Effect of Different Wound Dressings on Cell Viability and Proliferation

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Background: Many new dressings have been developed since the early 1980s. Wound healing comprises cleansing, granulation/vascularization, and epithelialization phases. An optimum microenvironment and the absence of cytotoxic factors are essential for epithelialization. This study examines the effect of extracts of different wound dressings on keratinocyte survival and proliferation.
Methods: Keratinocyte cultures were exposed for 40 hours to at least three extracts of each of the following wound dressings, which were tested in octuplicate: Acticoat, Aquacel-Ag, Aquacel, Algisite M, Avance, Comfeel Plus transparent, Contreet-H, Hydrasorb, and SeaSorb. Silicone extract provided the reference material. Controls were included of cells cultured in medium that had been incubated under conditions identical to those used with the extracts. Cell survival (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide reduction) and proliferation (5-bromo-2′-deoxyuridine incorporation) were measured.
Results: Extracts of silver-containing dressings (Acticoat, Aquacel-Ag, Contreet-H, and Avance) were most cytotoxic. Extracts of Hydrasorb were less cytotoxic but markedly affected keratinocyte proliferation and morphology. Extracts of alginate-containing dressings (Algisite M, SeaSorb, and Contreet-H) demonstrated high calcium concentrations, markedly reduced keratinocyte proliferation, and affected keratinocyte morphology. Extracts of Aquacel and Comfeel Plus transparent induced small but significant inhibition of keratinocyte proliferation.
Conclusions: The principle of minimizing harm should be applied to the choice of wound dressing. Silver-based dressings are cytotoxic and should not be used in the absence of infection. Alginate dressings with high calcium content affect keratinocyte proliferation probably by triggering terminal differentiation of keratinocytes. Such dressings should be used with caution in cases in which keratinocyte proliferation is essential. All dressings should be tested in vitro before clinical application. (*Plast. Reconstr. Surg.* 117 (Suppl.): 110S, 2006.)

Since the early 1980s, a great number of new dressings have been developed to promote wound healing. More than 200 new dressings were added to the British Drug Tariff in the period from 1988 to 2002.[1] The ideal dressing needs to ensure that the wound remains moist with exudate but not macerated; free of infection, excessive slough, toxic chemicals, particles, and fibers; at the optimum temperature and pH for healing; and undisturbed by the need for frequent changes.[2] There have been few clinical or experimental trials to establish the advantages of each individual dressing on the market. The choice of dressing is usually made on the basis of personal experience, availability, type, and state and site of the wound. Patient preference and tolerance may also be considered.

Wound healing generally comprises three stages: cleansing, granulation and vascularization, and epithelialization. The epithelialization phase requires an optimum microenvironment and the absence of any cytotoxic factors.

In this study, we examined a number of wound dressings commonly used in the Alfred Hospital Burns Unit (Table 1). Extracts of each dressing were tested in vitro by exposing cultured human

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Table 1. Dressings

Dressing	Manufacturer	Major Component	Silver
Acticoat	Smith & Nephew	Polyethylene mesh	Yes
Aquacel-Ag	ConvaTec	CM cellulose	Yes
Aquacel	ConvaTec	CM cellulose	No
Algisite M	Smith & Nephew	Alginate	No
Avance	SSL	Polyurethane foam	Yes
Comfeel	Coloplast	Hydrocolloid/CM cellulose	No
Contreet-H	Coloplast	Hydrocolloid/alginate	Yes
Hydrasorb	Kendall	Polyurethane	No
SeaSorb	Coloplast	Alginate/CM cellulose	No
Silicone	Corning	Silicone	No

keratinocytes to the extract for 24 hours. Cell survival was evaluated according to the reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) and cell proliferation by the incorporation of 5-bromo-2′-deoxyuridine (Br-dU).

MATERIALS AND METHODS

Materials

The cell culture medium used was basal keratinocyte serum-free medium supplemented with L-glutamine, bovine pituitary extract (25 mg/500 ml), and recombinant epidermal growth factor (2.5 μg/500 ml), all from Invitrogen, Grand Island, N.Y.

Cell Cultures

Primary cultures of human keratinocytes were originally obtained from two neonatal foreskins of tested donors by a modification of the method of Rheinwald and Green.[3] Secondary passage cells were grown in serum-free keratinocyte medium with bovine pituitary extract and epidermal growth factor and aliquoted into 1-ml cryovials for cryopreservation in liquid nitrogen. When required, the cells were thawed and cultured for 3 to 4 days in serum-free keratinocyte medium with bovine pituitary extract and epidermal growth factor at 37°C in a humidified atmosphere of 5% carbon dioxide/air before harvest and resuspension in fresh culture medium. All subsequent studies were performed using 96-well flat-bottom tissue culture plates (Costar). A uniform volume (200 μl) of the cell suspension (6000 cells) was dispensed into each well. Overnight culture ensured attachment before each experiment.

Extracts of Dressings

The following dressings (2 cm^2) were each incubated in 8 ml of culture medium in a polycarbonate tissue culture-safe tube at 37°C for 24 hours: Acticoat (Smith & Nephew), Aquacel with silver (Convatec), Aquacel (Convatec), Algisite M (Smith & Nephew), Avance (SSL), Comfeel Plus transparent (Coloplast), Contreet-H (Coloplast), Hydrasorb (Kendall), and SeaSorb (Coloplast). An extract of Silicone sheet (Corning) was included as a reference. The sterile dressings were cut under aseptic conditions with due care to prevent cross-contamination.

Test Conditions

Extracts of each dressing (in octuplicate) replaced the original culture medium in designated wells, and cells were cultured for a further 40 hours at 37°C. The controls were cells cultured in medium that had been incubated under conditions identical to those used for the extracts. Background controls comprised culture medium without the addition of cells.

Cell Survival by MTT Assay

Cell survival was determined by estimation of mitochondrial competence to reduce MTT. After the 40-hour incubation, the culture medium in each well was replaced by fresh medium containing 5 mg/ml MTT (Sigma) and incubated for a further 3 hours at 37°C. At this point, the medium was removed and replaced with 200 μl of dimethyl sulfoxide. After 1 hour of extraction with shaking, the plate was read in a microplate reader (model 3550; BioRad) at 490 nm.

Cell Proliferation by Br-dU Incorporation

Cell proliferation was determined by measuring the incorporation of Br-dU into nuclear DNA. This method was based on a Br-dU reagent kit (Roche). Br-dU labeling solution was added to each well after incubation for 24 hours, and the cells were cultured for an additional 16 hours at 37°C. The labeling medium was removed, FixDenat was added to denature the DNA, and cells were

incubated for 30 minutes at room temperature. The FixDenat solution was removed and the cells were then incubated for 90 minutes at room temperature with a solution of peroxidase-conjugated anti–Br-dU. Upon removal of this conjugate, the cells were washed three times with phosphate-buffered saline and then incubated with tetramethylbenzidine at room temperature for 10 minutes to develop color. The reaction was stopped with 1 M sulfuric acid. The plate was then read in a microplate reader (model 3550; BioRad) at 450 nm.

Cell Morphology

The effect of each extract on cell morphology was examined by phase contrast microscopy after cells were cultured for 40 hours at 37°C.

Composition of Extracts

The extracts of each dressing were analyzed for the following ions: sodium, potassium, chloride, bicarbonate, magnesium, and calcium (Department of Chemical Pathology, Alfred Hospital). Silver was analyzed by inductively coupled plasma/atomic emission spectroscopy (Australian Government Analytical Laboratories) after acidification of samples with nitric acid and filtration (0.45-μm filter).

Statistical Analysis

Each extract was compared with the control using Student's *t* test (two-tailed, unequal variance).

RESULTS

Cell Survival (MTT Reduction)

At least three extracts of each dressing were tested (Table 2). Three of the 10 dressings, Acticoat, Aquacel-Silver, and Contreet-H, tested were markedly cytotoxic. After exposure to these extracts, more than 95 percent activity was lost. The effect of extracts of Avance was more variable, ranging from 54 to 98 percent loss of activity. Hydrasorb also reduced cell viability by 40 to 50 percent. There was a significant small loss of cell viability with Algisite M, SeaSorb, and Aquacel, whereas that for Comfeel Plus transparent and silicone extracts was similar to cell viability loss in controls.

Cell Proliferation (Br-dU Incorporation)

At least three extracts of each dressing were tested (Table 3). Only the silicone extracts were similar to the controls. The proliferative ability of the cells after exposure to the other extracts was reduced compared with controls as follows: more than 90 percent loss: Acticoat, Aquacel-Ag, and Contreet-H; 70 to 90 percent loss: Avance and Hydrasorb; 60 to 70 percent loss: Algisite M and SeaSorb; and 10 to 20 percent loss: Comfeel Plus transparent and Aquacel.

Cell Morphology

Control and Silicone Extract

A confluent monolayer of cells was seen (Fig. 1). The cells had a polygonal shape with round to ovoid nuclei and prominent large nucleoli. In many areas, the cells showed a typical pattern of close apposition with no overlapping.

Aquacel and Comfeel Plus Transparent Extracts

There was approximately 90 percent confluence in these cultures (Fig. 2). The cells were similar to the control cells. The typical pattern of close apposition was maintained in many areas, particularly with the Aquacel extract.

Algisite M and SeaSorb Extracts

There were denuded areas of variable size seen in both of these cultures (Fig. 3). The cells had round to ovoid nuclei with variably sized nucleoli. Cytoplasmic and perinuclear vacuoles were seen

Table 2. Effect of Different Dressings on Cell Survival (MTT Reduction)

Sample	No. of Extracts	% Control (mean ± SEM)	% Control (range)	*p**
Control		100.0 ± 0.0	99.9–100.1	
Acticoat	4	1.3 ± 0.3	0.5–2.5	<0.00001
Aquacel-Ag	4	0.6 ± 0.1	0.3–1.4	<0.00001
Aquacel	4	95.0 ± 1.7	88.3–100.9	<0.05
Algisite M	4	89.5 ± 2.8	75.2–100.8	<0.01
Avance	4	15.2 ± 6.3	1.9–46.0	<0.00001
Comfeel	4	100.7 ± 1.5	92.5–106.8	0.7, NS
Contreet-H	3	1.0 ± 0.2	0.2–1.9	<0.00001
Hydrasorb	4	55.1 ± 1.4	48.6–59.9	<0.00001
SeaSorb	4	94.1 ± 1.9	83.6–101.5	<0.05
Silicone	5	96.8 ± 2.8	84.9–109.7	0.3, NS

*2-tailed t test comparing each extract with control (unequal variance)

Table 3. Effect of Different Dressings on Cell Proliferation (Br-dU Incorporation)

Sample	No. of Extracts	% Control (mean ± SEM)	% Control (range)	*p**
Control		105.4 ± 2.9	100.0–124.8	
Acticoat	4	8.5 ± 0.8	5.2–11.4	<0.00001
Aquacel-Ag	3	5.6 ± 1.6	0.0–8.7	<0.00001
Aquacel	4	87.5 ± 3.3	76.6–98.7	<0.01
Algisite M	4	35.0 ± 6.6	16.0–63.6	<0.0001
Avance	4	12.5 ± 1.8	4.7–18.4	<0.00001
Comfeel	4	81.9 ± 6.9	46.2–98.5	<0.05
Contreet-H	6	9.8 ± 3.0	0–34.2	<0.00001
Hydrasorb	4	10.0 ± 1.1	5.5–13.7	<0.00001
SeaSorb	4	40.6 ± 6.9	16.5–61.5	<0.0002
Silicone	4	94.2 ± 5.1	74.7–109.7	0.09, NS

*Two-tailed t test comparing each extract with control (unequal variance)

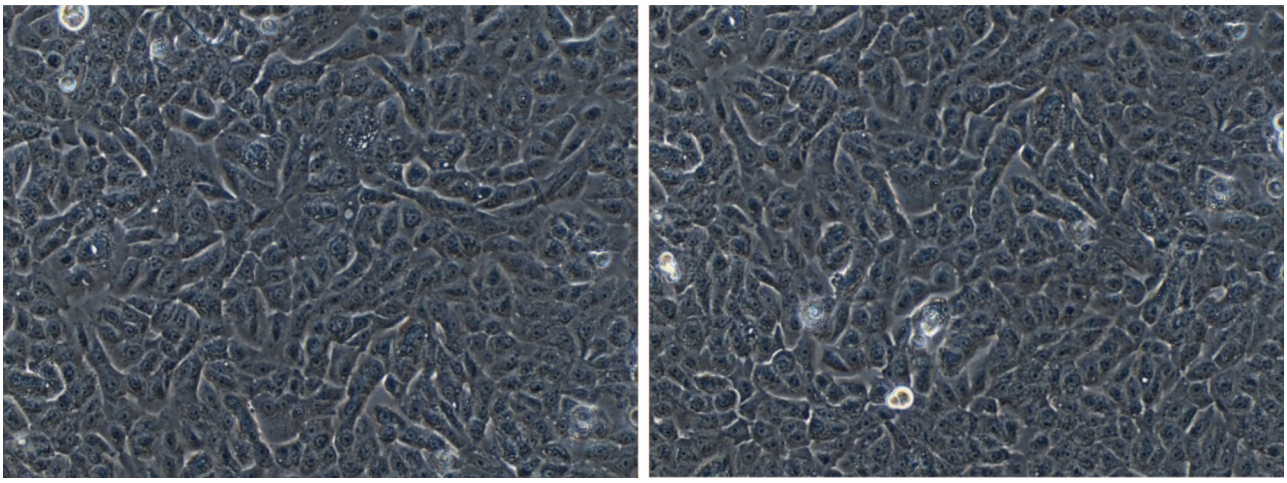

Fig. 1. Appearance of keratinocytes after incubation in extracts of each dressing for 40 hours: (*left*) control; (*right*) silicone.

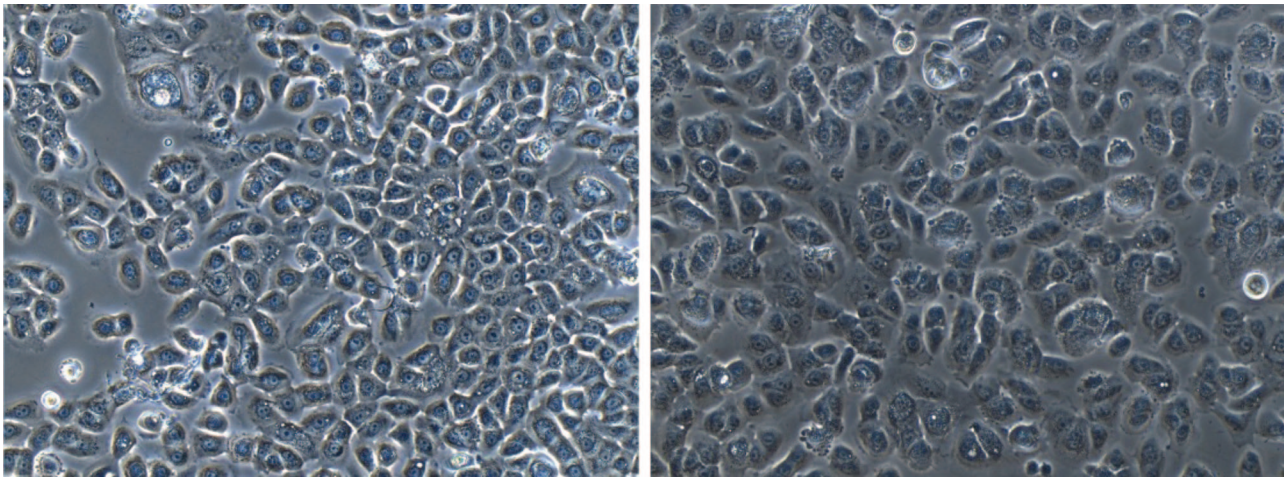

Fig. 2. Appearance of keratinocytes after incubation in extracts of each dressing for 40 hours: (*left*) Aquacel; (*right*) Comfeel Plus transparent.

in virtually all cells. The cytoplasmic borders of the cells were indistinct. The typical pattern of apposition was absent.

Hydrasorb Extract

Despite the cell loss observed, there were small patchy areas with the typical pattern of close ap-

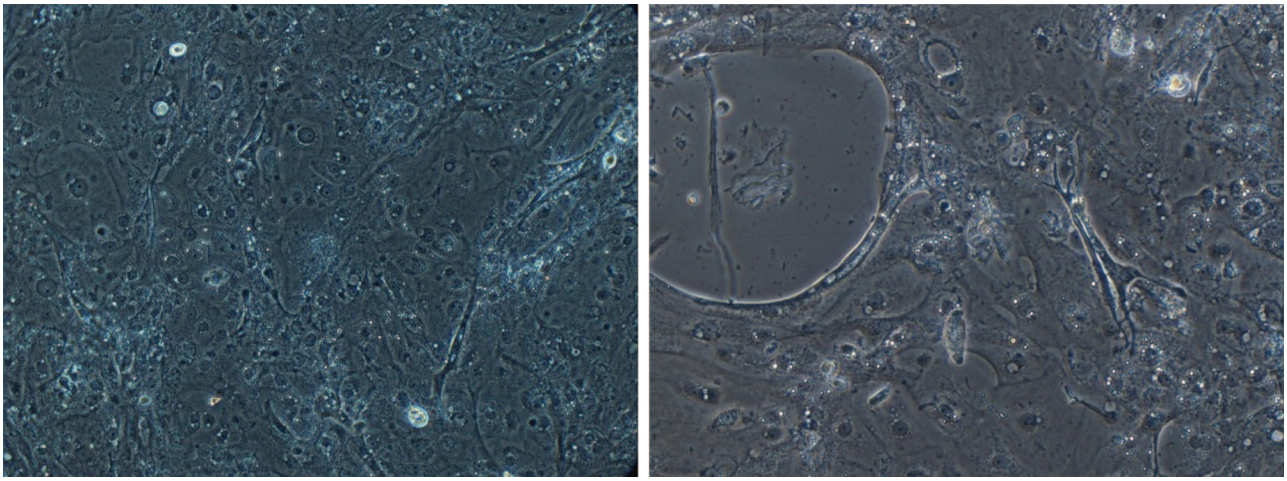

Fig. 3. Appearance of keratinocytes after incubation in extracts of each dressing for 40 hours: (*left*) Algisite M; (*right*) SeaSorb.

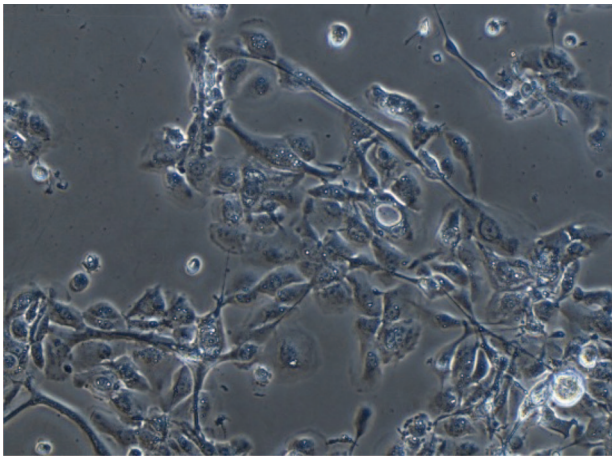

Fig. 4. Appearance of keratinocytes after incubation in extracts of each dressing for 40 hours: Hydrasorb.

position (Fig. 4). In two areas there was a small degree of cell overlap. The cells were polygonal in shape and larger than those in control cultures. Many of the cells had ovoid nuclei, small nucleoli, and cytoplasmic and nuclear vacuoles. Many of the cells showed short and thick cytoplasmic projections.

Contreet-H and Avance Extracts

The typical pattern of cell apposition was absent, with no evidence of a monolayer seen (Fig. 5). The cells were three times the size of control cells and larger than cells cultured with Hydrasorb extract. They had abundant polygonal cytoplasm, with many cells showing cytoplasmic projections. Nuclei were inconspicuous and nucleoli were absent. Particulate cell debris and rounded nonviable cells were more prominent in the background in the cultures with Contreet-H extract.

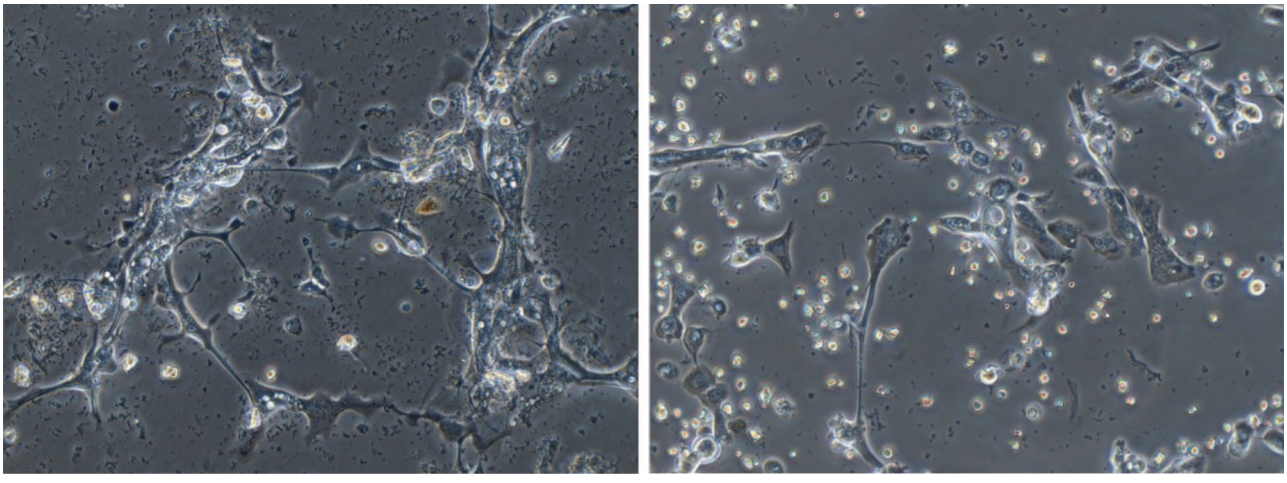

Fig. 5. Appearance of keratinocytes after incubation in extracts of each dressing for 40 hours: (*left*) Contreet-H; (*right*) Avance.

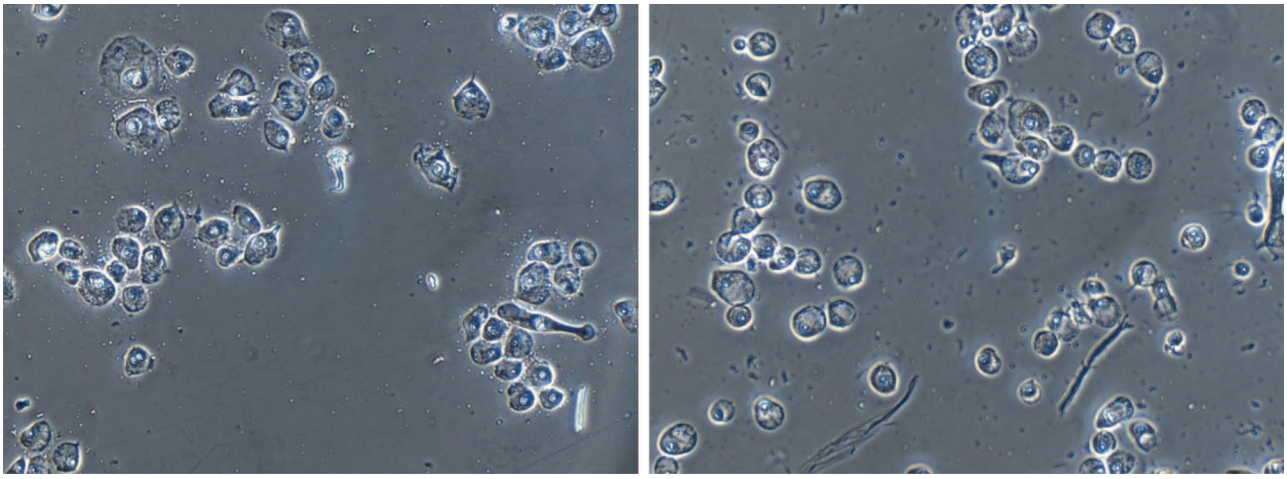

Fig. 6. Appearance of keratinocytes after incubation in extracts of each dressing for 40 hours: (*left*) Aquacel-Ag; (*right*) Acticoat.

Aquacel-Ag and Acticoat Extracts

The typical pattern of cell apposition was absent, with no evidence of a monolayer seen (Fig. 6). The cells had a round to ovoid cytoplasm with inconspicuous nuclei, and nucleoli were absent. Virtually all cells had cytoplasmic vacuoles. Particulate and irregularly shaped cell debris was seen.

Composition of Extracts

Extracts of Aquacel, Avance, Comfeel Plus transparent, Hydrasorb, and silicone had electrolyte composition similar to that of the control extract (Table 4). Potassium concentration was elevated in the Contreet-H extract. Magnesium concentration was significantly elevated in extracts of Algisite-M. Extracts of dressings containing alginate as the main component (Algisite M, Contreet-H, and SeaSorb) had higher concentrations of calcium than the control or the other cell extracts did. The silver content of extracts of dressings containing silver varied with each dressing. Contreet–H, Aquacel-Ag, and Acticoat extracts had measurable concentrations of silver, whereas the silver content of the Avance extract was below the detectable range.

DISCUSSION

This study demonstrated that all the extracts of wound dressings tested, except the extract of Comfeel Plus, had a variable but significant effect on cell survival. Similarly, there was a variable and significant effect on cell proliferation after exposure to extracts of all dressings.

Extracts of the four silver-containing dressings (Acticoat, Aquacel-Ag, Avance, and Contreet-H) induced the greatest cytotoxicity and disordered morphology. The effect of silver ions on proliferation and cellular metabolic activity has been tested in a system similar to that used in the current study.[4] Silver was toxic to keratinocytes, fibroblasts, and 3T3 feeder cells. The toxic dose of silver nitrate for keratinocytes was greater than 78 mg/liter; at 12.5 mg/liter there was 50 percent loss of metabolic activity after 3 hours of exposure.

Table 4. Composition of Extracts (in mmol/liter)*

Sample	Sodium	Potassium	Chloride	Bicarbonate	Magnesium	Calcium	Silver
Control	142	2.0	116	8	0.91	0.10	<0.005
Acticoat	141	2.0	114	8	0.91	<0.5	0.011
Aquacel-Ag	143	1.9	116	7	0.86	0.09	0.035
Aquacel	143	1.9	116	8	0.84	0.09	–
Algisite M	138	1.9	115	7	1.04	4.89	–
Avance	140	2.0	115	9	0.95	0.14	<0.005
Comfeel	148	1.9	116	8	0.87	0.12	–
Contreet-H	162	1.8	118	1	0.84	3.06	0.111
Hydrasorb	140	2.0	115	8	0.92	0.11	–
SeaSorb	138	1.9	116	7	0.92	3.42	–
Silicone	139	1.9	119	8	0.90	0.12	–

*Two extracts of each dressing, silicone, and control were tested. Silver assays were performed in triplicate on duplicate samples. All values are expressed as mmol/liter.

Silver-containing dressings have been developed in response to the many studies demonstrating the adverse effect of topical silver on micro-organisms. Topical silver was originally introduced to inhibit bacterial growth, on the premise that its action on bacterial DNA should not affect mammalian cells that contain 100-fold more DNA than bacteria.[5] The silver content (milligrams per 100 cm^2) of different dressings varied considerably: Aquacel-Ag, 19.7 mg/100 cm^2 [6] ; Acticoat, 109, Avance, 1.6 mg/100 cm^2; and Contreet-H, 31 to 32 mg/100 cm^2.[7] The nature of the silver complex was also different in these dressings: Aquacel-Ag incorporated ionic silver throughout the hydrofiber mesh. Silver is released into the wound over a period of 14 days. Acticoat was a nanocrystalline silver-coated material. The dressing consisted of a core of absorbent rayon and polyester coated on each side with a polyethylene mesh on which silver was deposited. This dressing was designed to deliver a dose of 50 to 100 mg/liter of silver ions to the wound for up to 48 hours.

Contreet-H is a hydrocolloid dressing that was also designed to release silver ions as it absorbed exudate from the wound.[8] The nature of the silver complex in this dressing has not been disclosed by the manufacturer. Avance is a silver-impregnated polyurethane foam film dressing with no glues to hold different layers.[9] Avance utilizes a zirconium phosphate–based ceramic ion exchange resin containing silver. It was not designed to release silver into the wound to treat infections. The silver content of the extracts in this study reflected differences in the content and release rate of silver. Three of the extracts of silver-containing dressings (Acticoat, Aquacel-Ag, and Contreet-H) contained measurable quantities of silver and induced the death of almost all the keratinocytes exposed to the extracts for 40 hours. The extract of Avance did not contain measurable silver but was also cytotoxic, albeit at a reduced level. Because this dressing contained polyurethane and no glues, it was difficult to assign a basis for its cytotoxicity other than the silver content. A number of studies have applied silver-containing dressings in vivo, but results have been conflicting: Aquacel-Ag was found to be effective in the treatment of superficial and partial-thickness burns.[6] Acticoat was shown to increase the rate of epithelialization in a group of patients with deep burns who were grafted with meshed grafts.[10] In another study, however, Acticoat was shown to significantly delay re-epithelialization of donor sites when compared with Allevyn, a hydrophilic polyurethane dressing that contained no silver.[11] The findings of our study support the suggestion that "as silver-based products cannot discriminate between healthy cells involved in wound-healing and pathogenic bacteria, silver-based products should be used with caution in situations where rapidly proliferating cells may be harmed as in donor sites, superficial burns, and applications of cultured cells, particularly in suspension."[4]

Extracts of the three alginate-containing dressings (Algisite M, Contreet-H, and SeaSorb Soft) each demonstrated a high calcium concentration. Contreet-H also contained silver. Exposure of keratinocytes for 24 hours to extracts of Algisite M and SeaSorb Soft markedly impaired cell proliferation, had profound effects on cell morphology, and had a small but significant cytotoxic effect. Calcium alginate has been applied in wound care since the mid-1980s. The calcium ions in this type of dressing are exchanged for sodium, and a soluble calcium/sodium alginate gel is formed. This type of dressing is most suitable for moderately to heavily exuding wounds, where its benefit is to provide a moist wound gel environment to promote epithelialization. Calcium ions promote hemostasis, but calcium also affects basic cellular activities, such as adhesion, locomotion, proliferation, and differentiation. An earlier study, evaluating survival (MTT reduction) of fibroblasts or HeLa cells after incubation with extracts of calcium alginate dressings for 48 hours, showed no significant effect.[12] Keratinocytes were not tested, and the effect on cell proliferation was not evaluated in that study. The authors concluded that calcium alginate dressings "present no obvious toxic risk," ignoring studies showing induction of morphological changes in cultured keratinocytes[13,14] and inhibition of proliferation of keratinocytes.[14] Moreover, exposure to different concentrations of calcium alginate for 1 to 7 days showed that all concentrations of calcium alginate completely inhibited proliferation of keratinocytes, whereas proliferation of fibroblasts and microvascular endothelial cells was concentration-dependent.[14] Keratinocytes need only a small amount of Ca^{++} (0.05 to 0.1 mmol/liter) to proliferate in contrast to fibroblasts, hepatocytes, and lymphoblasts (1 to 1.5 mmol/liter).[15] The epidermis maintains a steep, increasing Ca^{++} gradient. Increasing external Ca^{++} (>1 mmol/liter) will stop proliferation and trigger differentiation. In response to

the differentiation signal induced by a high (1 mmol/liter) calcium concentration, keratinocytes cease to proliferate and develop morphological features of terminally differentiated keratinocytes.[16] Keratinocyte chemotaxis and adhesion may also be compromised. Differentiation of keratinocytes into stratified squamous cells has frequently resulted in cell sloughing, leaving barren regions in the culture in other studies[14] as well as in this study. Calcium alginate and hydrocolloid have consistently demonstrated more rapid healing compared with other conventional dressings.[17–19] It has also been suggested that alginate oligosaccharides may support and, perhaps, stimulate proliferation of stem cells.[20] The development of a novel alginate dressing with 80 percent less calcium may presage a dressing with all the benefits of alginate and a reduced inhibition of keratinocyte proliferation.[21] Until such a dressing becomes commercially available, alginate dressings should be used with caution in clinical situations in which keratinocyte proliferation may be harmed with no other optional benefit (e.g., donor sites, superficial burns, or epithelial cell grafts).

Extracts of Hydrasorb, a polyurethane dressing, were cytotoxic (mean, 45 percent inhibition of MTT reduction), inhibited cell proliferation (90 percent), and affected cell morphology. Other studies have shown that polyurethane per se is not toxic.[13,22,23] Processing of polymeric material can produce small molecules capable of diffusing into cells and altering cellular metabolism. All polymeric material should be tested in vitro before use.

Extracts of Aquacel and Comfeel Plus transparent induced only small but significant inhibition of keratinocyte proliferation in this study. These findings may indicate possible low-level contamination of the material during processing.

CONCLUSIONS

The choice of a suitable wound dressing should involve the principle of minimizing harm. Silver-based dressings are cytotoxic and should not be used unless wound infection is a significant risk. Current alginate dressings with high calcium content should be used cautiously in situations in which optimal keratinocyte proliferation is essential. All dressings should be tested in vitro before clinical application to ensure the absence of cytotoxic molecules produced during manufacture.

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REFERENCES

1. Morgan, D. Wounds: What should a dressings formulary include? *Hosp. Pharmacist* 9: 261, 2002.
2. British Medical Association and Royal Pharmaceutical Society of Great Britain. *Wound Dressings.* Joint Formulary Committee, British National Formulary, 51st ed. Available at http://www.bnf.org/bnf/.
3. Rheinwald, J. G., and Green, H. Serial cultivation of human epidermal keratinocytes: The formation of keratinizing colonies from single cells. *Cell* 6: 331, 1975.
4. Poon, V. K. M., and Burd, A. In vitro cytotoxicity of silver: Implication for clinical wound care. *Burns* 30: 140, 2004.
5. Fox, C. L. Topical therapy and the development of sulfadiazine. *Surg. Gynecol. Obstet.* 157: 82, 1983.
6. Caruso, D. M., Foster, K. N., Hermans, M. H. E., and Rick, C. Aquacel Ag in the management of partial-thickness burns: Results of a clinical trial. *J. Burn Care Rehabil.* 25: 89, 2004.
7. Thomas, S., and McCubbin, P. A comparison of the antimicrobial effects of 4 silver-containing dressings on three organisms. *J. Wound Care* 12: 101, 2003.
8. Lansdowne, A. B. G., Jensen, K., and Jensen, M. Q. Contreet Foam and Contreet Hydrocolloid: An insight into two new silver-containing dressings. *J. Wound Care* 12: 205, 2003.
9. Ballard, K., and McGregor, F. Avance: Silver hydropolymer dressing for critically colonised wounds. *Br. J. Nurs.* 11: 206, 2002.
10. Demling, R. H., and DeSanti, L. The rate of epithelialisation across meshed skin grafts is increased with exposure to silver. *Burns* 28: 264, 2002.
11. Innes, M. E., Umraw, N., Fish, J. S., Gomez, M., and Cartotto, R. C. The use of silver coated dressings on donor site wounds: A prospective controlled matched pair study. *Burns* 27: 621, 2001.
12. Lin, S.-S., Ueng, S. W. N., Lee, S.-S., et al. In vitro elution of antibiotic from antibiotic-impregnated biodegradable calcium alginate wound dressing. *J. Trauma* 47: 136, 1999.
13. Rosdy, M., and Clauss, L.-C. Cytotoxicity testing of wound dressings using normal human keratinocytes in culture. *J. Biomed. Mater. Res.* 24: 363, 1990.
14. Doyle, J. W., Roth, T. P., Smith, R. M., Li, Y.-Q., and Dunn, R. M. Effect of calcium alginate on cellular wound healing processes modelled in vitro. *J. Biomed. Mater. Res.* 32: 561, 1996.
15. Whitfield, J. F., Bird, R. P., Chakravarthy, B. R., Isaccs, R. J., and Morley, P. Calcium: Cell cycle regulator, differentiator, killer, chemopreventor, and maybe, tumor promoter. *J. Cell Biochem. Suppl.* 22: 74, 1995.

16. Marchese, C., Rubin, J., Ron, D., et al. Human keratinocyte growth factor activity on proliferation and differentiation of human keratinocytes: Differentiation response distinguishes KGF from EGF family. *J. Cell Physiol.* 144: 326, 1990.
17. Davey, R. B., Sparnon, A. L., and Bayard, R. W. Unusual donor site reactions to calcium alginate dressings. *Burns* 26: 393, 2000.
18. Vanstraelen, P. Comparison of calcium sodium alginate (KALTOSTAT) and porcine xenograft (E-Z DERM) in the healing of split-thickness skin graft donor sites. *Burns* 18: 145, 1992.
19. Porter, J. M. A comparative investigation of re-epithelialisation of split skin graft donor areas after application of hydrocolloid and alginate dressings. *Br. J. Plast. Surg.* 44: 333, 1991.
20. DeGraffenried, L. A., and Isseroff, R. R. Wound dressings alter colony-forming efficiency of keratinocytes in cultured sheet grafts. *Cell Transplant.* 10: 749, 2001.
21. Suzuki, Y., Nishimura, Y., Tanihara, M., et al. Evaluation of a novel alginate gel dressing: Cytotoxicity to fibroblasts in vitro and foreign-body reaction in pig skin in vivo. *J. Biomed. Mater. Res.* 39: 317, 1998.
22. Dover, R., Otto, W. R., Nanchahal, J., and Riches, D. J. Toxicity testing of wound dressing materials in vitro. *Br. J. Plast. Surg.* 48: 230, 1995.
23. van Luyn, M. J. A., van Wachem, P. B., Nieuwenhuis, P., and Jonkman, M. F. Cytotoxicity testing of wound dressings using methylcellulose cell culture. *Biomaterials* 13: 267, 1992.