Research Compact

Tags Wound model, micro-environment

Title **Composition of Challenge Substance in Standardized Antimicrobial Efficacy Testing of Wound Antimicrobials Is Essential to Correctly Simulate Efficacy in the Human Wound Micro-Environment**

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Source 2022, Biomedicines, https://doi.org/10.3390/biomedicines10112751

Aim of the study

National and international standards have been introduced to test antimicrobial and antiseptic agents for specific indications in terms of effect and cytotoxicity on an *in vitro* level to establish baseline efficacy profiles. These standards insufficiently acknowledge the influence of the wound micro-environment on the efficacy of antimicrobial agents. Studies demonstrated significant and relevant variations between standard conditions, challenged conditions, artificially constructed wound conditions, and conditions using human material to simulate the acute/chronic wound micro-environment. The intention was a broad and comprehensive *in vitro* comparison of current standard test conditions, a modified peptide-based challenge, and the simulated acute/chronic wound conditions on the efficacy of most used antimicrobial and antiseptic solutions to determine which form of challenge most likely mimics the performance to be expected in a real-world scenario.

Methods

To address this, six antimicrobial and/or antiseptic wound irrigation solutions with octenidine/phenoxyethanol, polyhexanide, povidone-iodine, and sodium-hypochloride/hypochlorous acid solutions were submitted to standard-based (DIN-EN-13727) or modified peptide-based challenges and compared to a simulated clinical reference using human acute or chronic wound exudate (AWF/CWF). Antimicrobial efficacy against *S. aureus* and *P. aeruginosa* was compared using a quantitative suspension method. Agreement between methods were investigated using Bland-Altman analysis with AWF and CWF considered as comparison standard.

Setup Challenge Conditions

Volume Fraction	DIN-EN-13727 Challenges			Wound Challenges		Peptide/Protein Challenges		
	DS w/o	*DS + LB*	*DS + HB*	*AWF*	*CWF*	*TSB w/o*	*TSB + LB*	*TSB + HB*
100 µL	BS-DS	BS-DS	BS-DS	BS-DS	BS-DS	BS-TSB	BS-TSB	BS-TSB
100 µL	H_2O d.d.	0.3% BSA	3% BSA, 3% SE	AWF	CWF	H_2O d.d.	0.3% BSA	3% BSA, 3% SE
800 µL	TS	TS	TS	TS	TS	TS	TS	TS

(TS: Test solution; DS: Diluent solution; BS-TSB: Bacterial suspension in tryptone soy broth (TSB); BS-DS: Bacterial suspension in diluent solution; LB: Low burden; HB: High burden; AWF: Acute wound exudate; CWF: Chronic wound exudate; H_2O: Water; BSA: Bovine serum albumin; SE: Sheep erythrocytes).

Baseline solution	concentration [mg/ml]
DS w/o	0.379 ± 0.015
TSB w/o	1.762 ± 0.039
LB	3.598 ± 0.241
HB	46.173 ± 6.287
AWF	51.776 ± 4.484
CWF (~ 37%)	15.871 ± 1.793

Results

Different substances and challenges demonstrated diverging results, depending on class and concentration of agent and challenge. Highly concentrated antiseptics maintained a high efficacy under complex challenges, while especially chlorine-based irrigation solutions showed a remarkably reduced antimicrobial effect. Composition of challenge substance proved more relevant than pure concentration.

<0.005% NaOCl/HOCl: High efficacy and significantly reduced initial bacterial counts within 1 min under the standards (DIN-EN-13727) unchallenged and low burden conditions. When a higher level of challenge and biological burden was introduced, no overall antimicrobial effect was detected.

<0.08% NaOCl: Reduction in detectable counts was achieved within 1 min for unchallenged and low burden conditions, only a marginal reduction could be observed under high burden challenge conditions. Increased exposure to 15 min improved the antimicrobial effect.

0.2% NaOCl/SS: Overall high and significant antimicrobial efficacy within 1 min regardless of the challenge condition. Only for the modified peptide-challenge a prolonged exposure of 5 min (low burden) and 15 min (high burden) were necessary to achieve complete reduction.

0.04% PHMB: Highly effective results were observed against both bacterial species within 5 min the latest. With an increasing biological burden, reduction in initial bacterial counts was reduced at the individual time-points and overall delayed over time.

0.1%/2% OCT/PE and 10% PVP-IOD: Significant and high efficacy under all investigated challenge conditions.

Bland-Altman analysis revealed differences between the test conditions and the simulated wound environment. When comparing AWF with modified peptide challenge conditions, there was high agreement between the test methods and at low and no burden. Compared with other test conditions, the efficacy of the antiseptics tested was mostly overestimated. When CWF was compared, all models showed significant bias and thus over- or underestimation of antimicrobial efficacy.

Conclusion

Current standard challenge conditions did not adequately reflect the wound micro-environment with over- or underestimating antimicrobial efficacy, whilst the modified peptide-challenge showed a higher level of agreement with simulated realistic conditions (AWF/CWF). The results emphasize that a “one-fits-all” approach is not feasible to generalize antimicrobial efficacy, as certain aspects of the complex micro-environment pose a differing influence on varying agents.