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# A cytotoxic analysis of antiseptic medication on skin substitutes and autograft

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**Conflicts of interest**
*None declared.*

## Summary

*Background* There is an increasing demand for the clinical application of human skin substitutes (HSSs) for treating ulcers, burns and surgical wounds. Due to this increasing demand and due to the simultaneous requirement for the administration of topical antiseptic medications, there is a need to determine potential cytotoxic effects of these medications on HSSs compared with autograft skin.

*Objectives* To perform such an evaluation.

*Methods* Two different HSSs were used (autologous reconstructed epidermis on fibroblast-populated human dermis and allogeneic reconstructed epidermis on a fibroblast-populated rat collagen gel) and were compared with conventional full-thickness autograft. Twelve different antiseptics were applied topically to the stratum corneum *in vitro* for 24 h. The degree of cytotoxicity was analysed as detrimental changes in histology, metabolic activity (MTT assay) and RNA staining of tissue sections.

*Results* The antiseptic medications tested showed different degrees of cytotoxicity. Acticoat®, Aquacel Ag®, Dermacyn®, Fucidin®, 0·5% silver nitrate solution and chlorhexidine digluconate were not cytotoxic for either HSS or autograft, and can therefore be used as required. Flamazine® and zinc oxide cream resulted in moderate cytotoxicity. However, application of Betadine®, cerium-silver sulfadiazine cream, silver sulfadiazine cream with 1% acetic acid and Furacine® resulted in a substantial decrease in cell viability and a detrimental effect on tissue histology when applied to autograft and especially to HSS.

*Conclusions* Due to the potential cytotoxic effect of some antiseptics on HSS, it is advised that clinicians balance the cytotoxicity of the medication, its antiseptic properties and the severity of colonization in choosing which one to apply.

Medical specialists are still challenged in finding the optimal treatment for difficult-to-heal wounds, e.g. ulcers, trauma-induced wounds and deep burns. Open wounds are susceptible to invading pathogens such as bacteria and fungi. Therefore optimal wound healing is dependent on the type of antiseptic medication used in combination with the method of wound closure. In the case of small, acute wounds and large burn/trauma wounds an autograft (split-skin or full-thickness) is conventionally applied to close the wound directly.[1] Use of autograft skin requires relatively large amounts of donor skin which is often limited and the resulting scar formation, particularly when meshed or split-skin is used, is suboptimal. Ulcer patients are conventionally treated with wound debridement followed by wound dressings and/or compression therapy and in some cases autografts (punch biopsies or split-skin).[2,3] However, wound closure is a lengthy process and a therapy-resistant group of ulcers remains open.[3]

In all areas of wound healing, advances are being made in the field of tissue engineering as it is now thought that optimal wound healing may be achieved by application of tissue-engineered skin products. Developing areas of application include ulcers (venous, diabetic foot, decubitus), burns, trauma-induced wounds, tumour excision sites and treatment of bullous disease.[4] Application of tissue-engineered skin constructs has certain advantages over conventional methods: allogeneic human skin substitute (HSS) requires no donor skin and is an off-the-shelf product; autologous HSS requires less donor skin than autograft skin and the use of the patient's own cells ensures a good take. Moreover, tissue engineering is aimed at the modification of skin substitutes towards the

 

treatment of specific problems in wounds, for instance the stimulation of granulation tissue when applied to nonhealing ulcers and the prevention of scar formation when applied to burns.

As most wound beds are prone to colonization with pathogens such as bacteria and fungi, wounds treated with HSS will often also require antiseptics. Ulcers and large burns contain in the majority of cases aerobic Gram-negative (*Pseudomonas aeruginosa* and anaerobic cocci) and Gram-positive bacteria (*Staphylococcus aureus*) (for review see Pruitt *et al.*[5] and Jones *et al.*[6]). The colonization in a wound can impair the take of the HSS or autograft and in turn impair optimal wound healing. If left inadequately treated colonization can lead to morbidity and even mortality.

As antiseptics often have to be applied together with HSS treatment, it is important to evaluate the possible cytotoxicity of these antiseptics on HSS. For this study we used two different full-thickness HSSs, which are constructed in our laboratory. The first HSS is autologous reconstructed epidermis on fibroblast-populated human dermis (HSS-auto). This HSS is routinely used in our hospital for closing chronic wounds.[7] The second HSS is allogeneic (five pooled foreskin donors) reconstructed epidermis on a fibroblast-populated rat collagen gel (HSS-allo). This model is based on that of Bell *et al.*[8] A similar model is also routinely used for closing ulcers[9] and has also been described in the treatment of burns.[10] Both HSSs have a fully differentiated epidermis consisting of a basal layer (BL), stratum spinosum (SS), stratum granulosum (SG) and, importantly, a stratum corneum (SC). The presence of the SC means that, similar to autograft, HSSs exhibit barrier competency. Therefore antiseptics can be applied topically and must first penetrate the nonviable SC in order to exert a cytotoxic effect on the living cell layers below. These HSSs were compared with full-thickness autograft skin in this study. Twelve antiseptics that are routinely used in ulcer and burn wound treatment were analysed for their cytotoxic effect upon topical application *in vitro* to the two HSSs and the full-thickness autograft.

## Materials and methods

### Human skin substitute culture and autograft preparation

#### HSS-allo culture

Dermal fibroblasts were isolated from neonatal foreskins and cultured as described by Ponec *et al.*[11] in Dulbecco's modified Eagle's medium (DMEM) (ICN Biomedicals, Irvine, CA, U.S.A.) containing 1% ultroserG and penicillin/streptomycin (Invitrogen, Paisley, U.K.). Fibroblasts from five independent donors were pooled and incorporated into collagen gels ($1 \times 10^5$ cells $mL^{-1}$) essentially as described by Smola *et al.*[12]

Epidermal keratinocytes were isolated from neonatal foreskins, essentially as described earlier.[11,13] The subconfluent, second-passage cultures of five independent keratinocyte donors were pooled and seeded on to fibroblast-populated collagen gels. After culturing overnight in medium containing DMEM/Ham's F12 (3 : 1), 1% ultroserG, 1 μmol $L^{-1}$ hydrocortisone, 1 μmol $L^{-1}$ isoproterenol and 0·1 μmol $L^{-1}$ insulin, cultures were lifted to the air–liquid interface and cultured for a further 4 days in standard HSS culture medium [DMEM/Ham's F12 (3 : 1) (ICN Biomedicals), 1 μmol $L^{-1}$ hydrocortisone, 1 μmol $L^{-1}$ isoproteronol, 0·1 μmol $L^{-1}$ insulin, $1{\cdot}0 \times 10^{-5}$ mol $L^{-1}$ L-carnitine, $1{\cdot}0 \times 10^{-2}$ mol $L^{-1}$ L-serine, 1 μmol $L^{-1}$ DL-α-tocopherol, penicillin and streptomycin acetate] and enriched with a lipid supplement [containing 25 μmol $L^{-1}$ palmitic acid, 15 μmol $L^{-1}$ linoleic acid, 7 μmol $L^{-1}$ arachidonic acid and 24 μmol $L^{-1}$ bovine serum albumin] supplemented with 0·2% ultroserG. Hereafter, HSS-allo was cultured in standard keratinocyte culture medium supplemented with 50 μg $mL^{-1}$ of ascorbic acid for an additional 14 days.

#### HSS-auto culture

For each 4 $cm^2$ HSS-auto to be constructed, three 3-mm diameter punch biopsies were required. Epidermal sheets and dermal fibroblasts were separated from human adult abdominal skin by incubation in dispase II (Roche, Mannheim, Germany) overnight at 4 °C. HSS was constructed exactly as previously described.[7] In short: epidermal sheets were placed SC side upwards on dead de-epidermized dermis and cultured air-exposed in standard HSS medium supplemented with 0·2% ultroserG. After 7 days of culturing the primary fibroblast culture (in DMEM supplemented with 1% ultroserG and penicillin and streptomycin) and epidermal sheet apart, the fibroblasts were placed in contact with the reticular surface of the de-epidermized dermis in order to allow fibroblast migration into the dermis and then the HSS-auto was further cultured for 2 weeks. During the 3-week culture period, the epidermis expanded to cover the dermis, resulting in approximately 20-fold amplification of the original surface area of the epidermal sheet.

#### Autograft

Full-thickness skin obtained from human abdominal reduction within 12 h after surgery was washed in phosphate-buffered saline, removed of fat and cut into pieces of 4 $cm^2$.

All chemicals were derived from Sigma-Aldrich Chemie BV (Zwijndrecht, the Netherlands) unless otherwise stated.

### Antiseptic exposure

All experiments were performed with three independent donors for autograft and HSS-auto, and three independent cultures (originating from different donor pools) for HSS-allo, each being performed in duplicate within each single donor or donor pool. Autograft and HSSs were placed upon a 24-mm, 3-μm pore size transwell (Corning Inc., Corning, NY, U.S.A.). The bottom compartment of the transwell was filled with 1·5 mL standard keratinocyte culture medium supplemented with 50 μg $mL^{-1}$ ascorbic acid. In the case of autograft and HSS-auto, culture medium was additionally supplemented with

 

**Table 1** Composition of antiseptics used

| Name | Composition | Supplier |
|---|---|---|
| Acticoat® | Absorbent dressing with nanocrystalline silver | Smith & Nephew BV, Hoofdorp, NL |
| Aquacel Ag® | Hydrofibre dressing containing silver | ConvaTec, Woerden, NL |
| Dermacyn® | Superoxidized aqueous solution | Oculus Innovative Sciences Netherlands BV, Sittard, NL |
| Fucidin® | Ointment; 20 mg $g^{-1}$ sodium fusidate in lanolin, cetanol, liquid paraffin and white vaseline | Leo Pharma BV, Breda, NL |
| Furacine® | Ointment; nitrofurazone 2 mg $g^{-1}$ in macrogol 300, 1000, 3000 and water | Norgine BV, Amsterdam, NL |
| Chlorhexidine digluconate | Solution; chlorhexidine digluconate 0·5% in alcohol 70% | Lommerse Pharma BV, Oss, NL |
| Flamazine® | Cream; silver sulfadiazine 10 mg $g^{-1}$ cream (polysorbate 60, 80, glyceryl monostearate, cetylalcohol, liquid paraffin; propylene glycol, water) | Solvay Pharma BV, Weesp, NL |
| Betadine® | Ointment; povidone-iodine, macrogol 400, 4000, 6000 and water | Viatris Manufacturing BV, Diemen, NL |
| Zinc oxide cream (local brand) | Cream; zinc oxide 5% in cetomacrogol | In-house pharmacy, Red Cross Hospital, Beverwijk, NL |
| Cerium-silver sulfadiazine cream (local brand) | Cream; cerium (III) nitrate 2·2%, silver sulfadiazine 1% in Cetiol V, cetomacrogol, sorbitol 70% | In-house pharmacy, Red Cross Hospital, Beverwijk, NL |
| Silver sulfadiazine containing 1% acetic acid (local brand) | Ointment; silver sulfadiazine 1% with 1% acetic acid | In-house pharmacy, Red Cross Hospital, Beverwijk, NL |
| Silver nitrate solution (local brand) | Aqueous solution of silver nitrate 0·5% in macrogol 400 30% and sorbitol 20% | In-house pharmacy, Red Cross Hospital, Beverwijk, NL |

NL, Netherlands.

0·2% ultroserG. Antiseptic ointments and creams were spread evenly in a thin film over 18-mm filter paper discs (Epitest Ltd, Tuusula, Finland). For antiseptic solutions (see Table 1) and sodium dodecyl sulphate (SDS), 200 μL of the solution was used to soak the filter paper discs completely. SDS concentrations used were 10% for autograft and 1% for HSS. These SDS concentrations resulted in total loss of cell viability in autograft and HSS, respectively. Dressings were cut in appropriate 3-$cm^2$ pieces. Antiseptics were placed on HSS and autograft so that they were in direct contact with the SC in a similar manner to applying the antiseptics *in vivo*. Therefore the antiseptic could only penetrate via the SC. Care was taken that no leakage occurred around the edges of the HSS or autograft into the culture medium. Duplicate autograft or HSS cultures were exposed within a single experiment for 24 h at 37 °C in an atmosphere containing 7·5% $CO_2$.

## Measurement of cytotoxicity

### MTT assay

The MTT assay measures mitochondrial metabolic activity and was essentially performed as described by Mosmann.[14] Biopsies were taken from autograft and both HSSs with a biopsy punch 3 mm in diameter (Microtek Medical BV, Zutphen, the Netherlands) and transferred to a 96-well Microlon 200™ flat-bottom microtitre plate (Greiner Bio-one GmbH, Frickenhausen, Germany) filled with 200 μL per well of 2 mg $mL^{-1}$ MTT labelling reagent (Roche). After 2 h at 37 °C the biopsies were transferred to a 96-well microtitre plate containing 200 μL per well isopropanol, acidified with 0·04 mol $L^{-1}$ HCl 3 : 1, and incubated overnight at 37 °C. One hundred microlitres of the extractant solution was transferred to a 96-well microtitre plate for optical density measurements at 550 nm and a reference wavelength of 650 nm.

### Histology and RNA staining

To assess cytotoxicity with respect to changes in tissue architecture, samples were fixed in 4% paraformaldehyde and processed for conventional paraffin embedding. Sections (5 μm) were cut and stained with haematoxylin and eosin for light microscopic examination. Epidermal cytotoxicity, measured as a decrease in keratinocyte RNA, was assessed by pyronine Y staining as described previously.[15] Five-micrometre sections were deparaffinized and then incubated for 30 min at room temperature in a fresh pyronine Y staining solution [0·1% pyronine Y (Fluka Chemie GmbH, Buchs, Switzerland) in 0·2 mol $L^{-1}$ sodium acetate buffer, pH 4·0]. The sections were washed three times in water, air dried, and embedded in Depex mounting medium (BDH, Poole, U.K.). Results were analysed by two independent observers.

## Statistical analysis

The unpaired Mann–Whitney test (nonparametric) was used for statistical evaluation (GraphPad Prism, San Diego, CA, U.S.A.). $P < 0·05$ was considered statistically significant.

 

## Results

In order to determine the cytotoxic effect of antiseptic agents on autograft and the two different HSS models, twelve antiseptic agents were applied topically and cytotoxicity was determined by histology, metabolic activity (MTT) and detection of cellular RNA. For each factor, the degree of cytotoxicity was compared with that in unexposed cultures and in cultures exposed to extreme toxic concentrations of SDS.

Unexposed HSS and autograft consist of fully differentiated epidermis (BL, SS, SG and SC present) on a fibroblast-populated dermal matrix (Fig. 1a). Detrimental changes in tissue histology, for example vacuole formation, condensed nuclei, and separation of the epidermis from the dermal matrix, are directly related to cytotoxicity (Table 2, Fig. 1b). Exposure of autograft and both HSSs to Acticoat®, Aquacel Ag® and Dermacyn® had no detrimental effects on tissue histology with the exception of very mild vacuole formation in the upper layers of the epidermis in HSS-allo cultures exposed to Dermacyn®. Exposure to Fucidin® and silver nitrate solution resulted in mild vacuole formation in both HSSs, while autograft was still unaffected. Chlorhexidine digluconate exposure resulted in similar mild vacuole formation in all three exposed models. Antiseptics that resulted in partial separation of the epidermis from the dermal matrix in addition to mild vacuole formation in autograft and both HSSs were Flamazine® and zinc oxide cream. Severe detrimental effects on tissue histology (e.g. separation of the epidermis from the dermal matrix, condensed nuclei, vacuole formation) were found in all models after exposure to cerium-silver sulfadiazine cream, silver sulfadiazine cream containing 1% acetic acid, Furacine® and

**Table 2** Histological analysis of cytotoxicity

| Antiseptic | Autograft | HSS-auto | HSS-allo |
|---|---|---|---|
| Unexposed | – | – | – |
| Acticoat® | – | – | – |
| Aquacel Ag® | – | – | – |
| Dermacyn® | – | – | + |
| Fucidin® | – | + | + |
| Silver nitrate solution | – | + | + |
| Chlorhexidine digluconate | + | + | + |
| Flamazine® | ++ | ++ | ++ |
| Zinc oxide cream | ++ | ++ | ++ |
| Cerium-silver sulfadiazine cream | +++ | +++ | +++ |
| Furacine® | ++ | +++ | +++ |
| Silver sulfadiazine containing 1% acetic acid | +++ | +++ | +++ |
| Betadine® | ++ | +++ | +++ |
| SDS 10%/1% | +++ | +++ | +++ |

SDS, sodium dodecyl sulphate. Haematoxylin and eosin-stained sections were analysed with respect to epidermal cytotoxicity, taking into account detrimental effects on tissue architecture compared with control, intactness of basal layer, vacuole formation, and condensed nuclei (see Fig. 1b). Cytotoxicity was indexed as follows: – (nontoxic) → +++ (toxic). Data are derived from six samples from three independent donors.

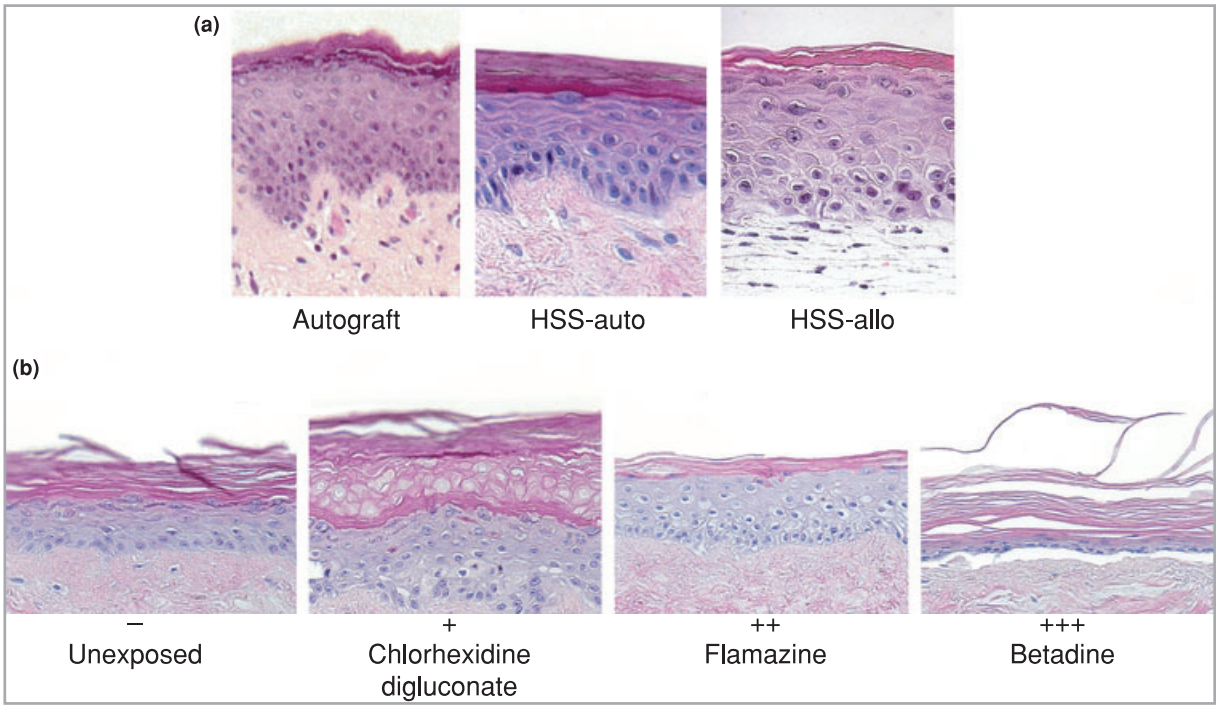


**Fig 1.** Histological analysis of cytotoxicity. (a) Tissue architecture of autograft, HSS-auto and HSS-allo. Haematoxylin and eosin staining of 5-μm paraffin-embedded sections is shown. (b) Tissue architecture of HSS-auto after a 24-h topical exposure to antiseptics showing increasing degrees of cytotoxicity. Cytotoxicity was indexed as follows: – → +++ indicating increasing cytotoxicity with – = unexposed culture, + = chlorhexidine digluconate, ++ = Flamazine®, +++ = Betadine® (see also Table 2). Data are representative of six samples from three independent donors.

 

Betadine®. These effects were slightly less pronounced for autograft exposed to Furacine® and Betadine®

Cytotoxicity can also be determined by the degree of decrease in cell viability. Because metabolic activity is representative of cell viability, the effect of the antiseptic agents on autograft and both HSS models was analysed in an MTT assay that measures mitochondrial activity (Fig. 2). The antiseptics that resulted in MTT levels comparable with those in unexposed models were Acticoat®, Aquacel Ag® and Dermacyn®. MTT levels for this group were > 70% (unexposed cultures = 100%), which implies a very mild cytotoxic effect of these antiseptics on all three models. This result corresponds to our histological observations described above. Antiseptics that resulted in a decrease in MTT in the range of 30–70% compared with unexposed models were Fucidin®, silver nitrate solution, chlorhexidine digluconate, Flamazine®, zinc oxide cream and cerium-silver sulfadiazine cream. Within this group a certain degree of variation was observed between the two HSS models, reflecting the intrinsic differences with respect to penetration and cytotoxicity between the two constructs. The antiseptics that resulted in the lowest MTT levels in HSSs were Furacine®, silver sulfadiazine cream containing 1% acetic acid and Betadine®. MTT values decreased to 0–20% compared with unexposed models. These antiseptics resulted in a similar degree of cytotoxicity to that observed after exposure to toxic concentrations of SDS. For this most toxic group of antiseptics, autograft was affected to a lesser degree than either HSS with values decreasing to 30–55% compared with unexposed models.

If a cell is viable, RNA will be present within the cell. Therefore an early indication of the cytotoxicity of a substance penetrating the epidermis before detrimental tissue damage occurs can be visualized by RNA staining of tissue sections (Table 3, Fig. 3). As a toxic substance penetrates the epidermis, a reduction of RNA staining will occur from the SG down towards the BL. Exposure to Acticoat®, Aquacel Ag®, Dermacyn®, Fucidin® and silver nitrate solution resulted in only a slight reduction of RNA staining in the SG. Cytotoxicity caused by these antiseptics was considered to be extremely mild and cultures were comparable with unexposed cultures. Antiseptics which resulted in a substantial decrease in RNA staining in SG and SS of HSSs after exposure were chlorhexidine digluconate and Flamazine®. For autograft these effects were less pronounced where only minor RNA reduction in SG occurred. Exposure to the antiseptics zinc oxide cream, cerium-silver sulfadiazine cream, Furacine®, silver sulfadiazine cream containing 1% acetic acid and Betadine® resulted in a strong decrease in RNA staining in all layers of both HSSs and autograft, indicating a deep penetration and strong cytotoxicity upon topical exposure. Within this group, Furacine® and Betadine® were less cytotoxic to autograft as observed by less reduction in RNA when compared with either HSS.

In summary, this study shows from all three cytotoxic determinations that Acticoat®, Aquacel Ag® and Dermacyn® are noncytotoxic. Fucidin®, silver nitrate solution, chlorhexidine digluconate, Flamazine®, zinc oxide cream and cerium-silver sulfadiazine cream are moderately cytotoxic. Furacine®, silver sulfadiazine cream containing 1% acetic acid and Betadine® are very cytotoxic. Our results demonstrate that both HSSs

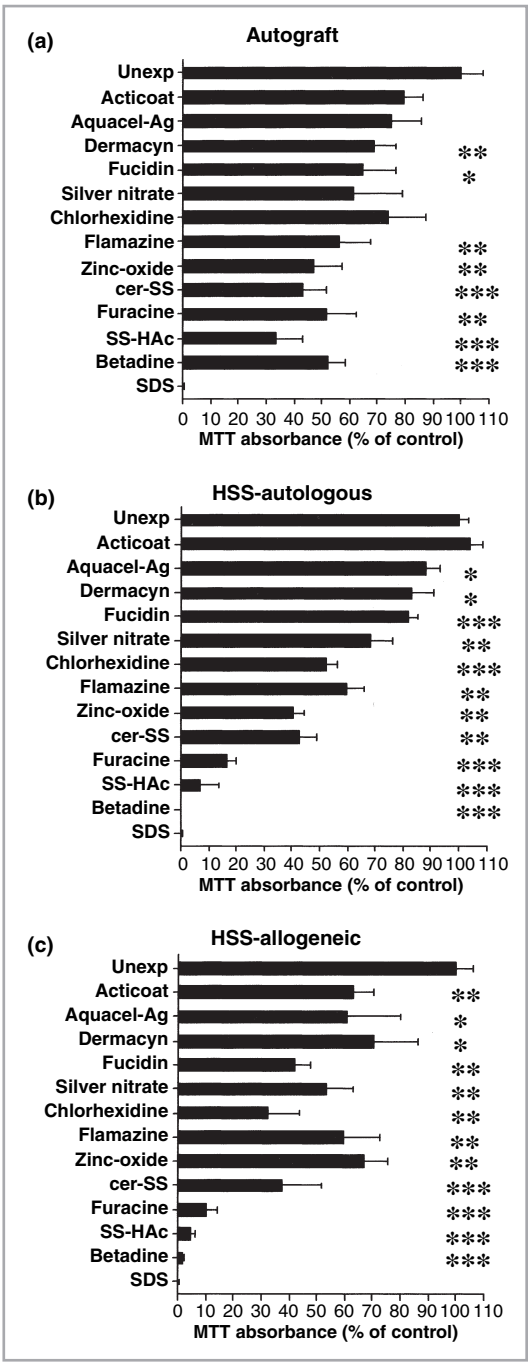


**Fig 2.** Metabolic activity after exposure to antiseptics. MTT levels of autograft, HSS-auto and HSS-allo after exposure to antiseptics. For each individual experiment, MTT levels of unexposed cultures are set at 100% and MTT levels of sodium dodecyl sulphate (SDS)-exposed cultures (total cell death) are set at 0%. Data are presented as mean ± SEM of six experiments, from three independent donors. ***$P < 0{\cdot}001$, **$P < 0{\cdot}01$, *$P < 0{\cdot}05$ vs. unexposed (control) cultures of equivalent model. cer-SS, cerium-silver sulfadiazine cream; SS-Hac, silver sulfadiazine cream containing 1% acetic acid.

 

**Table 3** Detection of cellular RNA

| Antiseptic | Autograft | HSS-auto | HSS-allo |
|---|---|---|---|
| Unexposed | + | + | + |
| Acticoat® | + | + | + |
| Aquacel Ag® | + | + | + |
| Dermacyn® | + | + | + |
| Fucidin® | + | + | + |
| Silver nitrate solution | + | + | + |
| Chlorhexidine digluconate | − SG | −SG, SS | − SG, SS |
| Flamazine® | − SG | − SG, SS | − SG, SS |
| Zinc oxide cream | − SG, SS, BL | − SG, SS, BL | − SG, SS, BL |
| Cerium-silver sulfadiazine cream | − SG, SS, BL | − SG, SS, BL | − SG, SS, BL |
| Furacine® | − SG, SS | − SG, SS, BL | − SG, SS, BL |
| Silver sulfadiazine containing 1% acetic acid | − SG, SS, BL | − SG, SS, BL | − SG, SS, BL |
| Betadine® | − SG, SS | − SG, SS, BL | − SG, SS, BL |
| SDS 10%/1% | − SG, SS, BL | − SG, SS, BL | − SG, SS, BL |

SDS, sodium dodecyl sulphate. Overview showing the presence or absence of RNA in different cell compartments of the epidermis: stratum granulosum (SG), stratum spinosum (SS) and basal layer (BL). + indicates RNA staining in all compartments. − indicates absence/strongly decreased RNA staining in SG, SS and/or BL. Paraffin-embedded sections (5 μm) were stained with pyronine Y as described in Materials and methods. Data are derived from six samples from three independent donors.

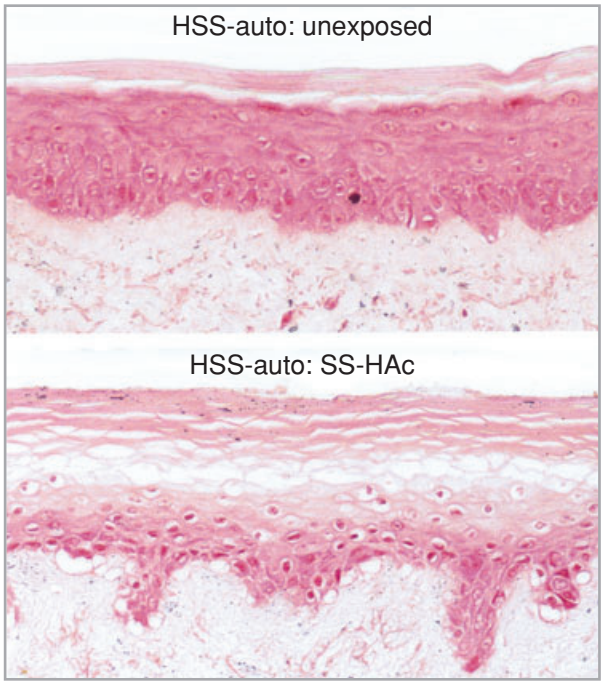


**Fig 3.** Detection of cellular RNA. RNA (dark pink) staining of HSS-auto unexposed and exposed to silver sulfadiazine cream containing 1% acetic acid (SS-HAc) for 24 h. Five-micrometre paraffin-embedded sections were stained with pyronine Y as described in Materials and methods.

were affected to a greater degree than the autograft, with this being especially apparent after exposure to the most cytotoxic antiseptics.

## Discussion

Exposure of autograft and HSSs to 12 antiseptics showed that some antiseptics were not cytotoxic whereas others were extremely cytotoxic. The degree to which a substance was found to be cytotoxic was verified by all three analytical methods: histology, metabolic activity (MTT) and RNA staining. In general, the autograft showed less cytotoxicity than either HSS after exposure to antiseptics. This is most probably due to the fact that although HSSs have a fully differentiated epidermis and SC the barrier function is still slightly less competent than autograft[16] and therefore the rate of penetration of the antiseptics is greater for HSS than for autograft.

Chlorhexidine digluconate solution, Flamazine® cream, Aquacel Ag® dressing and Betadine® ointment are routinely used for the treatment of ulcers (Table 4). Within this group of antiseptics, chlorhexidine digluconate solution is used for superficial and immediate cleaning of the wound area. Because this solution is extremely volatile (ethanol based) and only mildly toxic for HSS, we can conclude that chlorhexidine digluconate can be used together with HSS. Flamazine® cream, Aquacel Ag® dressing and Betadine® ointment, in contrast to the volatile chlorhexidine digluconate, remain in contact with the ulcer for a substantial period of time. The major factor determining which of these three antiseptics is applied to an ulcer is the degree of exudation from the wound. For ulcers with relatively modest exudation, the use of Flamazine® is preferred and occasionally Betadine®. Both of these antiseptics are used as a preventative antiseptic to reduce general microbial burden. However, Betadine® remains active in the wound

**Table 4** Summary of results

| Antiseptic | Wound type | Safe to apply |
|---|---|---|
| Acticoat® | Burn | Autograft/HSS |
| Aquacel Ag® | Ulcer | Autograft/HSS |
| Dermacyn® | Burn | Autograft/HSS |
| Fucidin® | Burn | Autograft/HSS |
| Silver nitrate solution | Burn | Autograft/HSS |
| Chlorhexidine digluconate | Ulcer | Autograft/HSS |
| Flamazine® | Ulcer | Autograft/HSS |
| Zinc oxide cream | Burn | Autograft/HSS |
| Cerium-silver sulfadiazine cream | Burn | No |
| Furacine® | Burn | Autograft only |
| Silver sulfadiazine containing 1% acetic acid | Burn | No |
| Betadine® | Ulcer/burn | Autograft only |

Antiseptics are listed in order of increasing cytotoxicity to human skin substitute (HSS), with Acticoat® being nontoxic and Betadine® being severely cytotoxic.

bed for a relatively short time compared with Flamazine®. As we have shown that Betadine® is severely cytotoxic on HSS whereas Flamazine® only exhibited moderate cytotoxicity, the use of Flamazine® together with HSSs instead of Betadine® is very strongly advised. The dressing Aquacel Ag® is exclusively applied on ulcers with considerable exudation. This is due to the ability of this dressing to gel on contact with wound fluid, thus creating a large fluid-absorption capacity.[17] Furthermore, it contains the antimicrobial active silver in the dressing that is only released in a moist environment. This last property makes Aquacel Ag® less suitable for wounds with only minor exudation. As our data do not show any cytotoxic effect after exposure of Aquacel Ag® to all three models this dressing can be used together with application of either HSS or autograft.

Autografts (full-thickness or meshed split-skin) are often required for the treatment of burns.[1] The use of HSS in burn wound treatment is still infrequent, but there are reports describing the use of the full-thickness HSS, Apligraf® (Organogenesis Inc., Canton, MA, U.S.A.), alone or in combination with meshed split-thickness autograft.[10,18] It is to be expected that the number of reports will increase considerably in the coming years and therefore prior knowledge concerning the administration of antiseptics together with HSS will be beneficial to patient care. The antiseptics used in burn wound care are listed in Table 4. Three different procedures are common within the 5–7 day period after application of an autograft: (i) a solution of Betadine® is applied when burn wounds do not exceed 10% of total body area; (ii) silver nitrate solution is applied when burn wounds exceed 10% of total body area or when wounds are infected with *P. aeruginosa*; and (iii) Furacine® is applied when wounds are infected with *S. aureus*. Betadine® and Furacine® are moderately cytotoxic for autograft and severely cytotoxic for both HSSs. Therefore, Betadine® and Furacine® can be applied to an autograft but it is not recommended to apply either Betadine® or Furacine® to HSS when treating burns. The antiseptic silver nitrate solution showed only mild cytotoxicity upon exposure to any of the three models. Therefore silver nitrate is safe to use when applying autograft or HSS and may be used to replace Betadine® or Furacine® if an HSS is required.

Dermacyn® is used to clean burn wounds. As described above for ulcer treatment, it is only mildly cytotoxic for autograft and the HSSs and therefore can be used to clean wounds prior to application of autograft and HSS.

Antiseptic ointments which are used preoperatively to acute surgical, trauma and burn wounds are Betadine®, Fucidin®, Furacine®, cerium-silver sulfadiazine cream and silver sulfadiazine cream containing 1% acetic acid (Table 4). This study shows that Fucidin® is only mildly cytotoxic to autograft and both HSSs and can therefore be used as required. Furacine® and Betadine® both show moderate and severe cytotoxicity on autograft and HSS, respectively. Therefore their use before autograft transplantation is relatively safe. However, upon HSS application care should be taken to remove all traces of Furacine® and Betadine® prior to placing the HSS. Fucidin® is less cytotoxic than Furacine® and is used to treat the same wide range of cocci infection. As these two antiseptics are often used in an alternating therapy to prevent sensitization it is advised to use the less cytotoxic antiseptic Fucidin® directly prior to transplantation. The antiseptics cerium-silver sulfadiazine cream and silver sulfadiazine cream containing 1% acetic acid were extremely cytotoxic to autograft and HSS, and therefore care should be taken to remove all traces of these antiseptics prior to placing an autograft or HSS.

Our results can be used to advise clinicians in their choice of which antiseptic is best to use when applying autograft or HSS during burn and ulcer care. The results are summarized in Table 4. Due to ethical reasons our study is an *in vitro* study and therefore carries certain limitations in the experimental set-up. Importantly, *in vivo*, quick inactivation or dilution of an antiseptic may occur if a great deal of wound exudate is present. Dilution of an antiseptic *in vivo* will result in the antiseptic being less cytotoxic than as determined in our *in vitro* assay. Moreover, the epidermis regenerates from the basal proliferative layer. Therefore even if a great deal of tissue damage is observed in histological sections (or a decrease in RNA staining) due to cytotoxicity in the upper cell layers, as long as the basal cell layer remains intact and undamaged the HSS or autograft may survive and regenerate an epidermis. In this respect, the measurement of metabolic activity (MTT assay) is a very reliable measurement of cytotoxicity to the BL as it measures metabolic activity only in undifferentiated keratinocytes in the BL and first suprabasal layer of the epidermis[19] and the fibroblasts in the dermis.

In conclusion, antiseptics which are classed as safe to use in the *in vitro* study are almost certainly safe to use *in vivo*. Antiseptics which are classed as cytotoxic in our *in vitro* study may be slightly less cytotoxic *in vivo*, particularly if a great deal of wound exudate is present. This study gives an indication of the cytotoxic effect of antiseptics and, taken together with the antiseptic properties of the particular substances, a clinician may use these data to help decide on an optimal wound care procedure which takes into account the type of wound colonization and whether an autograft or HSS can be applied.

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