Antibacterial and antifungal effects of electrolyzed and non-electrolyzed hypochlorous acid (HOCl) against pathogenic microorganisms

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Content

Introduction/HOCl

- The concept of Electrolyzed water (EW)was first proposed in Russia, however, it has been widely used for various purposes including disinfection, and water decontamination in different areas such as the food industry, agriculture, livestock management, and clinical application

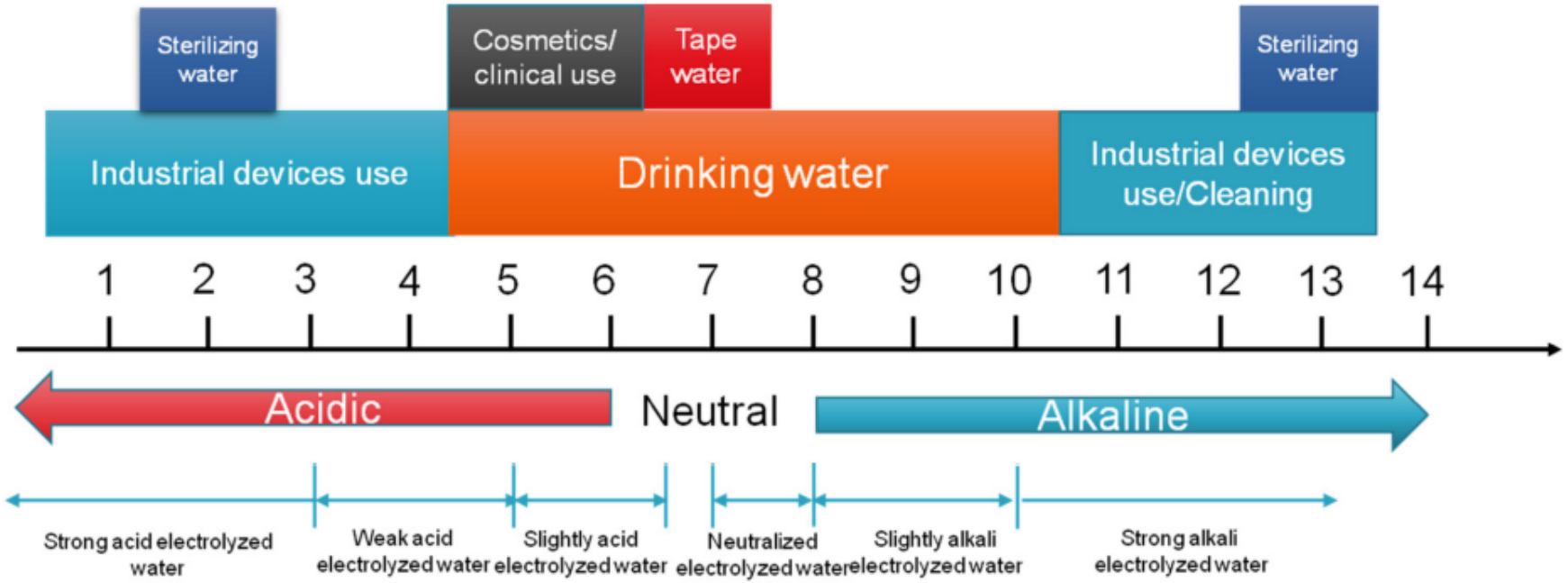
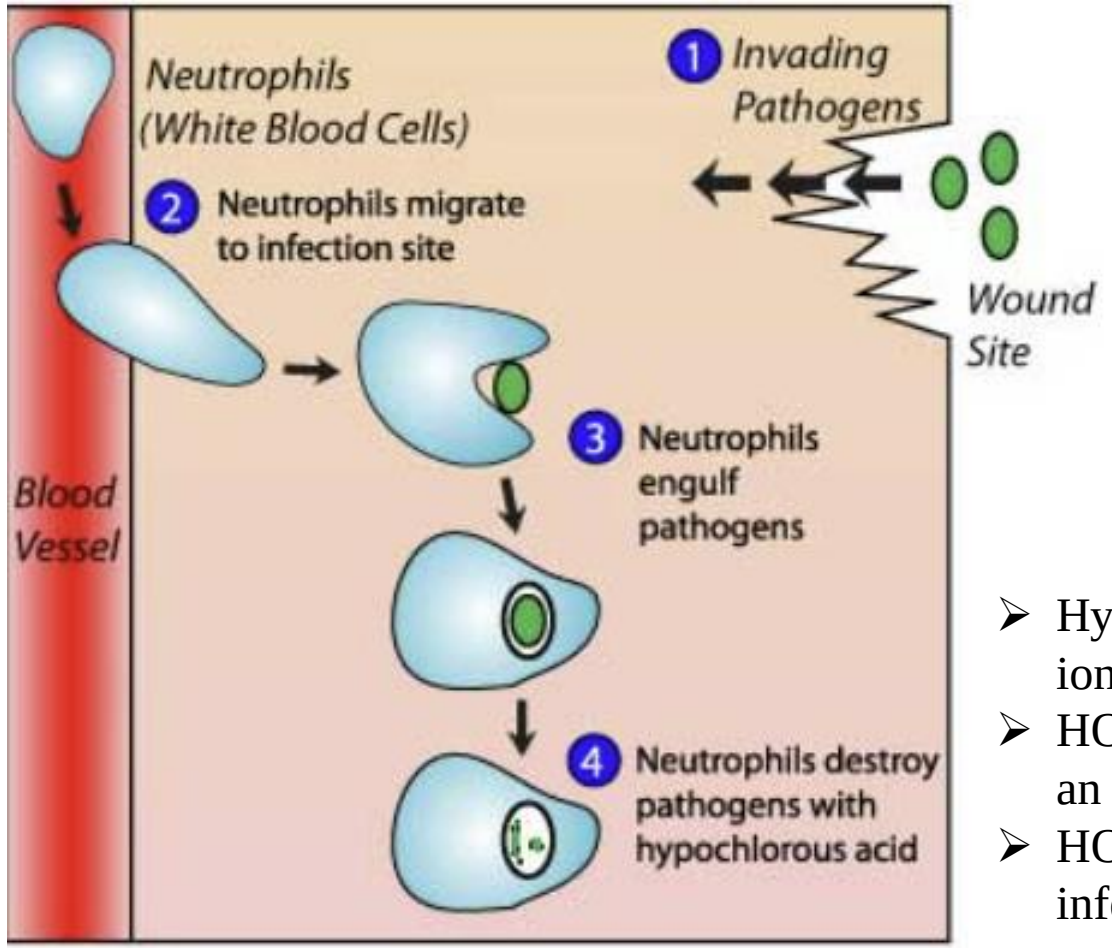
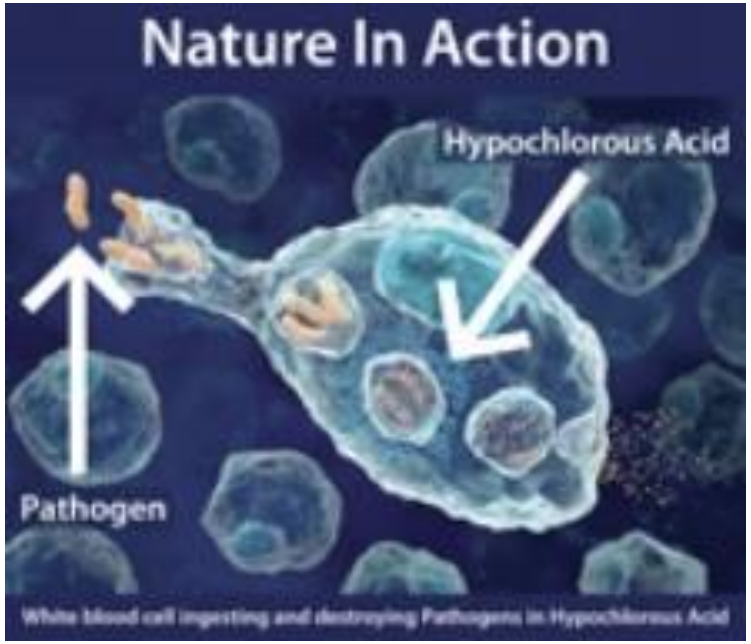


Figure 1. Application of electrolyzed water (EW) at different pH values in various fields.

Introduction/HOCl





- Hypochlorous acid that is produced by Chanson Water ionizers is also produced naturally by our body.
- HOCl is naturally produced by our white blood cells and is an essential part of our immune system.
- HOCl is a powerful antimicrobial that can fight off and kill infection, germs, and invading pathogens.

Sultana, S., Foti, A., & Dahl, J. U. (2020).

Introduction/HOCl

Properties of experimental water preparation

Parameter	TW	Non-HOCl	HOCl
pH	5.7	6.0	6.4
ORP (mV)	350	980	1000
Cl_2	0.6	50	50

ORP: Oxidation-reduction potential, **TW**: Tap water, Electrolyzed hypochlorous acid, non-electrolyzed hypochlorous acid, (**ppm)**, parts per million, (**Cl_2**)Chlorine

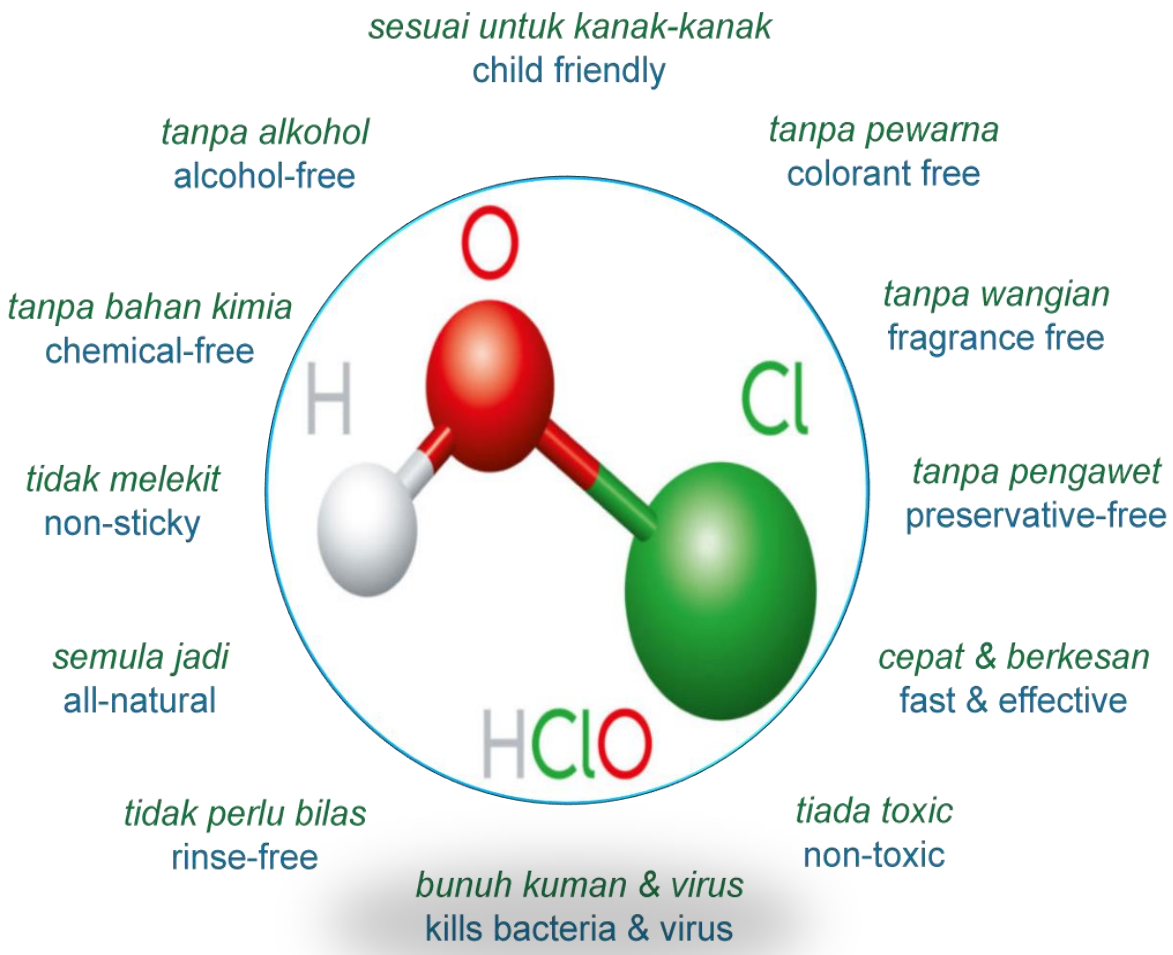
Comparison of applicability of HOCl and non-HOCl

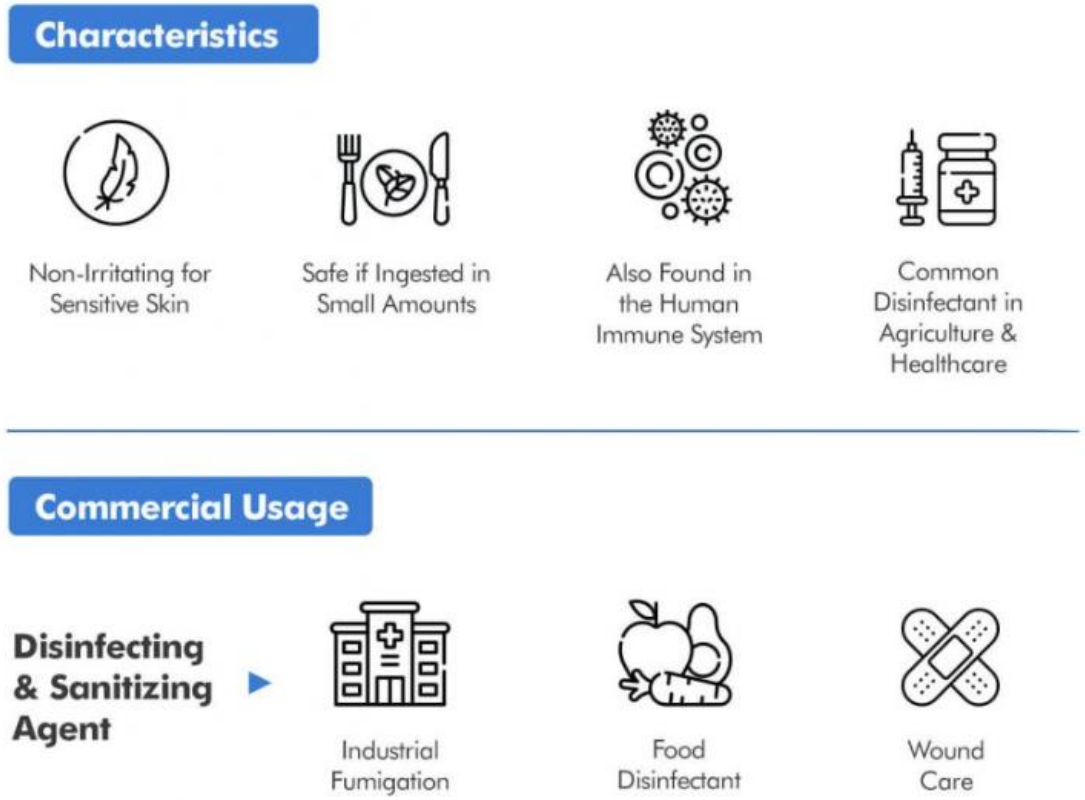
Applicability	E-HOCl (Electrolysis type)	HM-HOCl (Mixing type, non-electrolysis)
pH	5~6.5	5~6.5
Oxidative power, sterilization effect	Moderate	High
Washing metal devices	High	Low
Generation of harmful components	High	Low
Safety for drinking water disinfection	Low	High
AFC(Active free chlorine) stability with organic material mixing	Moderate	High
Production capacity of huge volume and high AFC concentration	Low	High
Using for livestock farming and pesticide free agriculture	Low	High

FAC: free active chlorine.

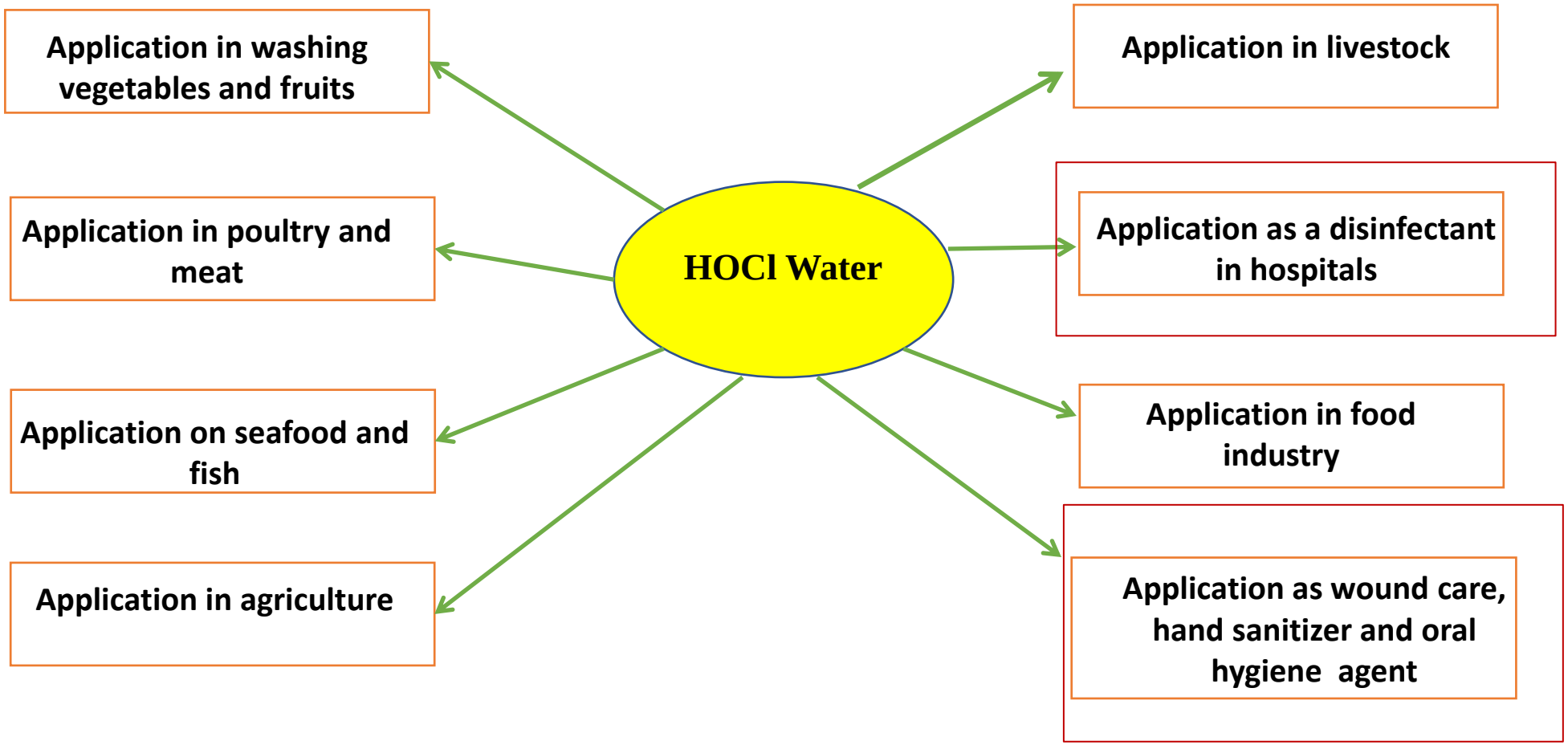
Bajgai, J., Kim, C. S., Rahman, M. H., Fadriquela, A., Thuy, T. T., Song, S. B., & Lee, K. J. (2020).

Properties of HOCl





Application of HOCl Water in Various Areas



HOCl application

Area	Usage	Purpose
medical	disinfection	Hand washing, endoscopy disinfection, wound, Ulcer, hospital disinfection, etc
dental	disinfection	Dental device disinfection, mouth washing, etc
agriculture	disinfection	Organic farming, sanitation, fruit vegetable washing
food	disinfection	Food washing, sanitation, kitchen disinfection
fishery	disinfection	Cleaning, bad smell removal, seafood disinfection,
veterinary	disinfection	Livestock sanitation, skin disease etc
household	disinfection	Toilet washing, utensil disinfection, hand washing
environmental	disinfection	Swimming pool disinfection, drinking water disinfection, disease prevention
veterinary	drinking	Tap water disinfection, diarrhea prevention, fecal odor removal

Table 1. Criteria of EW in different countries.

	Japan	The United States	EU	China
Administration	Ministry of Health, Labor, and Welfare	Administration of US Food and Drug	European Commission Directorate-General for Agriculture and Rural Development	Standardization administration
Application	Strong acid electrolyzed water (pH < 2.7): 20–60 ppm: hand washing in operation, cleaning and disinfection of endoscope and food additives.	Poultry Processing Facilities	Buildings and installations Aquaculture (only in the absence of animals)	Indoor air environment General object surface Medical equipment Surface of secondary water supply equipment and facilities
	Slightly acid electrolyzed water (2.7–5.0): 10–60 ppm: food additives and designation of specified pesticides (specific control materials)	Meat Processing	In general agriculture and in organic farming Plant and animal production Food processing	Vegetables and fruits
	Slightly acid electrolyzed water (ph:5.0–6.0): 10–80 ppm: food additives	Fruit and Vegetable Processing Facilities		Fabric
		Fish and Seafood Processing		Utensils
		Processed and Preformed Meat and Poultry		Hands
		Shell Egg Wash Organic Production and Handling		Skin and mucous membrane
ACC concentration	Strong acid electrolyzed water (ph < 2.7): 20–60 ppm Slightly acid electrolyzed water (2.7–5.0): 10–60 ppm Slightly acid electrolyzed water (pH:5.0–6.0): 10–80 ppm	<60 ppm Organic production and Handling(≤4 ppm)	Electrolyzed water usually contains 20–60 ppm (hypochlorite and hypochlorous acid, in a pH-dependent equilibrium).	Requirement of different application of toxicity
Requirement	Electrolyzed water must be decomposed or removed before completion of the final food	The treatment will be followed by either a 10 min drain step or a potable water rinse to remove		Non toxicity

Published Papers

SCIENTIFIC REPORTS

OPEN

Hypochlorous-Acid-Generating Electrochemical Scaffold for Treatment of Wound Biofilms

Received: 13 April 2018
Accepted: 7 January 2019
Published online: 25 February 2019

Mia Mae Kiamco[1], Hannah M. Zmuda[1], Abdelrhman Mohamed[1], Douglas R. Call[2], Yash S. Raval[3], Robin Patel[3,4] & Haluk Beyenal[1]

Biofilm formation causes prolonged wound infections due to the dense biofilm structure, differential gene regulation to combat stress, and production of extracellular polymeric substances. *Acinetobacter baumannii*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* are three difficult-to-treat biofilm-forming bacteria frequently found in wound infections. This work describes a novel wound dressing in the form of an electrochemical scaffold (e-scaffold) that generates controlled, low concentrations of hypochlorous acid (HOCl) suitable for killing biofilm communities without substantially damaging host tissue. Production of HOCl near the e-scaffold surface was verified by measuring its concentration using needle-type microelectrodes. E-scaffolds producing 17, 10 and 7 mM HOCl completely eradicated *S. aureus*, *A. baumannii*, and *P. aeruginosa* biofilms after 3 hours, 2 hours, and 1 hour, respectively. Cytotoxicity and histopathological assessment showed no discernible harm to host tissues when e-scaffolds were applied to explant biofilms. The described strategy may provide a novel antibiotic-free strategy for treating persistent biofilm-associated infections, such as wound infections.

In Vitro and *In Vivo* Antimicrobial Activity of Hypochlorous Acid against Drug-Resistant and Biofilm-Producing Strains

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Marta Palau, Estela Muñoz, Sergi Colominas, and Joan Gavaldà contributed equally to this work. Author order was determined on the basis of seniority.

ABSTRACT The aims of this study were as follows. First, we determined the antimicrobial efficacy of hypochlorous acid (HClO) against bacterial, fungal, and yeast strains growing planktonically and growing in biofilms. Second, we sought to compare the activity of the combination of daptomycin and HClO versus those of the antimicrobial agents alone for the treatment of experimental catheter-related *Staphylococcus epidermidis* infection (CRI) using the antibiotic lock technique (ALT) in a rabbit model. HClO was generated through direct electric current (DC) shots at determined amperages and times. For planktonic susceptibility studies, 1 to 3 DC shots of 2, 5, and 10 mA from 0 to 300 s were applied. A DC shot of 20 mA from 0 to 20 min was applied to biofilm-producing strains. Central venous catheters were inserted into New Zealand White rabbits, inoculated with an *S. epidermidis* strain, and treated with saline solution or ALT using daptomycin (50 mg/mL), HClO (20 mA for 45 min), or daptomycin plus HClO. One hundred percent of the planktonic bacterial, fungal, and yeast strains were killed by applying one DC shot of 2, 5, and 10 mA, respectively. One DC shot of 20 mA for 20 min was sufficient to eradicate 100% of the tested biofilm-producing strains. Daptomycin plus HClO lock therapy showed the highest activity for experimental CRI with *S. epidermidis*. HClO could be an effective strategy for treating infections caused by extensively drug-resistant or multidrug-resistant and biofilm-producing strains in medical devices and chronic wounds. The results of the ALT using daptomycin plus HClO may be promising.

Hydrogen Mineral Disinfectant Water and its Application in Agriculture and Livestock Farming

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Hypochlorous acid (HOCl) water was first introduced in Japan in 1987, and its antibacterial properties were first reported in 1992. HOCl water is utilized to promote public health all over the world, and it has only lately begun to be used in agriculture

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ORIGINAL ARTICLE

Journal of Applied Microbiology

Hypochlorous acid solution is a potent antiviral agent against SARS-CoV-2

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Abstract

Aim: A novel coronavirus, termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) suddenly appeared in Wuhan, China, and has caused pandemic. In this study, we evaluated antiviral activity of purified hypochlorous acid (HClO) against coronaviruses such as SARS-CoV-2 and transmissible gastroenteritis virus (TGEV) responsible for pig diseases.

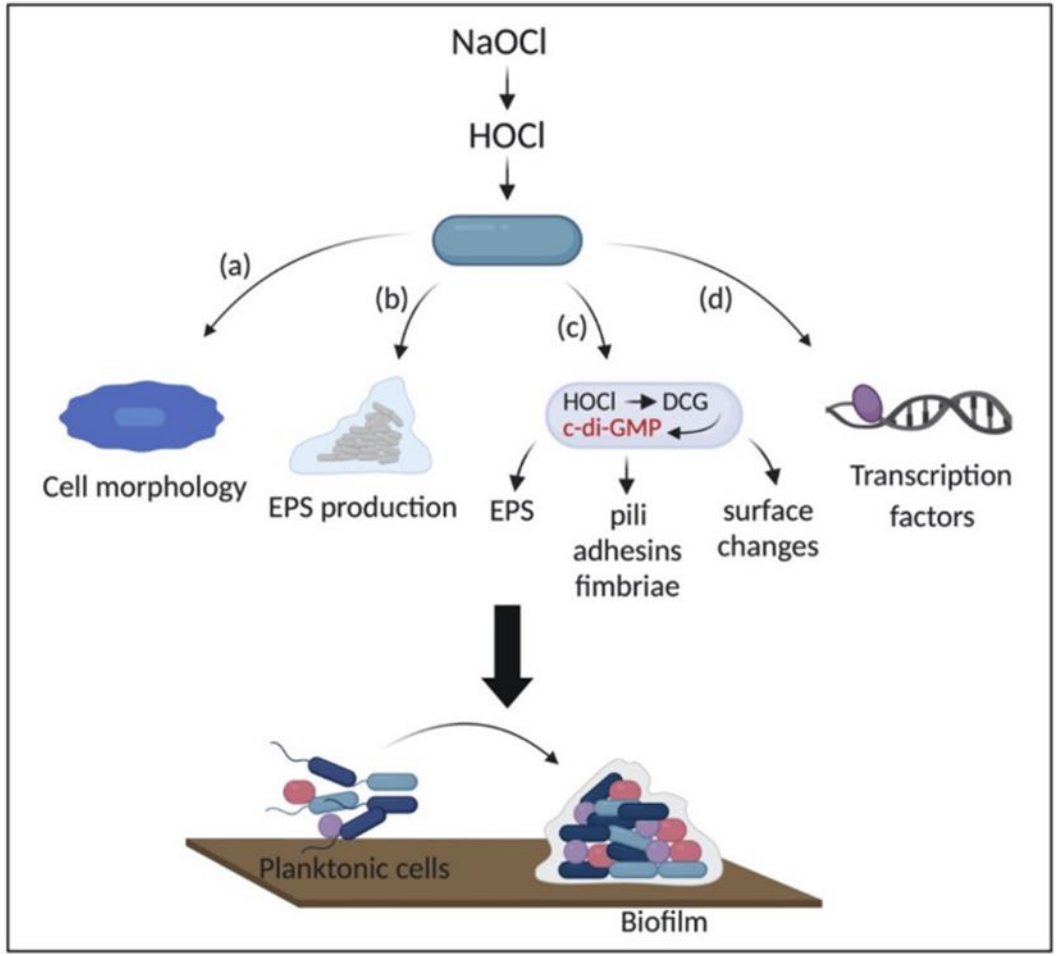
Materials and Results: In a suspension test, 28.1 ppm HClO solution inactivated SARS-CoV-2 in phosphate-buffered saline with the reduction of 10^4 of 50% tissue culture infectious dose per ml ($TCID_{50}$ per ml) within 10 s. When its concentration increased to 59.4 ppm, the virus titre decreased to below the detection limit (reduction of 5 logs $TCID_{50}$) within 10 s even in the presence of 0.1% foetal bovine serum. In a carrier test, incubation with 125 ppm HClO solution for 10 min or 250 ppm for 5 min inactivated SARS-CoV-2 by more than 4 logs $TCID_{50}$ per ml or below the detection limit. Because the titre of TGEV was 10-fold higher, TGEV was used for SARS-CoV-2 in a suspension test. As expected, 56.3 ppm HClO solution inactivated TGEV by 6 logs $TCID_{50}$ within 30 s.

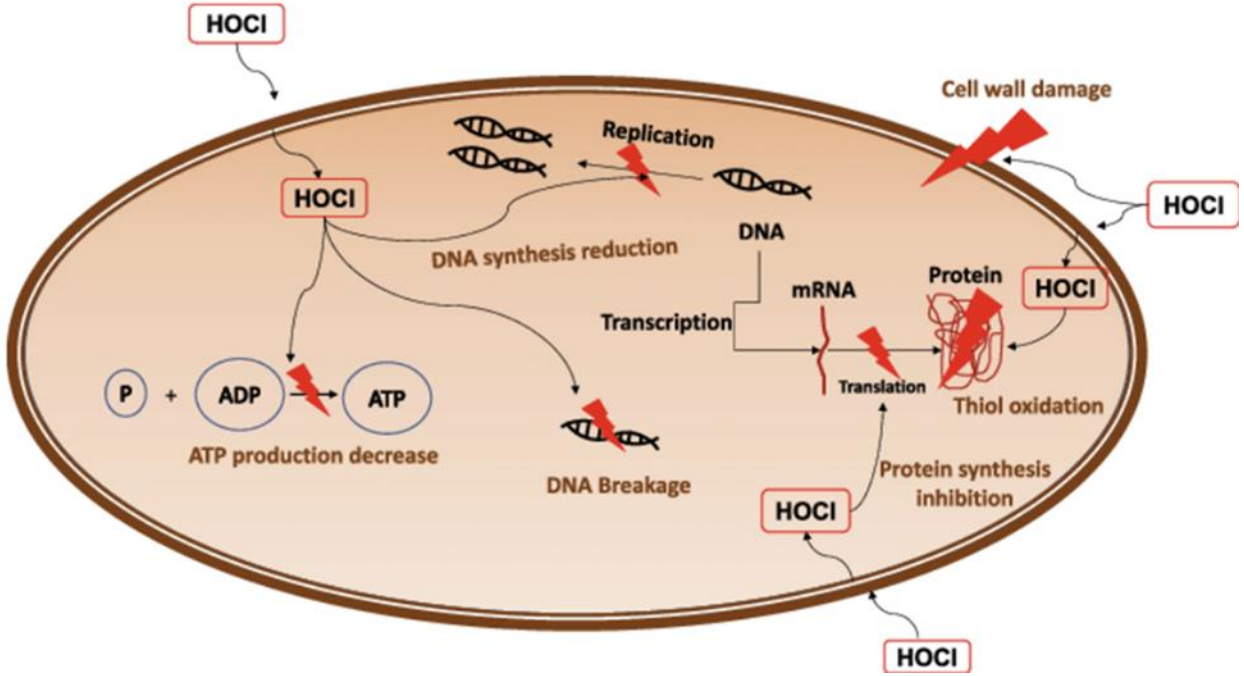
Conclusions: In a carrier test, 125 ppm HClO solution for 10 min incubation is adequate to inactivate 4 logs $TCID_{50}$ per ml of SARS-CoV-2 or more while in a suspension test 56.3 ppm HClO is adequate to inactivate 5 logs $TCID_{50}$ per ml of SARS-CoV-2 when incubated for only 10 s regardless of presence or absence of organic matter.

Significance and Impact of the Study: Effectiveness of HClO solution against SARS-CoV-2 was demonstrated by both suspension and carrier tests. HClO solution inactivated SARS-CoV-2 by 5 logs $TCID_{50}$ within 10 s. HClO solution has several advantages such as none toxicity, none irritation to skin and none flammable. Thus, HClO solution can be used as a disinfectant for SARS-CoV-2.

KEYWORDS
antiviral activity, COVID-19, hypochlorous acid, SARS-CoV-2, TGEV

Introduction/HOCl

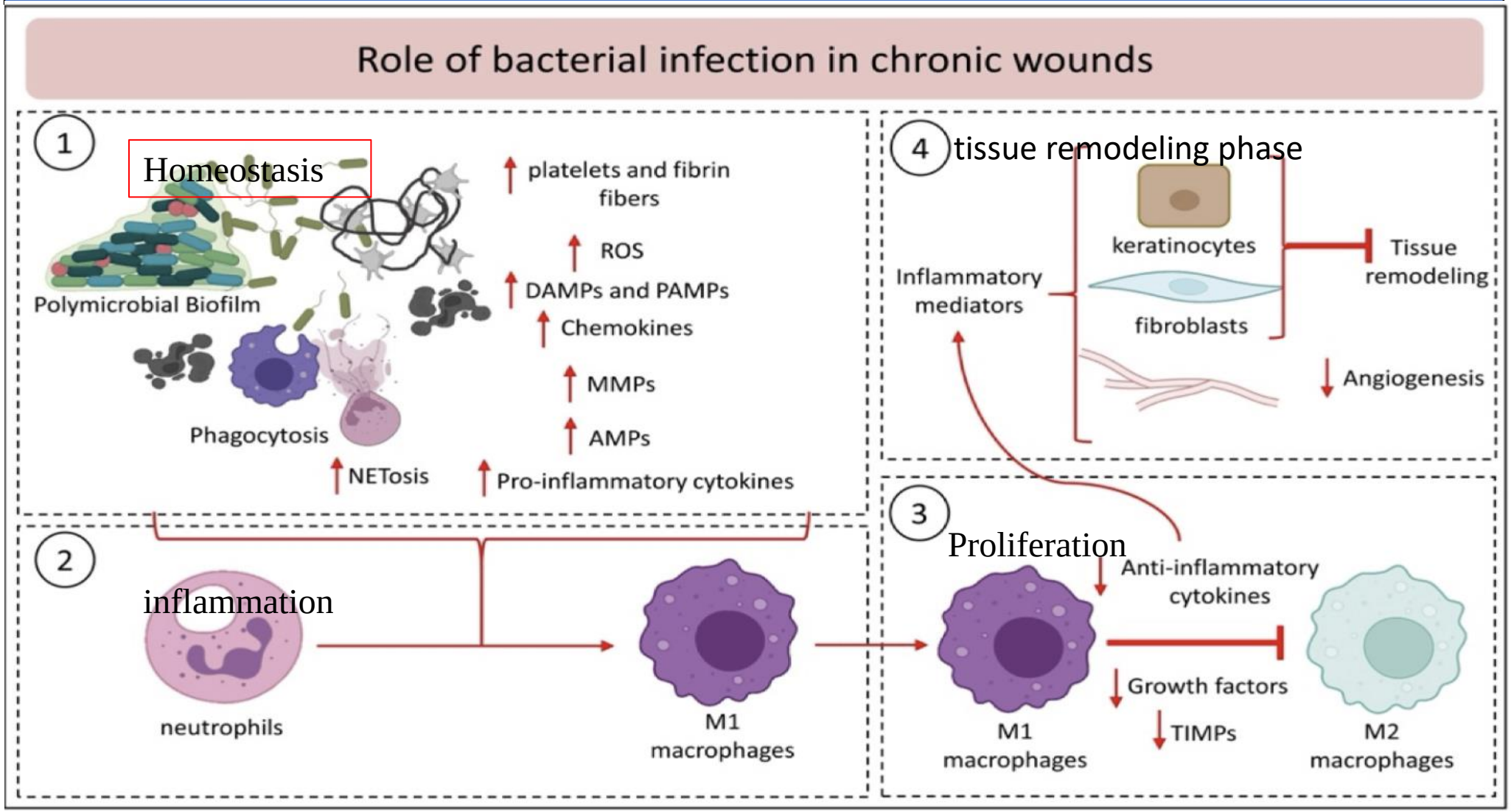




Sodium hypochlorite (**NaOCl**),hypochlorous acid (**HOCl**); extracellular polymeric substance (**EPS**), diguanylate cyclase; c-di-GMP: cyclic-di-GMP (**DCG**), Adenosine triphosphate (**ATP**),

da Cruz Nizer, W. S., Inkovskiy, V., & Overhage, J. (2020).

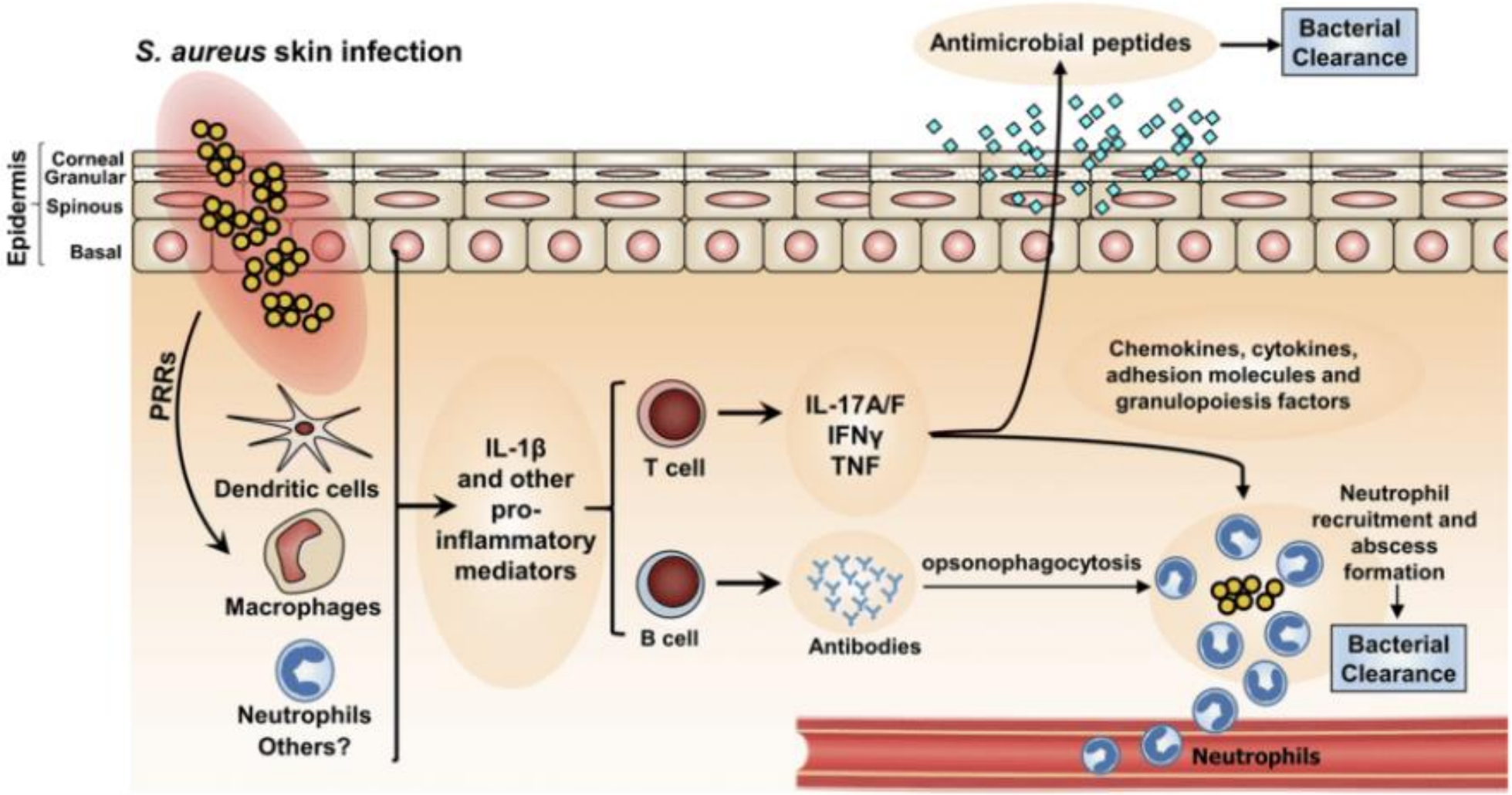
Introduction



Matrix metalloproteinases (**MMP**s), antimicrobial peptides and proteins (**AMPs**), damage-associated molecular patterns (**DAMPs**), pathogen-associated molecular patterns (**PAMPs**), neutrophil extracellular traps (**NET**), tissue inhibitors of metalloproteinases (**TIMPs**),

psoriasis and cardiovascular disease. Frontiers in immunology, 9, 1234.

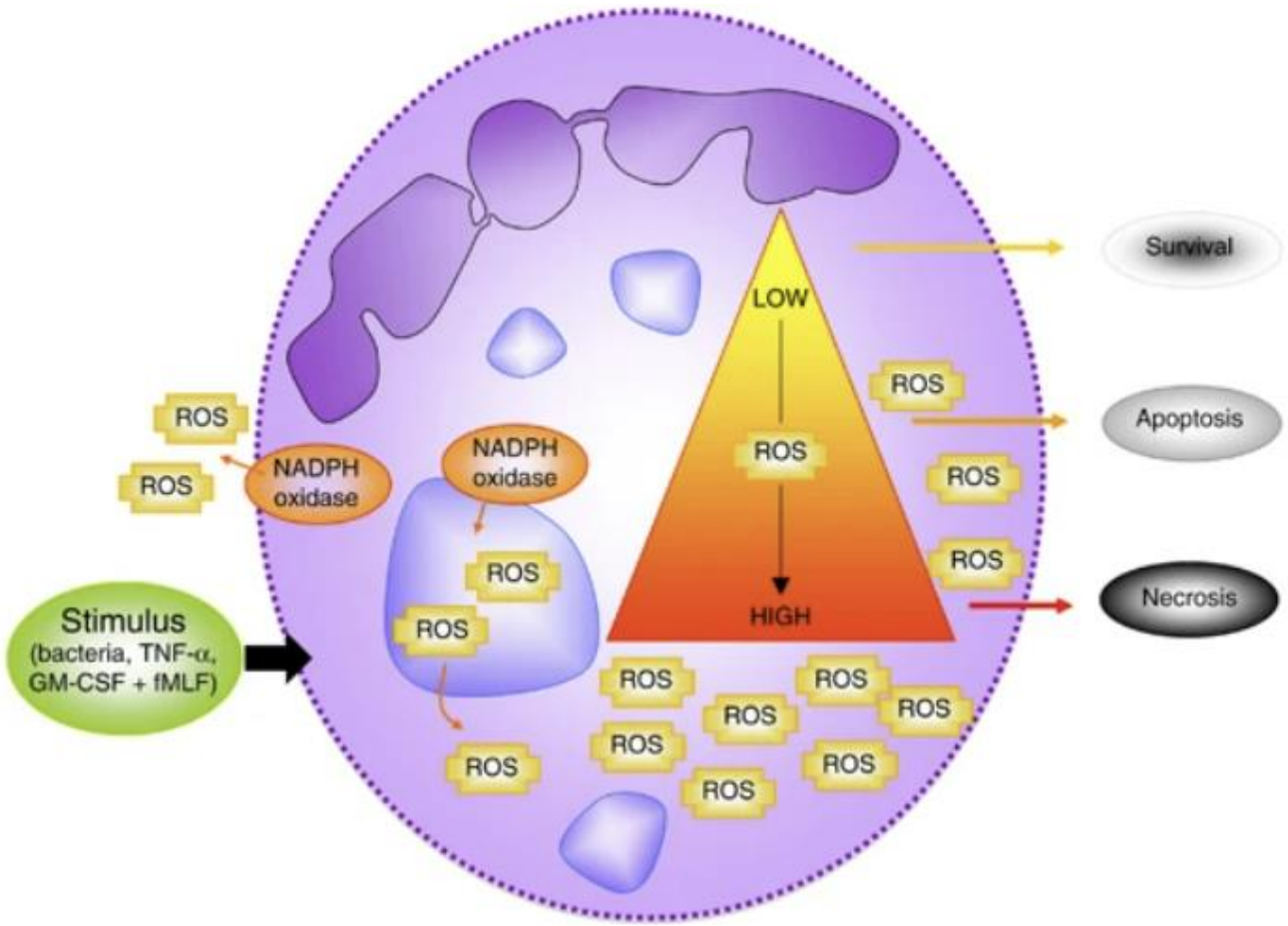
Introduction

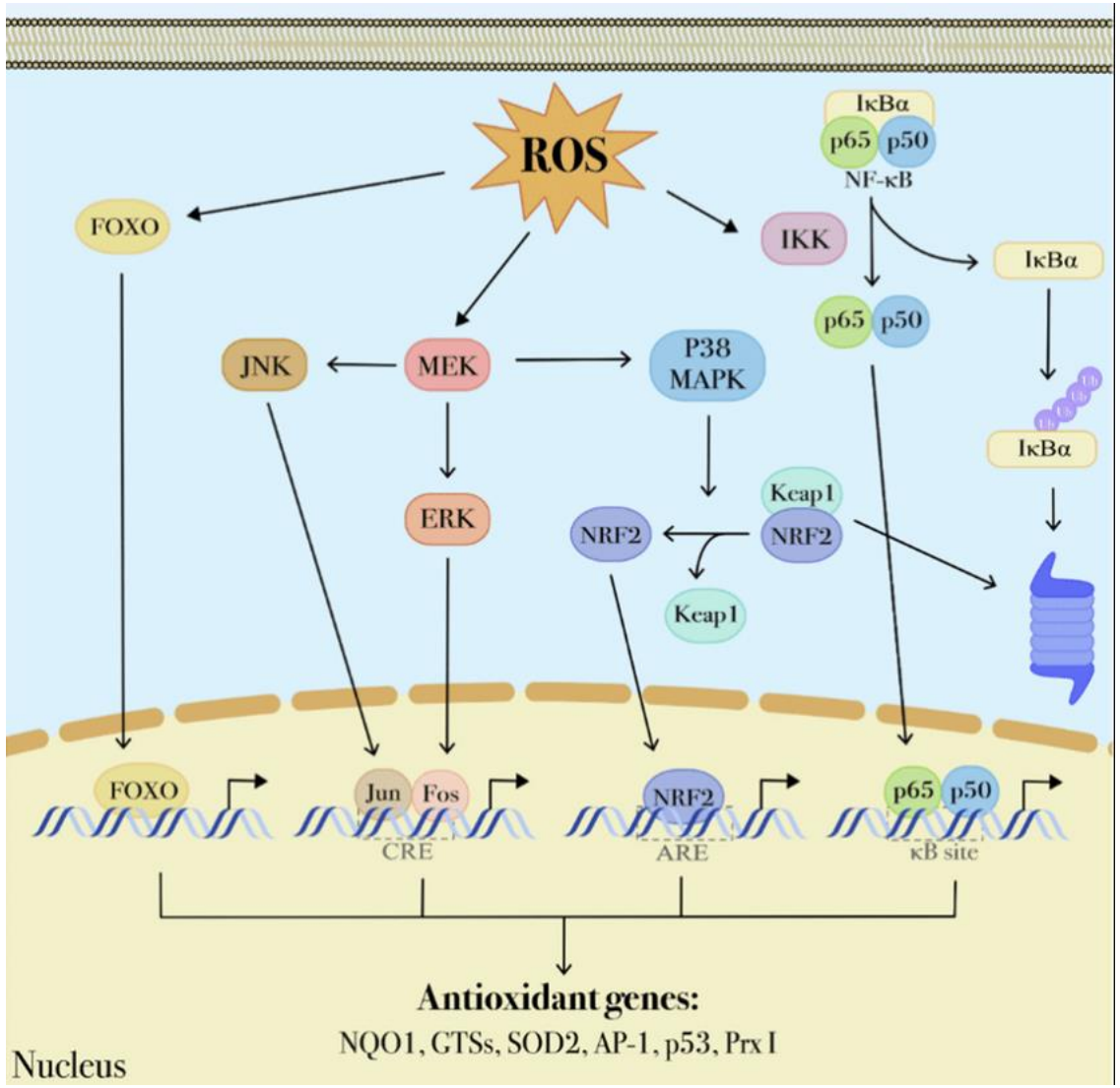


Pattern recognition receptors (**PRRs**)

McGuinness, W. A., Kobayashi, S. D., & DeLeo, F. R. .

Introduction



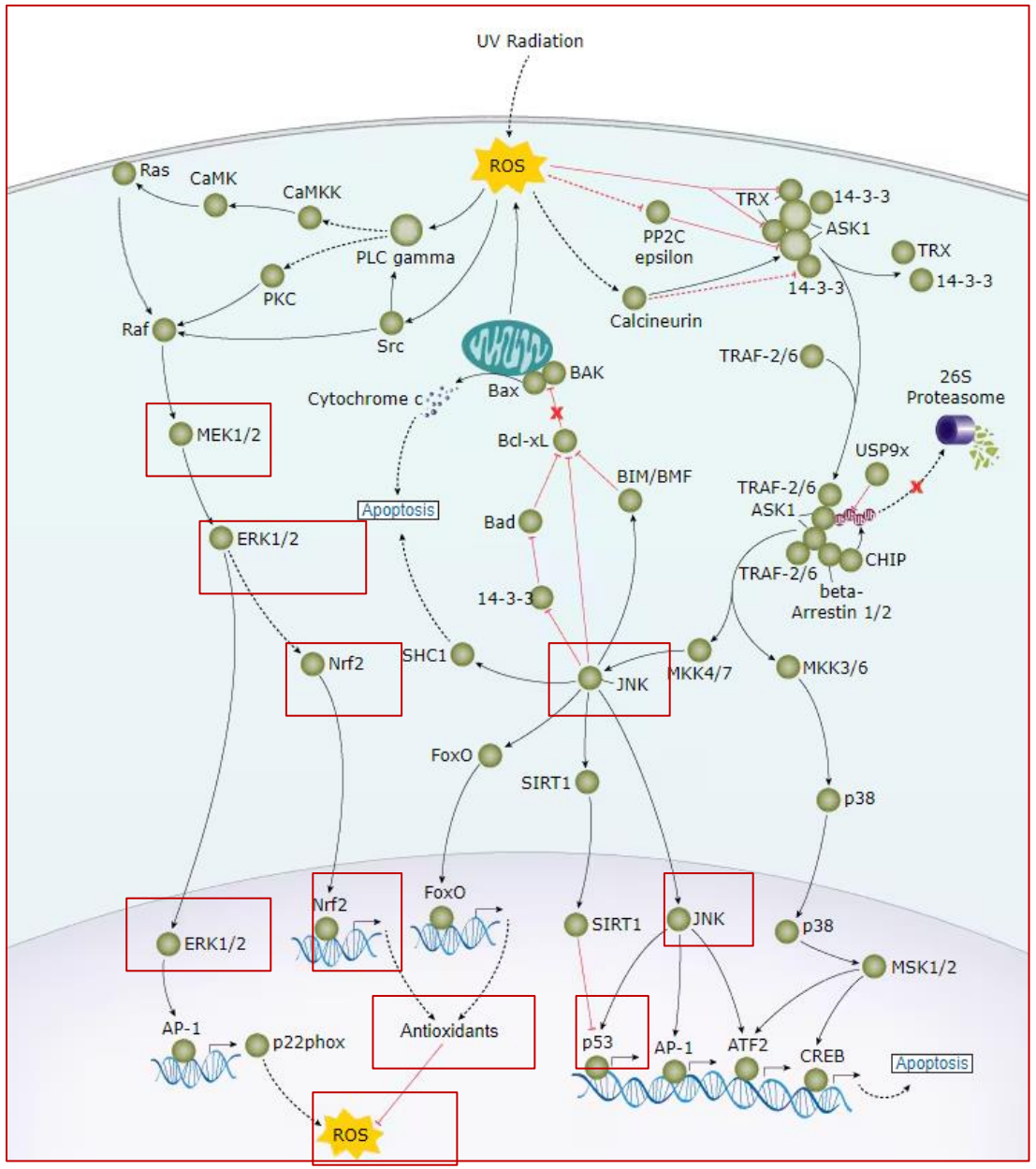


Granulocyte macrophage-colony stimulating factor (GM-CSF), N-Formylmethionine-leucyl-phenylalanine, activator protein 1 (AP-1), SOD2 superoxide dismutase 2,

New insight into the role of reactive oxygen species (ROS) in cellular signal-transduction processes. International review of cell and molecular biology,

Introduction

Mitogen-activated protein kinase (**MAPK**), Reactive oxygen species (**ROS**), MEK1, extracellular signal-regulated kinase 1 (**ERK1**), Nuclear factor erythroid 2-related factor 2 (**Nrf2**), BCL2 Associated X, Apoptosis Regulator (**Bax**), B-cell lymphoma-extra large (**Bcl-xL**), activator protein 1 (**AP-1**), Extracellular signal-regulated kinase (**ERK**), Jun N-terminal kinase (**JNK**), cAMP-response element binding protein (**CREB**), activating transcription factor 2 (**ATF2**) thioredoxin (**Trx**) , Protein phosphatase-2B (**PP2B**), apoptosis signal-regulating kinase 1 (**ASK1**), forkhead box O (**FoxO**), Bcl-2-associated death promoter(Bad), Bcl-2 homologous antagonist/killer (**BaK**), Bcl-2-associated X protein (**Bax**)



Yin, J., Ren, W., Wu, X., Yang, G., Wang,

Specific Aim

Aims this study was to examine the antibacterial and antifungal effects of electrolyzed and non-electrolyzed hypochlorous acid (HOCl) against pathogenic microorganisms.

Hypothesis

- We hypothesize that both electrolyzed and non-electrolyzed hypochlorous acid disinfectants will exhibit potent germicidal effects against a spectrum of infectious agents

- The electrolyzed hypochlorous acid, due to its enhanced oxidative potential, may demonstrate superior antimicrobial properties compared to non-electrolyzed hypochlorous acid.

- We anticipate that both forms of hypochlorous acid will effectively target the infectious agents associated with skin infection.

Research Design

Antibacterial and antifungal effects of electrolyzed and non-electrolyzed hypochlorous acid (HOCl) against pathogenic microorganisms

Bacteria, Fungi

S. aureus, P. aeruginosa and *E.coli*

C. albicans

Antibacterial activities of HOCl
Antifungal activities of HOCl

CFU/mL

OD

Biofilm

Cytotoxicity/LDH ↑

Inflammatory markers such as Chemokines/Cytokines

Cytokine+IL33,IL8,IL6,MMP3,TRL-2, TNF-α ↑ ↓
Chemokines+ CXCL8/CXCL4/CXCL12

In vitro

SK-MEL-1 cell line

Post-infection

Oxidative stress markers such as ROS, ↑ ↓
No, CAT, GPx, etc.

Western blot
Proteins Bax, Bcl-2

Treatment of HOCl and non-electrolyzed-HOCl

Skin Disease

Material and Methods

- Cell culture

-SK-MEL-1 cell lines

- Standard Microorganisms:

- *Staphylococcus aureus*
-*Escherichia coli*
-*Pseudomonas aeruginosa*
- *Candida albicans*

- Methods

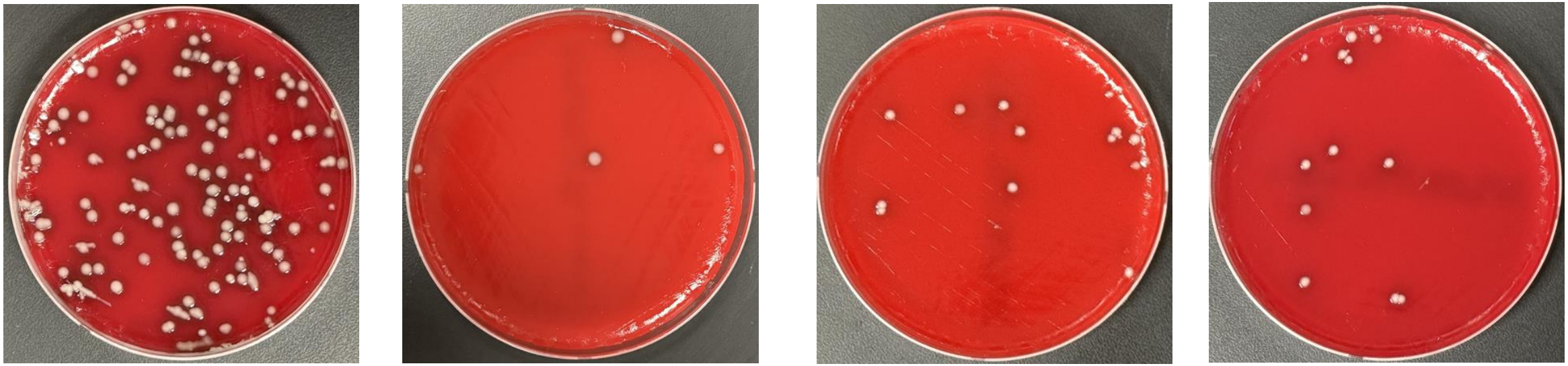
-CFU/ml(Colony forming unit/mL)
- OD (Optical Density)
-Biofilm assay

No	Materials	Remarks
1	LDH/Cell viability	Cytotoxicity
2	ROS, NO, GPX, CAT,	Oxidative stress marker
3	Bcl-2, Bax, ERK, MMP-3, MMP-9, JNK etc	Western blotting
4	IL-33,IL-8,IL-6/IFN- γ ,TNF-α	Cytokines (qPCR)
5	CXCL8,CXCL4 and CXCL12	Chemokines (qPCR)

Results

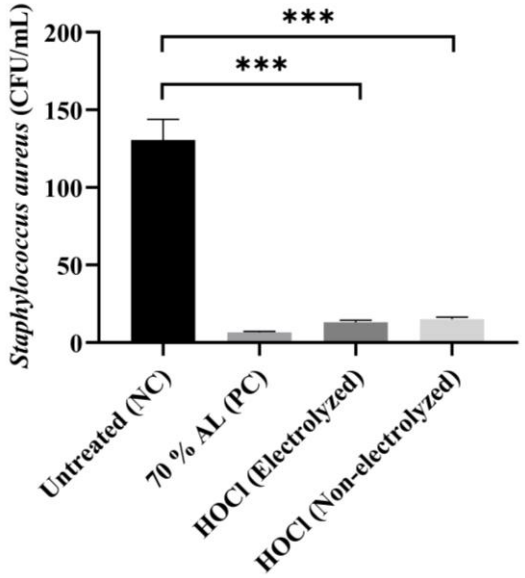
Figure 1

A



Staphylococcus aureus + Untreated (NC) *Staphylococcus aureus* + 70% AL (PC) *Staphylococcus aureus* + HOCl *Staphylococcus aureus* + non-HOCl

B



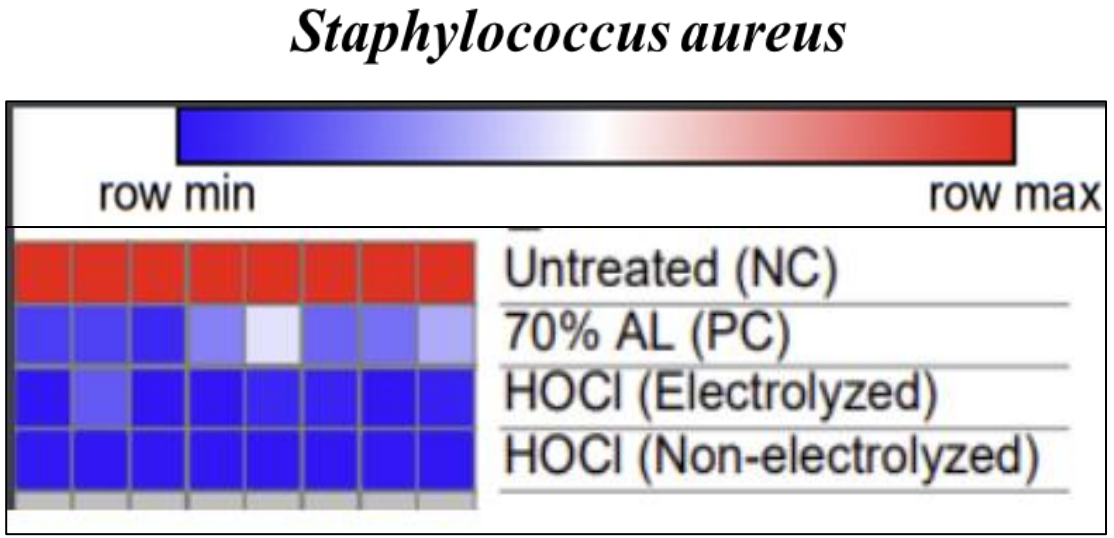
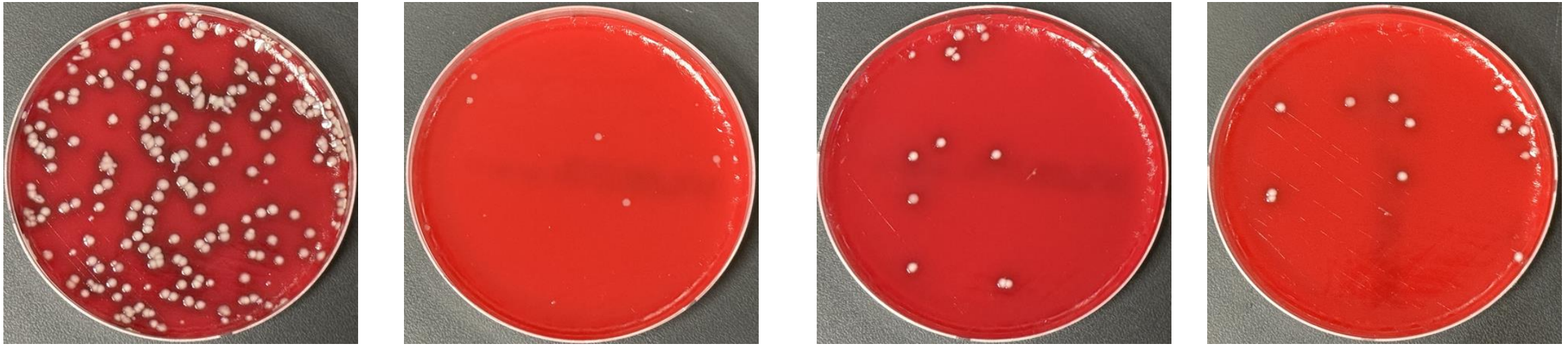


Fig. 1 The antibacterial activities of HOCl, on human pathogenic bacteria. A)The bacteria growth was completely inhibited after treatment of HOCl against *Staphylococcus aureus* at 37˚C for 24 hours of incubation with treatment. **(A)** CFU/mL, **(B),** Bacterial inhibition using biofilm methods. Abbreviation: HOCl, Hypochlorous acid; *S. areus*; St*aphylococcus aureus.*

Figure 2

A



Pseudomonas aeruginosa + Untreated (NC) *Pseudomonas aeruginosa* + 70% AL (PC) *Pseudomonas aeruginosa* + HOCl *Pseudomonas aeruginosa* + non-HOCl

B

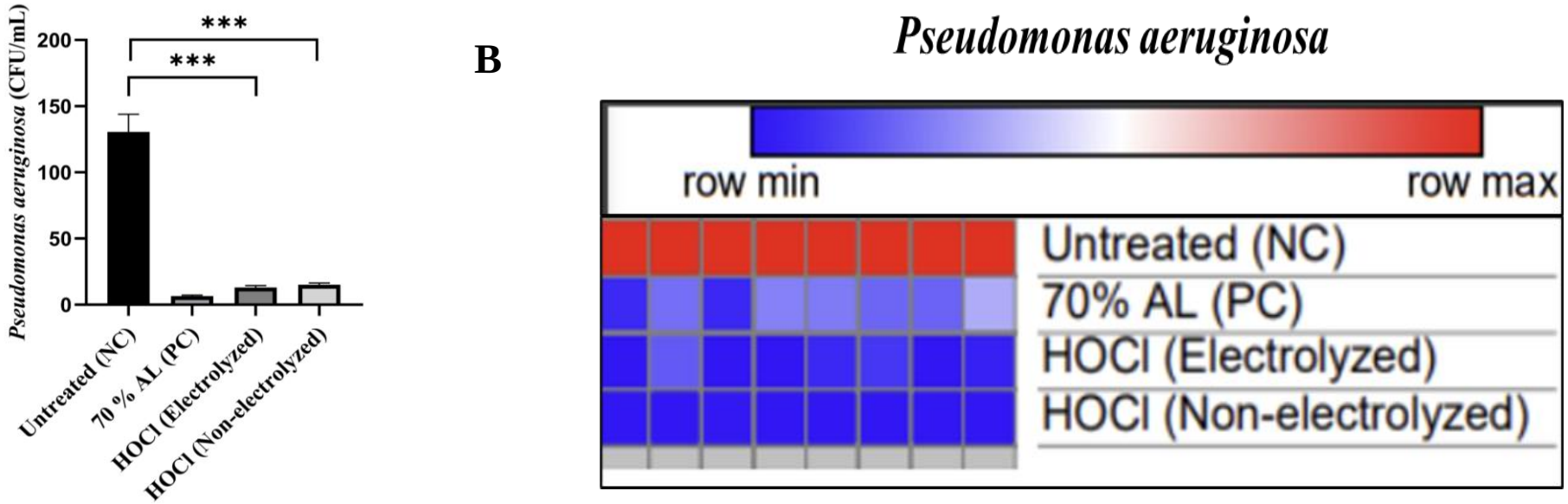
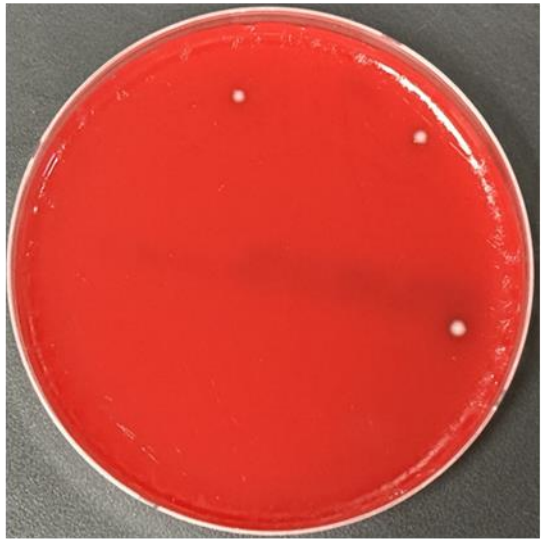


Fig. 2 The antibacterial activities of HOCl on human pathogenic bacteria. A) The bacteria growth was completely inhibited after treatment of HOCl against *Pseudomonas aeruginosa* at 37˚C for 24 hours of incubation with treatment. **(A)** CFU/mL, **(B),** Bac terial inhibition using biofilm methods Abbreviation: HOCl, Hypochlorous acid; *P. Aeruginosa*; *Pseudomonas aeruginosa*

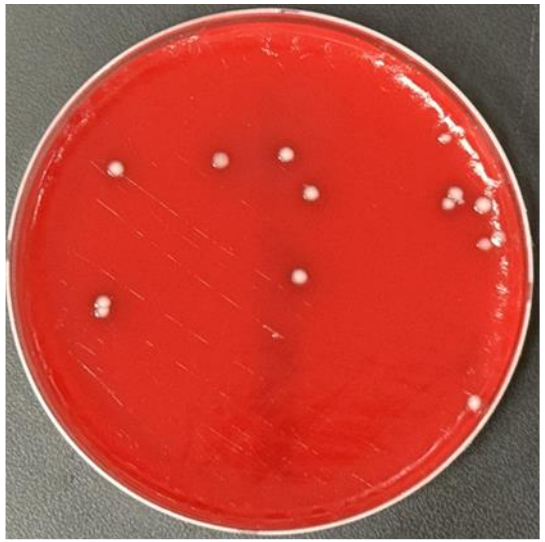
Figure 3

A

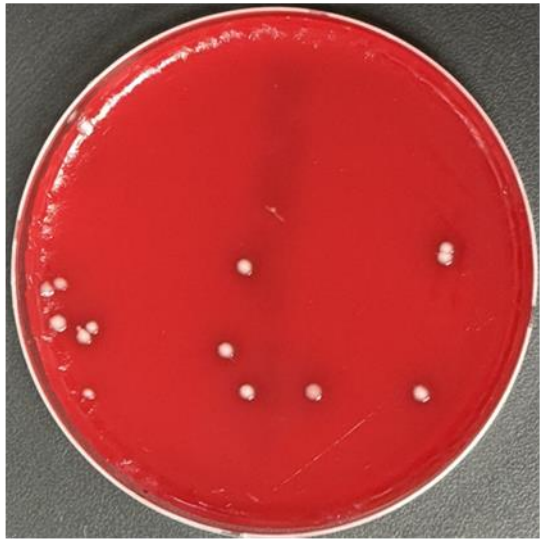
Escherichia coli + Untreated (NC)



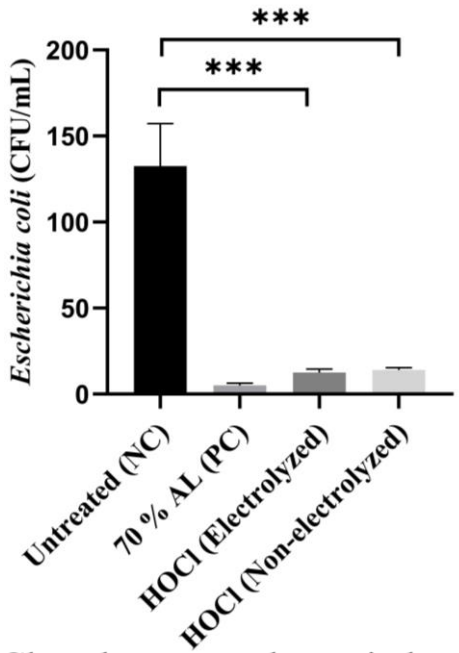
Escherichia coli + 70 % AL (PC)



Escherichia coli + HOCl



Escherichia coli + non-HOCl



B

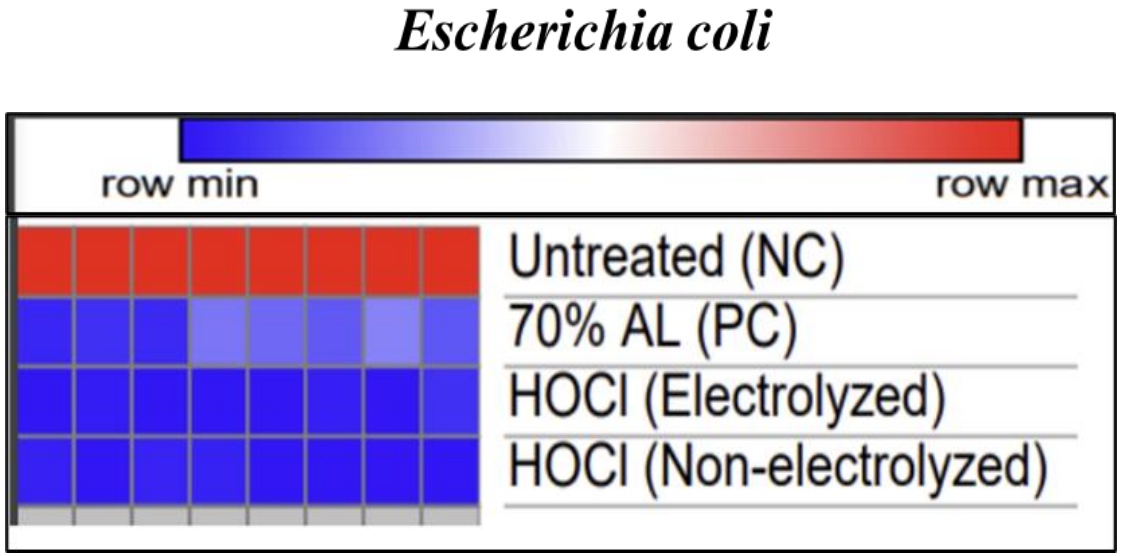
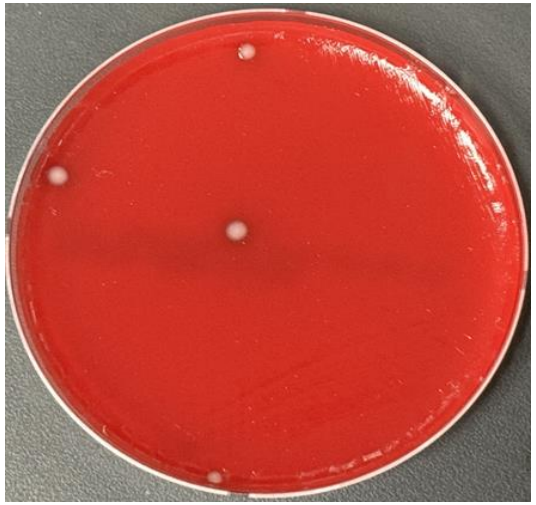


Fig. 3 The antibacterial activities of HOCl on human pathogenic bacteria. A)The bacteria growth was completely inhibited after treatment of HOCl against *Escherichia coli* at 37˚C for 24 hours of incubation with treatment. **(A)** CFU/mL, **(B),** Bacterial inhibition using biofilm methods Abbreviation: HOCl, Hypochlorous acid; *E. coli*; *Escherichia coli*

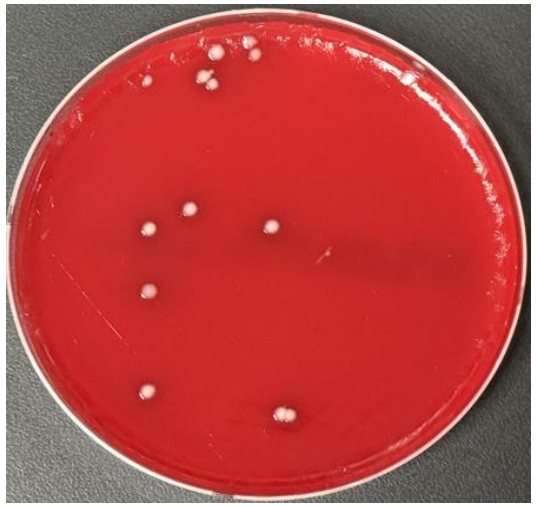
Figure 4

A

Candida albicans + Untreated (NC)



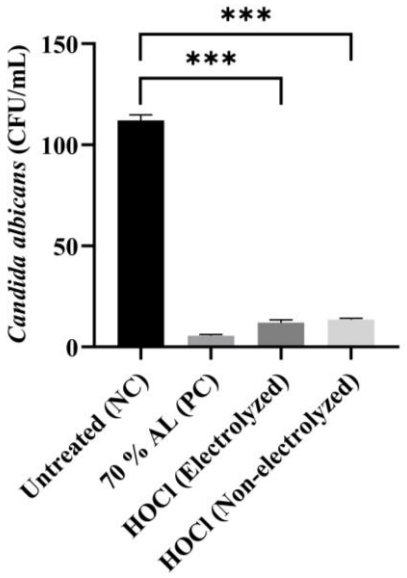
Candida albicans + 70 % AL (PC)



Candida albicans + HOCl

Candida albicans + non-HOCl

B



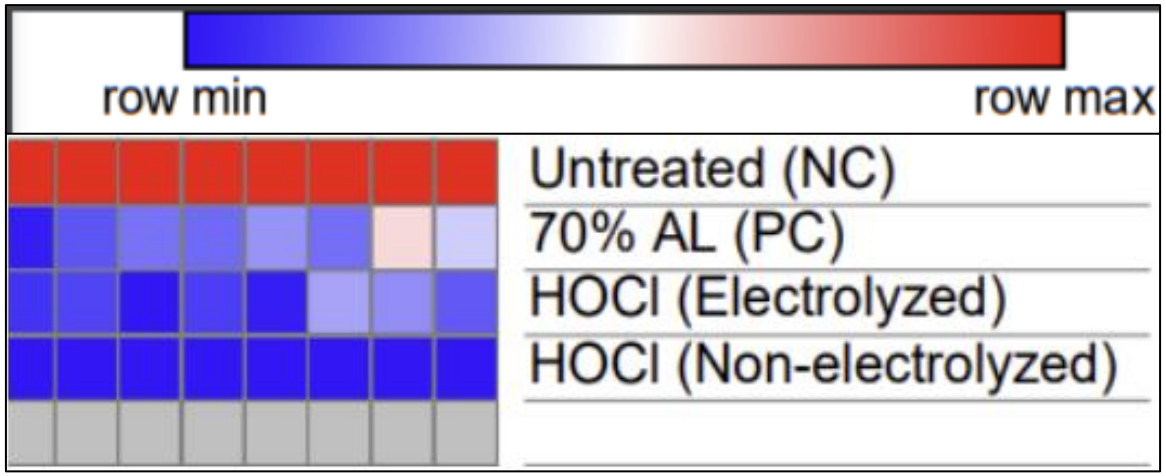


Fig. 4 The antibacterial activities of HOCl on human pathogenic Antifungal. A)The Antifungal growth was completely inhibited after treatment of HOCl against *Candida albicans* at 37˚C for 24 hours of incubation with treatment. **(A)** CFU/mL, **(B),** Bacterial inhibition using biofilm methods. Abbreviation: HOCl, Hypochlorous acid; *C. abicans*; *Candida albicans*

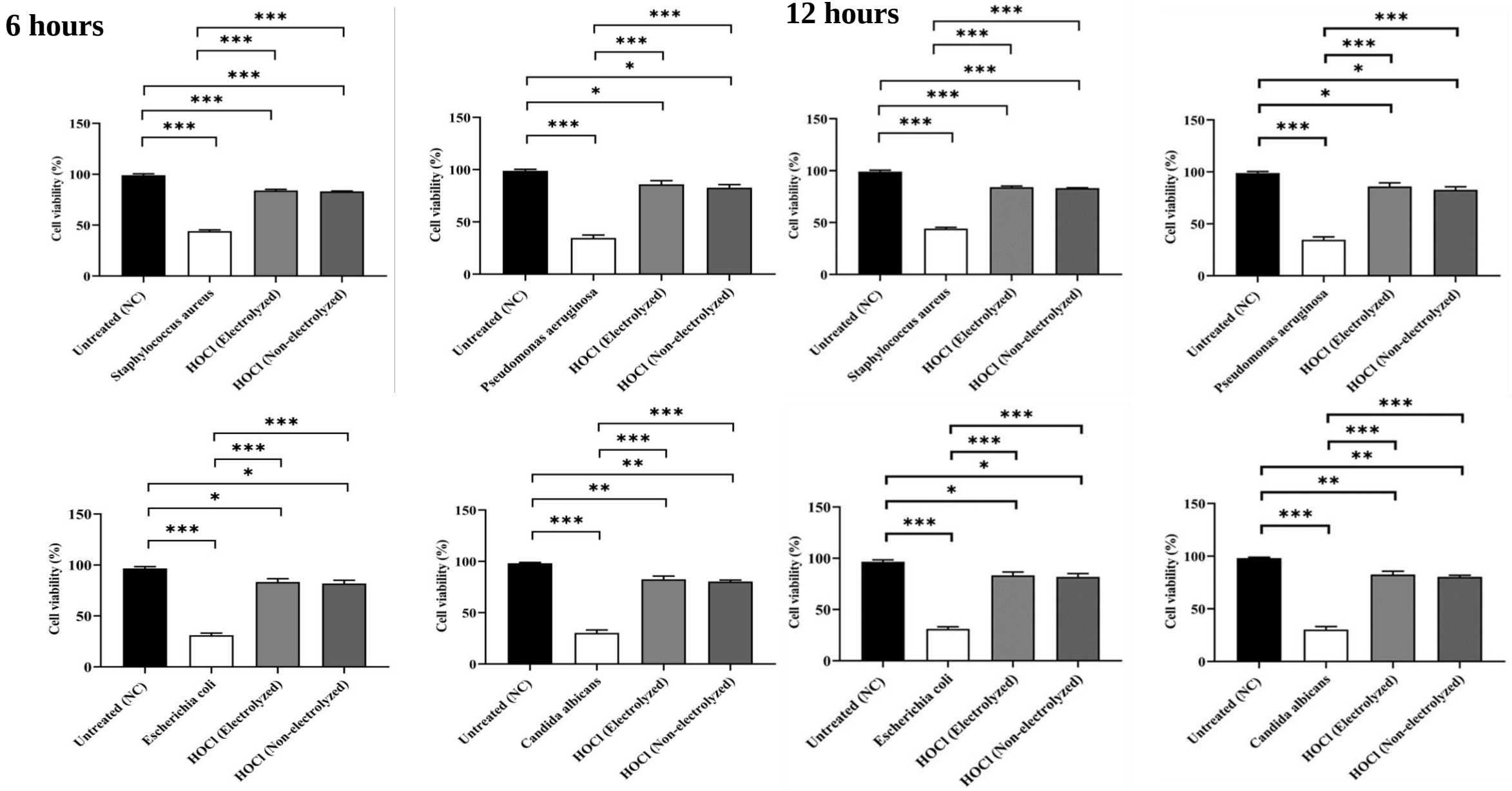


Figure 5.Cell viability after 6 and 12 hours; NC; treated with RPMI media; each pathogenic bacteria and fungal infection group; infection and treated HOCl; infection and treated and with Non-HOCl.

24 hours

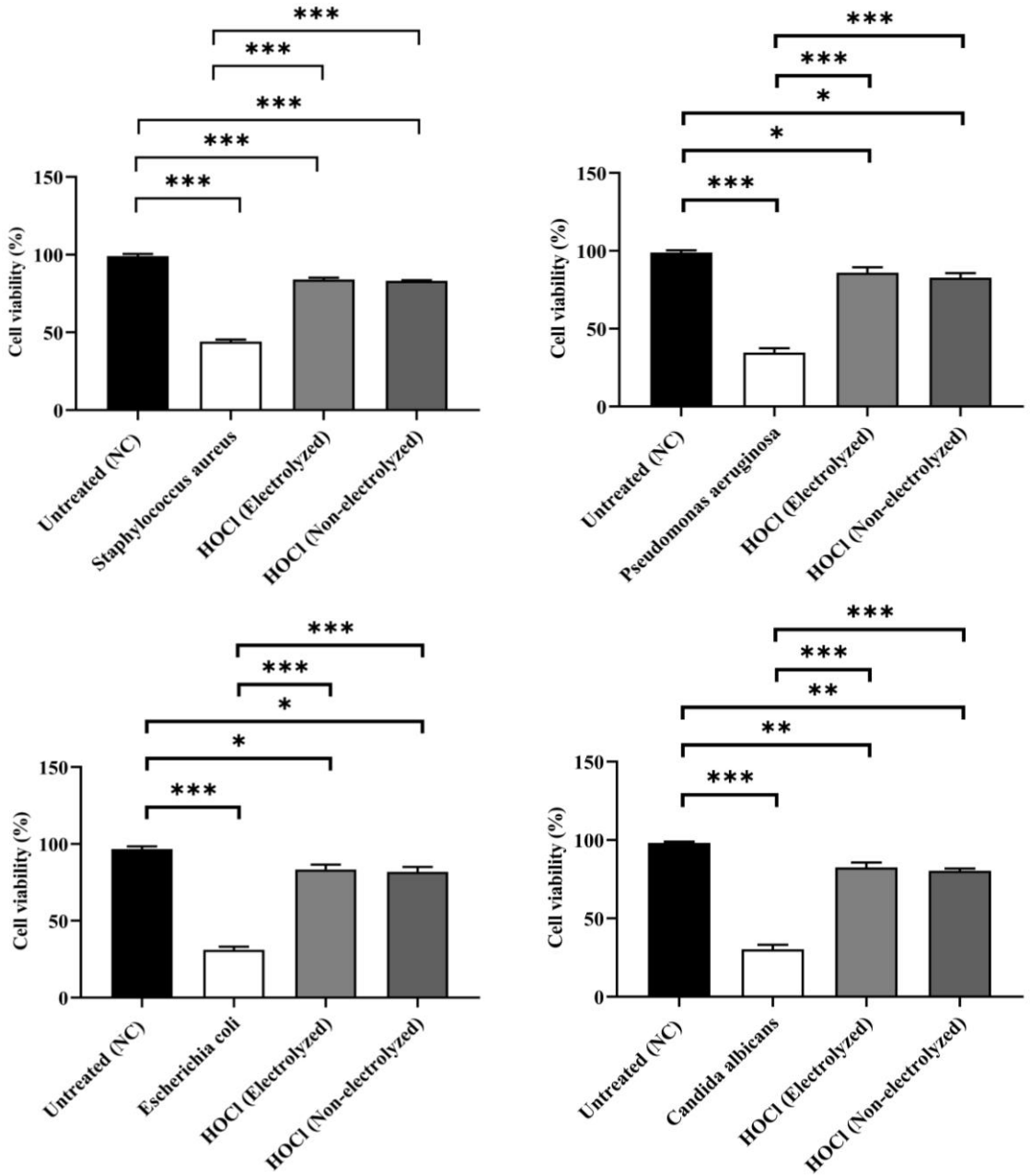


Figure 6.Cell viability after 24 hours; NC; treated with RPMI media; each pathogenic bacteria and fungal infection group; infection and treated HOCl; infection and treated and with Non-HOCl.

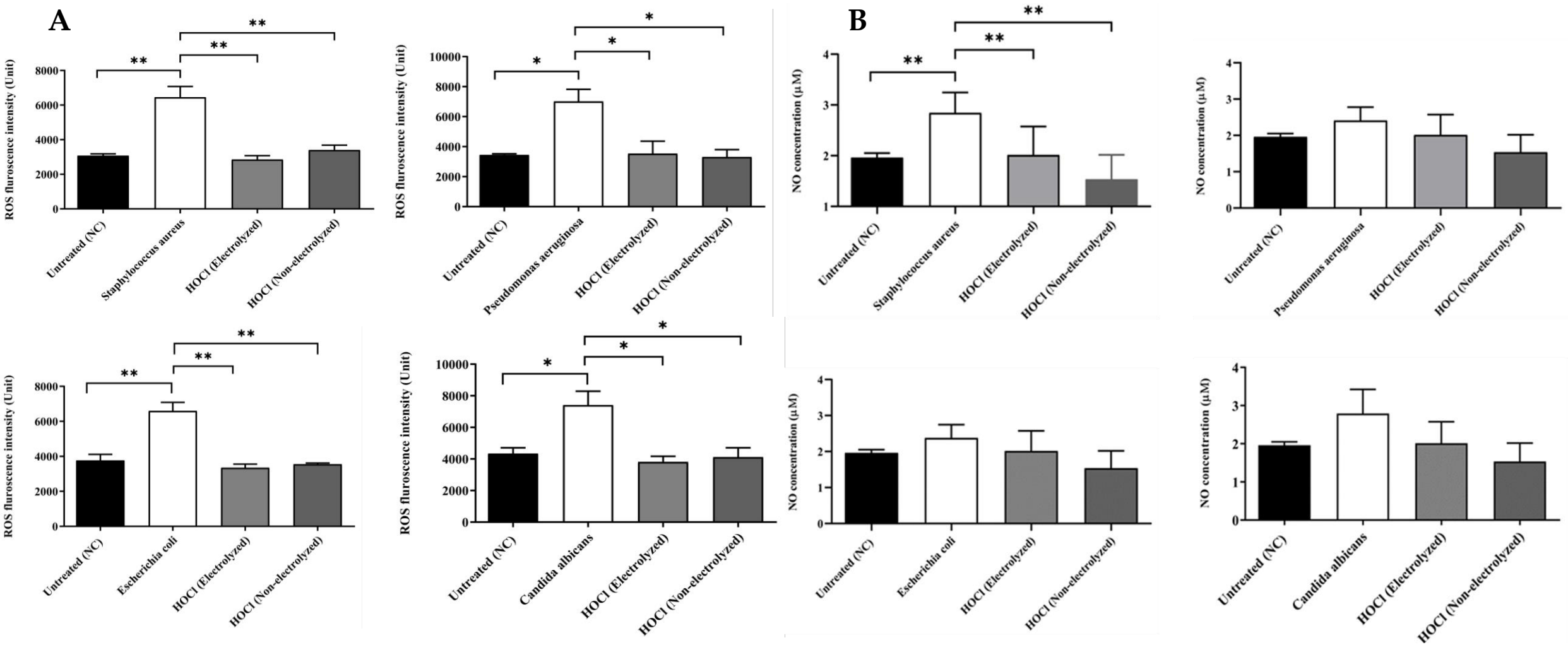


Figure 7. ROS and NO levels after HOCl and non-HOCl treatment on the pathogenic bacteria and fungi.

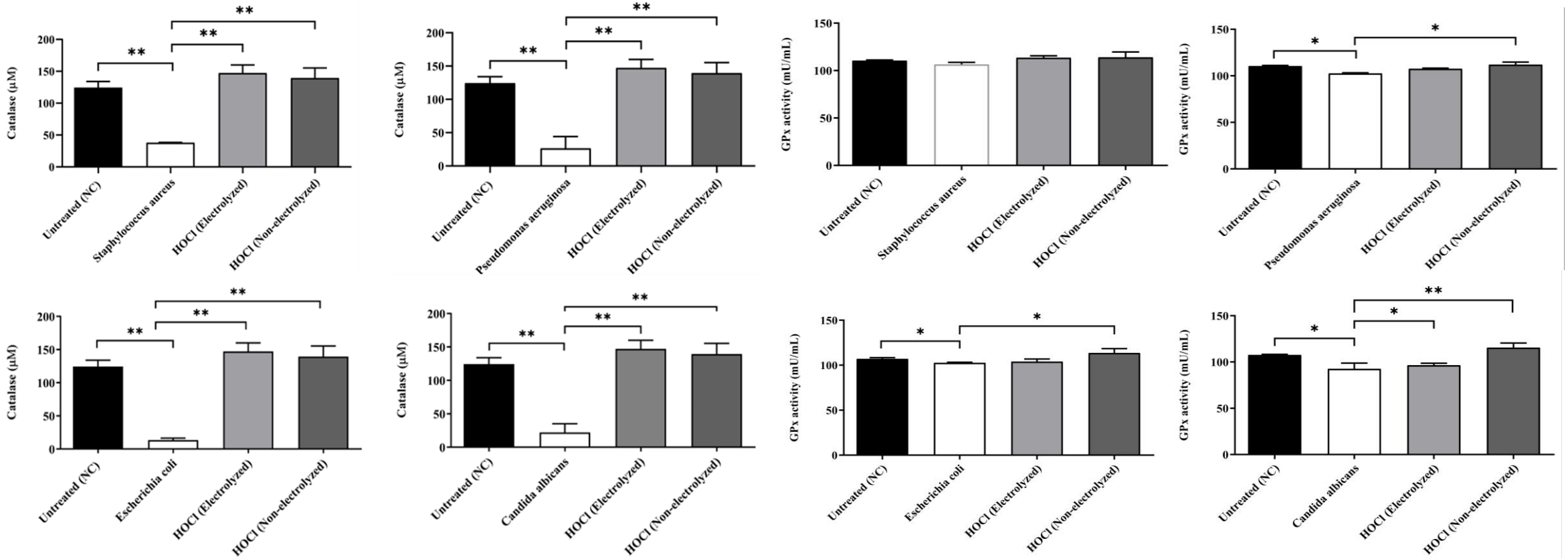


Figure 8. Catalase and GPx levels after HOCl and non-HOCl treatment on the pathogenic bacteria and fungi.

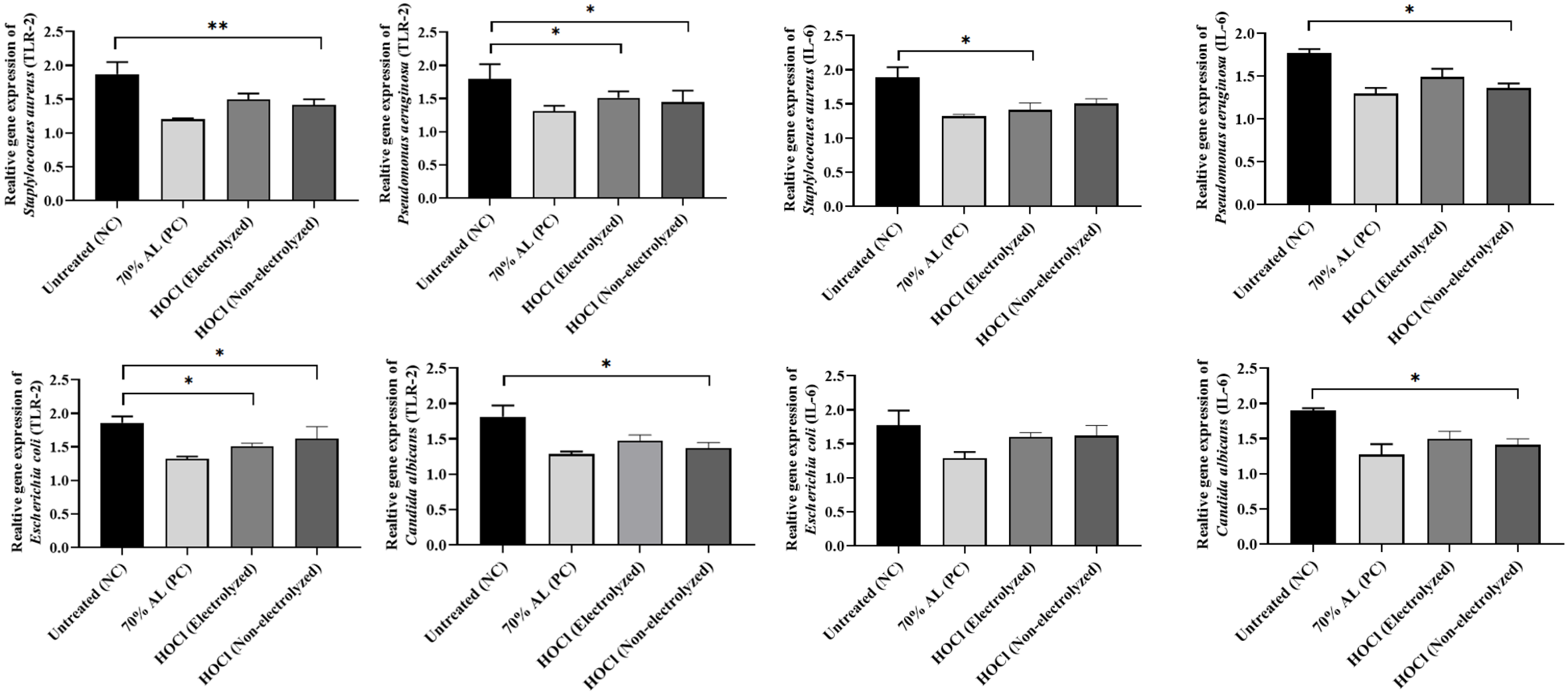


Figure 9. Anti-inflammatory effects of electrolyzed and non-electrolyzed HOCl on TLR-2 and IL-6 in SK-MEL-1 cells.

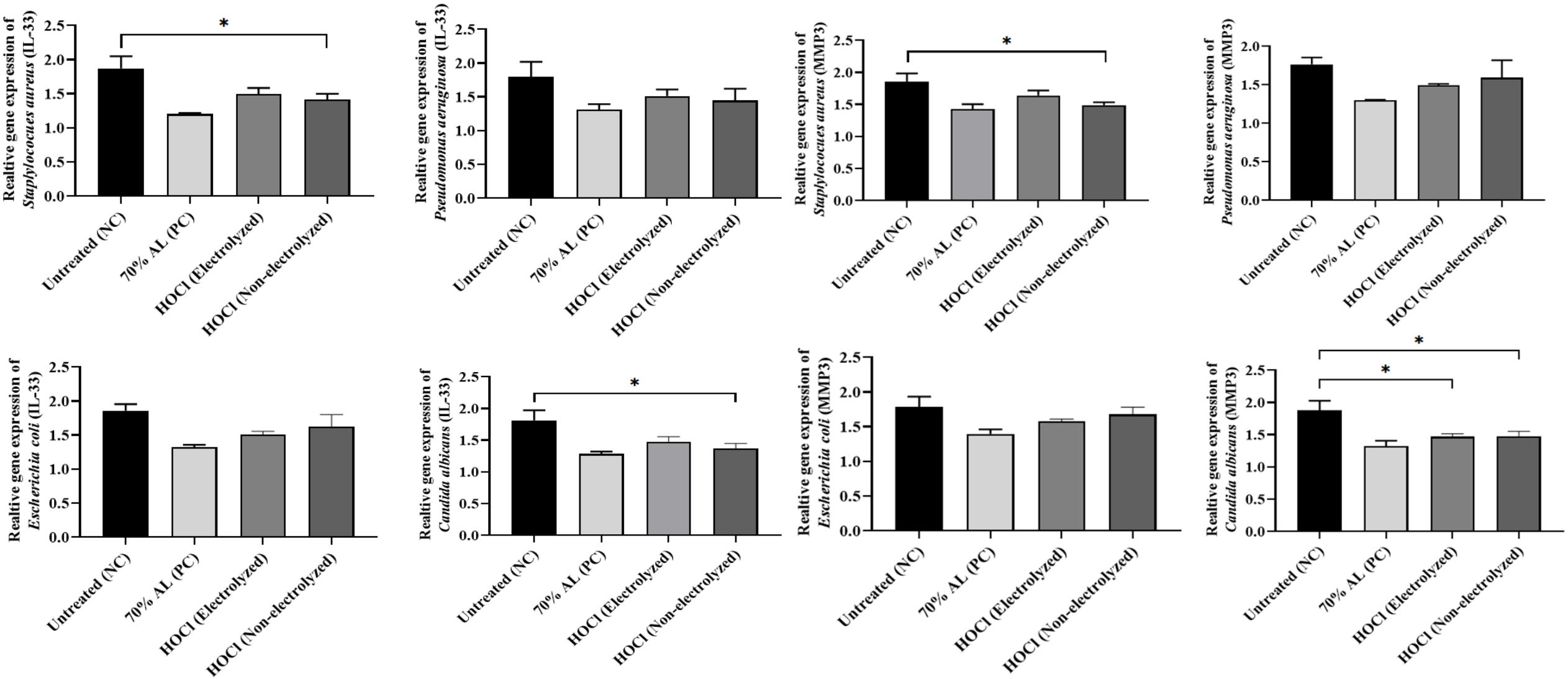


Figure 10. Anti-inflammatory effects of electrolyzed and non-electrolyzed HOCl on IL-33 and MMP-3 in SK-MEL-1 cells.

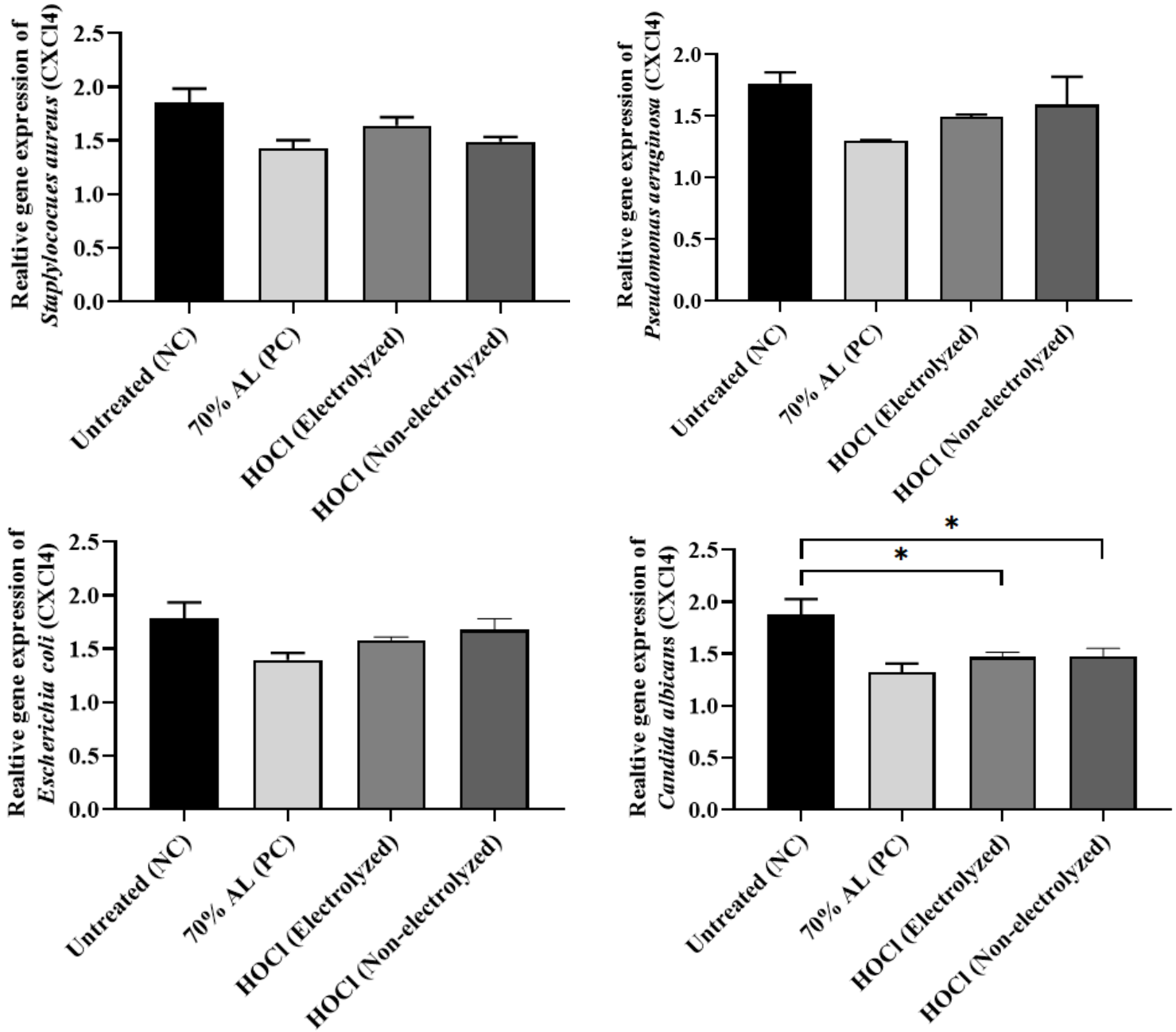


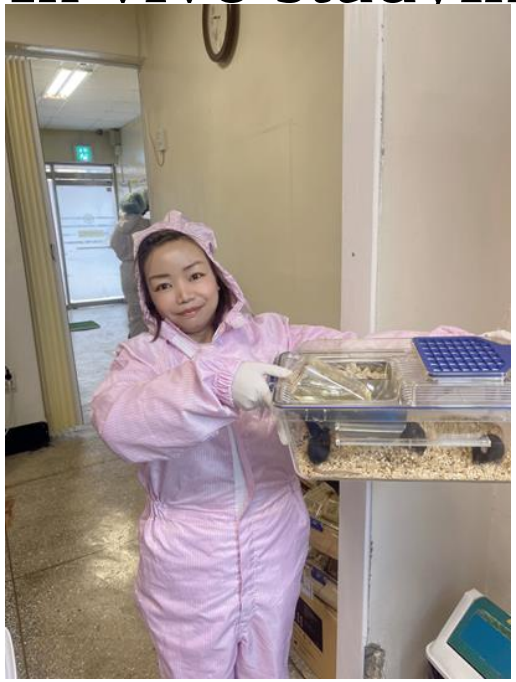
Figure 11. Anti-inflammatory effects of electrolyzed and non-electrolyzed HOCl on CXCl4 in SK-MEL-1 cells.

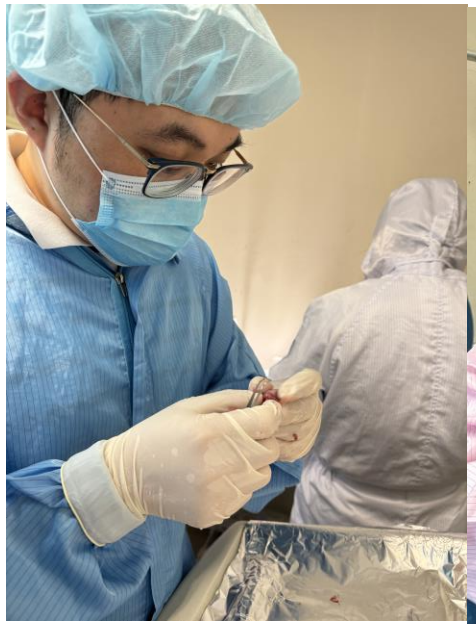
Conclusion

This study emphasizes the potential of non-electrolyzed HOCl as a therapeutic agent against bacteria and fungi, providing a promising avenue for future treatments. Continued research efforts are essential to fully understand its mechanism of action and further establish it.

Further, established in vivo studying

In vivo studying Mice Sacrifice





Thank you for your time!