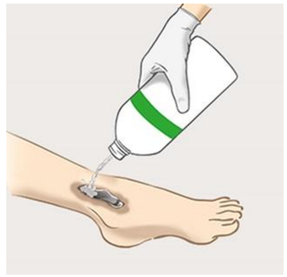
In vitro evaluation of cytotoxic and proliferative effects of wound irrigation solutions on human skin cells

Ismena Mardani, Malin Bjursten Brailsford, Tina Hamberg, Anette Svensson Henriksson
Mölnlycke Health Care, Mölndal, Sweden

INTRODUCTION

- Effective wound irrigation is essential, as removing microorganisms and debris from the wound bed can prevent delays in the healing process[1].
- Simple cleansing methods like soap and water are inadequate, while solutions such as those containing polyhexamethylene biguanide (PHMB) can be too harsh and damage healthy cells and tissue[2,3].
- A biocompatible solution can be crucial to ensure safe wound cleansing and to maintain proper moisture balance within the wounds.
- One way to assess the safety and quality of a wound irrigation solution is to evaluate its effect on cell viability and proliferation *in vitro*.



AIM To assess the cytotoxic and proliferative effects of wound irrigation solutions on human skin cells

METHODS

The cytotoxic and proliferative effects of five wound irrigation solutions containing hypochlorous acid (HOCl) ranging from 0.003% to 0.08%, and one solution containing polyhexamethylene (PHMB) at 0.1% were evaluated (Table 1).

Table 1. Wound irrigation solutions tested

Solution	Active substance	Conc. (%)
A	HOCl	0.003
B	HOCl	0.004
C	HOCl	0.005
D	HOCl	0.033
E	HOCl	0.08
F	PHMB	0.1

Cell preparation:

Normal Human Dermal Fibroblasts (NHDFs) and Normal Human Epidermal Keratinocytes (NHEKs) were seeded in a semi-confluent cell layer in low or serum-free medium. The cells were exposed to the wound irrigation solutions for 15 minutes, simulating a clinically worst-case scenario. The exposure was dose-dependent, ranging from undiluted (100%) down to 50% dilution.

Cytotoxicity measurement:

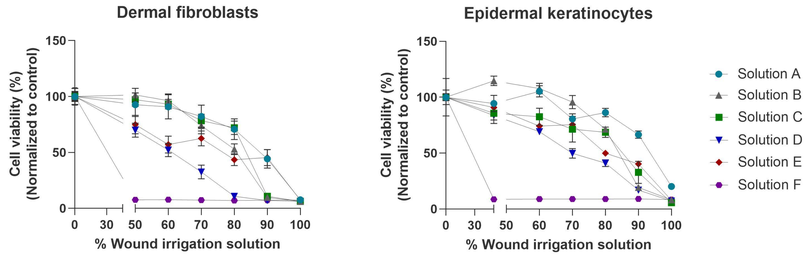
Cell viability was assessed with Cell Counting Kit-8 (CCK-8) assay promptly after 15-minutes exposure. The assay utilizes a tetrazolium salt which, when reduced in viable cells produces a dye. The amount of dye generated is directly proportional to the number of living cells, and its absorbance can be measured using a spectrophotometer.

Proliferation measurement:

Cell proliferation was measured with CyQUANT cell proliferation assay, 24h post-treatment to allow for cell division to occur. The assay utilizes a fluorescent nucleic acid dye that selectively binds to the DNA of living and dividing cells. In addition, a cell-impermeant background suppressor is included to prevent staining of dead cells or cells with compromised membranes ensuring that only live and healthy cells are measured using a fluorescent spectrophotometer.

RESULTS

Cell cytotoxicity



The results demonstrated superior cell viability (across both NHDF and NHEK cells) for the three wound irrigation solutions with the lower concentrations of HOCl (Solution A-C). In contrast, the higher concentrations of HOCl (Solution D and E) significantly reduced cell viability, e.g. by up to 60% ($p<0.0001$) in NHDF, particularly at lower dilutions (70-80%). The PHMB-based irrigation solution (Solution F) significantly reduced cell viability for both NHDF and NHEK across all dilutions ($p<0.0001$), (Figure 1).

Figure 1. Cell viability (%), normalized to untreated control, for dermal fibroblasts and epidermal keratinocytes, n=6, data presented as mean±.SD.

Cell proliferation

A comparable pattern was detected in the cell proliferation assays, where both PHMB and higher-concentrations of HOCl irrigation solutions (Solution D-E) markedly suppressed proliferation by as much as 70% ($p<0.0001$) independent of cell-type. This is in comparison to the wound irrigation solutions with lower concentration of HOCl (Solution A-C), (Figure 2).

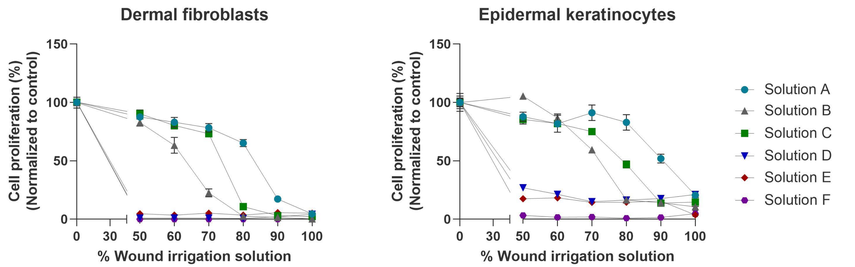


Figure 2. Cell proliferation (%), normalized to untreated control, for dermal fibroblasts and epidermal keratinocytes, n=6, data presented as mean±.SD.

CONCLUSIONS AND TAKE-HOME MESSAGES

This study highlights the importance of understanding how wound irrigation solutions affect tissue viability. Their impact on both cell survival and proliferation is critical, as these factors directly influence the wound healing process. Minimizing the potential toxicity of an irrigation solution towards skin cells can directly reduce the risk of impaired healing, which is an essential consideration when selecting an appropriate wound irrigation solution.

- Wound irrigation solutions containing higher concentrations of HOCl (≥0.033%) or PHMB (0.1%) adversely affect cell viability and proliferation in NHDF and NHEK.
- In comparison, solutions with lower HOCl concentrations (0.003-0.005%) exhibit improved biocompatibility with human skin cells.
- Selecting a wound irrigation solution that is biocompatible can mitigate disrupted wound healing.

References
[1]Edwards-Jones V, Flanagan M, and Wolcott R (2015) Technological advancements in the fight against antimicrobial resistance. Wounds Int 6(2):47-51. [2]Zhang M, Jin J, Liu Y, Ben C, Li H, Cheng D, et al. Analysis of povidone iodine, chlorhexidine acetate and polyhexamethylene biguanide as wound disinfectants: in vitro cytotoxicity and antibacterial activity. *BMJ Nutrition, Prevention & Health*. 2023;6. [3] Rembe JD, Fromm-Dornieden C, Schäfer N, Böhm JK, Stuermer EK. Comparing two polymeric biguanides: chemical distinction, antiseptic efficacy and cytotoxicity of polyaminopropyl biguanide and polyhexamethylene biguanide. J Med Microbiol. 2016 Aug;65(8):867-876.

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