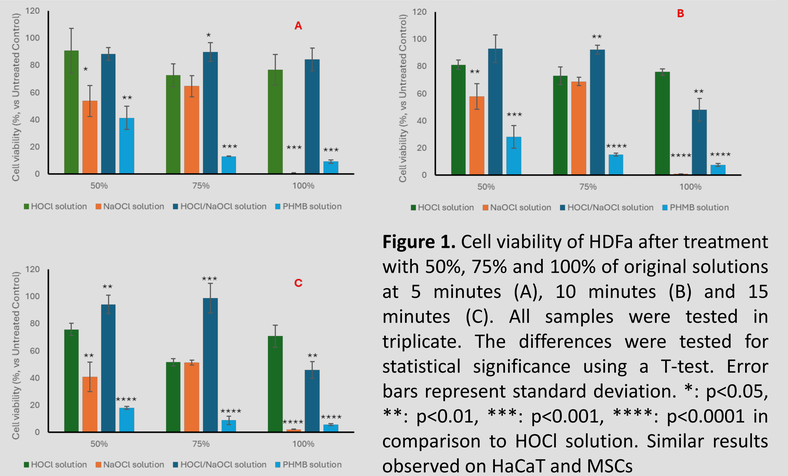

Figure 1. Cell viability of HDFa after treatment with 50%, 75% and 100% of original solutions at 5 minutes (A), 10 minutes (B) and 15 minutes (C). All samples were tested in triplicate. The differences were tested for statistical significance using a T-test. Error bars represent standard deviation. *: p<0.05, **: p<0.01, ***: p<0.001, ****: p<0.0001 in comparison to HOCl solution. Similar results observed on HaCaT and MSCs

DISCUSSION & CONCLUSIONS

The hypochlorous acid (HOCl) cleanser (~0.033%) demonstrated superior oxidative potential (ORP) compared to NaOCl (0.125%), HOCl/NaOCl (0.01%), and PHMB (0.1%) solutions, indicating excellent antimicrobial preservation. Even after a 50% dilution, HOCl maintained higher ORP values and strong antimicrobial activity, achieving complete elimination of *P. aeruginosa* (>6.08 Log_{10} reduction). At equivalent dilutions, HOCl exhibited minimal cytotoxicity toward HaCaT, HDFa, and MSCs during up to 15 minutes of exposure, outperforming NaOCl and PHMB.

Integrating data on ORP, cytotoxicity, and antibacterial efficacy, HOCl cleanser offers high preservative strength, robust antimicrobial efficacy, and low cytotoxicity among tested formulations, making it an optimal choice for wound care applications.

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