Title: Permeation of chlorhexidine from alcoholic and aqueous solutions within excised human skin

Short running title: Skin penetration of chlorhexidine

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Chlorhexidine digluconate (CHG) is widely used in the clinical setting for skin antisepsis prior to incision or insertion of medical devices for example central venous catheters (11-13), however, its permeation into skin is limited (6, 7, 9, 10, 16). In a recent study, we demonstrated the limited penetration of CHG in a skin model comprising full thickness excised human skin following application of 2% (w/v) aqueous CHG (9). The aim of this current study was to compare the penetration of chlorhexidine into skin following topical application of 2% (w/v) CHG in 70% (v/v) iso-propyl alcohol (IPA) compared to aqueous CHG.

Skin permeation studies were performed on full-thickness excised human skin as described previously (9). Many differences between animal models and human skin absorption have been shown to be permeant-specific, and due to the applied nature of this work, it was considered that human skin must be used (3, 5). Due to the limitation with availability of fresh human skin, frozen skin is most often used as a model to minimize interpersonal variability on the donor skin permeability. Human skin has benefits over animal skin and storage of human skin for a prolonged times has been shown not to have a significant effect on skin permeability (3, 5). In brief, excised human skin was exposed to aqueous and alcoholic CHG for clinically relevant time periods of 2 min and 30 min in a Franz cell diffusion model. The concentration of CHG in serial skin sections was determined by high-performance liquid chromatography.

Overall, following a 2 and 30 min exposure, skin penetration of CHG from both aqueous and alcoholic solutions was limited (Figures 1 and 2). At skin depths of $\geq$300
μm the concentration of CHG detected from both solutions was negligible (<0.0008 µg/mg tissue). In addition, CHG did not completely permeate through the full thickness human skin model.

The concentration of CHG recovered within the top 100 µm skin sections was significantly less following a 2 min exposure to alcoholic CHG than that following similar exposure to aqueous CHG [mean CHG concentration (± s.e.) of 0.023 (± 0.007) µg and 0.157 (± 0.047) µg per mg tissue for CHG/IPA and CHG respectively, (p=0.008)]. Following a 30 min exposure, there was no significant difference in skin penetration of CHG from alcoholic and aqueous solutions within the model (p>0.05).

The results from this study clearly demonstrate the limited permeation of CHG within a human skin model following application of either alcoholic or aqueous solutions. Moreover, the negligible concentrations of CHG detected at skin depths of >300 µm may indeed allow for microorganisms residing in the deeper layers, for example around hair follicles, to survive the skin antisepsis procedures recommended in the current EPIC guidelines (13).

Whilst chlorhexidine in alcoholic solution has clearly been shown to have superior antimicrobial activity compared to aqueous CHG (1, 8), their efficacy in reducing catheter colonization and infection is comparable (14). Alcohol, at a concentration of 70% (v/v) has rapid antimicrobial activity against a broad spectrum of microorganisms (2). However, it has also been shown to extract important lipid components of the stratum corneum (SC) and to cause dehydration of SC proteins, thus potentially compromising the permeation of CHG within the skin (4, 15). These results
clearly lay the foundation for further research within the field of skin antisepsis with a view to developing improved formulation strategies for use of CHG in clinical practice.


**Figure 1.** Penetration profile showing the location and concentration of chlorhexidine (µg/ mg tissue) in excised human skin after 2 min (n=15) exposure to 2% (w/v) chlorhexidine digluconate in 70% (v/v) isopropyl alcohol and aqueous 2% (w/v) CHG (mean ± s.e; *p=0.008).
**Figure 2.** Penetration profile showing the location and concentration of chlorhexidine (μg/ mg tissue) in excised human skin after 30 min (n=15) exposure to 2% (w/v) chlorhexidine digluconate in 70% (v/v) isopropyl alcohol and aqueous 2% (w/v) CHG (mean ± s.e).