The Effect of Copper-Iodine Complex Solution on Relevant Microbial Pathogens: An *in vitro* Time-Kill and Persistence Study

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Introduction

Copper-Iodine Irrigation Solution (CICS) is an FDA 510(k) cleared medical device. It is indicated in wound management, cleansing, irrigating, moisturizing and debriding of acute and chronic dermal lesions that are partial or full thickness wounds. The indications include 1st and 2nd degree burns, stage I–IV pressure ulcers, diabetic ulcers, stasis ulcers, abrasions and minor skin irritations, post-surgical wounds, grafted and donor sites. This unique Copper-Iodine Complex as a preservative in solution can neutralize a broad number of pathogens such as bacteria, fungi, yeast, and viruses without evoking bacterial resistance[1-4].

CICS has been proven to be safe, non-cytotoxic, non-pyrogenic, non-irritating, and non-sensitizing to dermal tissue[5-6]. It can be applied directly to the wound bed and can be used with static or pulsed lavage, low frequency ultrasonic debridement, hydrosurgical debridement, and negative pressure wound therapy, with or without instillation mode. Also, importantly, it does not need to be rinsed after treatment or application in either static or dynamic mode, resulting in reducing procedure times. An acute and especially chronic wounds and burns can be colonized in hours of exposure, and this effects the ability to maintain linear healing. This issue alters the pH of the wound bed towards alkalinity and allows pathogens to flourish. CICS has as a dermal pH of 5.0 and assists in improving the wound bed environment by decreasing pathologic microorganisms' survivorship. There are many gram-positive and gram-negative bacteria, yeast and fungi responsible for subclinical and clinical infection in the wound bed.

We identified 21 of the common microbial pathogens tested in this study. These microorganisms were tested with CICS *in vitro* with respect to the Time-Kill effect and the persistence of the antimicrobial activity as a preservative in solution against these known pathogens. Yeast infections that are produced by Candida spp. are common in chronic wounds and burns. Candida albicans-related infection has multiple treatment options, both topical and pharmacological. However, Candida auris is an emerging World Health Organization threat with no known pharmacologic treatment course and can lead to significant morbidity and mortality. The purpose of this *in vitro* study is to demonstrate the Time-Kill effect and antimicrobial persistence with microbial reinoculation without adding additional Copper-Iodine Complex Solution at the data points.

Materials and Methods

Persistent antimicrobial efficacy determination of CICS against clinically relevant pathogens in the face of pathogen re-inoculation at 5 different time points. Test followed the same culture and plating procedure as Time-Kill test with pathogen re-inoculations at 1 minute. The antimicrobial activity of the Copper-Iodine Complex Solution (CICS) and was determined using a time-kill assay. Tubes containing 4 mL each test substance was inoculated with ~105 CFU/mL microorganisms. The solution was mixed thoroughly and incubated at 37 °C. At each time point, 0.04 mL aliquots of the test substance were removed and added to 0.360 mL Dey-Engley Neutralizing Broth (DE broth) and mixed thoroughly. Samples were plated using the spiral plater and incubated for 48 hours under organism appropriate conditions. Titer controls were also prepared at each time point. Persistent antimicrobial efficiency determination of CICS against the same clinically relevant pathogens in the face of re-inoculation at 5 relevant time points. Test followed the same culture and plating procedure as Time Kill test, with pathogen re-inoculation at 10 minutes, 4, 24, 48, and 72 hours after initial inoculation.

The log reduction values were calculated using the following formula:

$$\text{Log reduction} = \log_{10} U - \log_{10} C$$

Where: U = Average titer control
C = Average recovered counts

The percent reduction values were calculated using the following formula:

$$\%\ \text{reduction} = (1 - 1/10^{(\log\ \text{reduction})}) \times 100\%$$

Results

Table 1: Time-Kill Data of Copper-Iodine Complex Solution (CICS) against 19 Relevant Pathogens

Microorganism	Exposure Time	% Reduction
Pseudomonas aeruginosa (ATCC 10145)	1 min	99.9999
Staphylococcus aureus (ATCC 6538)	1 min	100
Streptococcus pyogenes (ATCC 19695)	1 min	100
Escherichia coli (ATCC 25922)	1 min	100
Acinetobacter baumannii (ATCC 19606)	1 min	100
Klebsiella pneumoniae (ATCC 4352)	1 min	100
VRE (ATCC 70021)	1 min	100
Staph. aureus MRSA (ATCC 33592)	1 min	99.9979
Candida albicans (ATCC 10231)	1 min	99.9251
Candida auris (ATCC B11221)	1 min	96.6024
Candida tropicalis (ATCC 750)	1 min	96.8063
Proteus mirabilis (ATCC 29906)	1 min	100
Staphylococcus epidermidis (ATCC 35984)	1 min	100
Streptococcus salivarius (ATCC FUA35984)	1 min	99.9998
Enterococcus faecalis (ATCC 29212)	1 min	99.0064
Klebsiella aerogenes (ATCC 13048)	1 min	100
Enterococcus faecium (ATCC 19434)	1 min	99.3426
Trichophyton interdigitale (ATCC 9533)	1 min	100
Aspergillus brasiliensis ATCC 11232	1 min	99.9999
Cutibacterium acnes ATCC 11827	1 min	99.9999
Clostridium difficile spores (ATCC BAA1870)	30 min	67.2789
	1 hr	86.7129
	4 hrs	96.4405
	24 hrs	99.9992

Results

Table 2: Persistent Antimicrobial Efficacy of CICS against Clinically Relevant Pathogens. Sustained *in vitro* antimicrobial activity after pathogen re-inoculation over 72 hours

Microorganism	Re-challenge Interval	% Reduction
Pseudomonas aeruginosa (ATCC 10145)	10 min	>99.99997
	4 hours	>99.99997
	24 hours	>99.99997
	48 hours	>99.99997
	72 hours	>99.99997
Staphylococcus aureus MRSA (ATCC 33592)	10 min	>99.99998
	4 hours	>99.99998
	24 hours	>99.99998
	48 hours	>99.99998
	72 hours	>99.99998
Streptococcus pyogenes ATCC 19695	10 min	>99.99999
	4 hours	>99.99999
	24 hours	>99.99999
	48 hours	>99.99999
	72 hours	>99.99999
VRE ATCC 700221	10 min	>99.99999
	4 hours	>99.99999
	24 hours	>99.99999
	48 hours	>99.99999
	72 hours	>99.99999
Klebsiella pneumoniae ATCC 13048	10 min	>99.99999
	4 hours	>99.99999
	24 hours	>99.99999
	48 hours	>99.99999
	72 hours	>99.99999
Acinetobacter baumannii ATCC 19606	10 min	>99.99999
	4 hours	>99.99999
	24 hours	>99.99999
	48 hours	>99.99999
	72 hours	>99.99999
Candida albicans ATCC 10231	10 min	>99.99952
	4 hours	>99.99999
	24 hours	>99.99999
	48 hours	>99.99999
	72 hours	>99.99999
Candida auris B11221	10 min	>99.99993
	4 hours	>99.99999
	24 hours	>99.99999
	48 hours	>99.99999
	72 hours	>99.99999

Conclusion

Copper-Iodine Complex Solution (CICS) demonstrated an effective time-kill rate (neutralization) of 21 clinically relevant pathogens at I minute of exposure. Additionally, the antimicrobial persistence testing with re-inoculation using microbes of 105 CFU/mL over 5 time points up to 72 hours has significant positive implications for the management of acute and chronic wounds, trauma and burns. CICS has the equilibrium and synergy resulting in the ability to continue to release additional free iodine as needed (Chemistry in Reserve) to neutralize the microbes during reinoculation. Clinically, the wound bed wound never be challenged with a 105 CFU/mL in such short duration as demonstrated in the persistence phase of this *in vitro* trial. CICS was never refreshed throughout the 5 re-inoculations.

There are many other medical and surgical specialties that can benefit from this novel technology. Surgical specialties such as orthopedics, neurosurgery, plastics, general, colorectal, ENT, urology, GYN, oral maxillofacial, and others all can derive improved outcomes for their patients. The success of primary closure, delayed primary closure, autografts, allografts, and xenografts depend on excellent wound bed preparation for positive outcomes. Regardless of the medical or surgical discipline, excellence in wound bed preparation is essential and Copper-Iodine Complex Solution can be beneficial and improve outcomes as part of the treatment process.

References

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