Eur J Dermatol 2008; 18 (4): 416-21

Dave VAN DEN PLAS
Kris DE SMET
Dimitri LENS
Philippe SOLLIE

Research Department, Flen Pharma nv, Drie Eikenstraat 661, B-2650 Edegem, Belgium

Reprints: D. Van Den Plas <dave.vandenplas@flenpharma.com>

Differential cell death programmes induced by silver dressings *in vitro*

In past decades the gold standard for topical burn treatment was the use of silver sulfadiazine. Due to toxicity caused by the silver, the cream base itself, or a combination of both negatively influencing the wound healing process, the healthcare industry searched for alternatives. In recent years, various dressings containing silver have become available to wound professionals. Although these have been reported to be a significant improvement, the dressings still show residual cytotoxicity. Given the ongoing debate about whether and how these dressings influence cell survival, this article endeavours to clarify some of the mystique surrounding the subject. Various commercially available silver-type dressings were analysed *in vitro* and attention was paid to the cell death stage induced by these dressings on different cell lines. The results show that within 2 hours, for all dressings tested, cells undergo cell death and further analysis suggests that the death stage induced is dependent on the cell line and type of dressing investigated. Further, the antimicrobial activity of all dressings was analysed. The silver dressings tested have potent antimicrobial activity. Our results showed, however, that silver dressings induce rapid cell death of cells involved in wound healing. We therefore recommend the use of silver dressings only on critically contaminated wounds rather than use on a *de facto* basis.

Article accepted on 27/2/2008

Key words: silver, cytotoxicity, wound healing

The ultimate goal of wound management and therapy is fast healing and re-epithelialisation with minimal complications. In this way the risk of reduced functionality and aesthetic discomfort greatly diminishes. In order to realize this objective, a great deal of attention is paid to preventing infection of the wound. Provided that the patient's immune status can handle the bacterial burden, the presence of a certain number of micro-organisms generally does not impair wound healing. As a rule of thumb, infection could negatively influence wound healing once a microbial burden of 10^5 micro-organisms/g of tissue is reached [1, 2]. Signs of infection are most often swelling, redness, an increased amount of wound exudates and increased wound pain caused largely by the activation of immune cells, resulting in a delay in wound healing and general discomfort to the patient.

Micro-organisms impede wound healing by competing for nutritional compounds, by secreting toxins, disrupting the inflammatory cytokine balance in favour of pro-inflammatory mediators and degrading newly formed tissue, and in general give an unpleasant odour to the wound [3, 4]. One strategy gaining renewed attention for fighting the threat of microbial infection and preventing wound sepsis is the use of silver. Traditionally, two main products have been used for this purpose. Silver-nitrate is active against a variety of micro-organisms and is used in a concentration of 0.5% on patients with extensive burns [5]. Silver sulfadiazine combines the inhibitory action of the silver with the antibacterial effect of sulfadiazine [6].

In the years following the introduction of these products, however, more concerns arose about their safety and disadvantages. The main complication occurring during treatment with $AgNO_3$ was a drop in serum sodium and chlorine due to ion exchange between Ag^+ and Cl^-, HCO_3^-, CO_3^- and protein anions, leading to the production of very slightly soluble or insoluble salt solutions. Secondly, it was seen that during $AgNO_3$ treatment all objects which came into contact with the $AgNO_3$ coloured black on exposure to light [7]. Thirdly, Bader [8] found elevated silver levels in the kidneys, spleen, liver and muscles of two patients on post-mortem examination.

To resolve these problems, wound-care companies searched for improved products combining the strength of silver with technological advances in wound dressings. The new products offer the opportunity of a slow release of silver enabling less frequent changes of dressings. The result is a myriad of Ag-dressings on the market (for review see [3]). Although the cytotoxicity of these dressings is reported to be less pronounced, some authors do describe reduced viability of cells after contact with such silver compounds [9-11].

In order to assess the ambiguity surrounding the subject, we decided to check the cytotoxicity of various commercially available Ag-dressings, all profoundly different in both composition and type of silver. Comfeel®-Ag (Coloplast) is a sticky hydrocolloid plate containing a silver complex which, on contact with wound exudates, releases the silver. Seasorb®-Ag (Coloplast) is an alginate dressing containing

doi: 10.1684/ejd.2008.0437

calcium alginate (fibres), sodium carboxymethylcellulose (CMC) and an ionic silver complex which, in the presence of wound exudates, releases silver ions for a period of 7 days. Acticoat® 7 (Smith&Nephew) is a silver dressing consisting of 5 layers (2 internal absorbing layers and 3 silver-containing polyethylene layers) in which the silver is present in the form of Nanocrystalline™ silver. The dressing is reported to be active for 7 days. Biatain®-Ag (Coloplast) is a polyurethane foam dressing with an antibacterial silver complex homogeneously dispersed in its structure.

Materials and methods

Materials

Silver dressings were purchased from the respective manufacturers. Comfeel®-Ag, Seasorb®-Ag and Biatain®-Ag were bought from Coloplast and Acticoat® 7 from Smith&Nephew. DMEM, FBS, Penicillin/Streptomycin, L-Glutamine were all purchased from Sigma (Bornem, Belgium). The growth medium in which eukaryotic cells were grown consisted of DMEM supplemented with 10% heat inactivated calf serum, 4 mM L-glutamine, 100 U/mL streptomycin and 100 µg/mL penicillin. Bacteria were routinely grown on Tryptic Soy Agar (TSA) and yeasts on Sabouraud Dextrose Agar (SDA) (Becton Dickinson (BD), Erembodegem, Belgium) plates. Tryptic Soy (TS) broth was used for liquid cultures. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was bought from Sigma (Bornem, Belgium) and a stock solution was made (5 mg/mL). The final concentration in medium for cytotoxicity tests was 0.5 mg/mL. HaCaT keratinocytes were kindly provided by Prof. Dr J. Merregaert (Lab Molecular Biotechnology, University of Antwerp). Mouse fibroblast NCTC clone 929 cells were purchased from ECACC (Salisbury, UK) Polycarbonate cell culture transwell inserts for 6-well plates (0.4 µ) Millicell®-PCF were purchased from Millipore (Brussels, Belgium). *Staphylococcus aureus* (ATCC6538), *Escherichia coli* (ATCC8739) and *Candida albicans* (ATCC10231) were purchased from the Belgian Coordinated Collection of Micro-organisms (Brussels, Belgium).

Cell death analysis in the presence of silver dressings

Cells were seeded subconfluently into 6 well plates and were grown overnight at 37 °C/5% CO_2 until 90%-100% confluency was reached. Silver dressings were divided into 1.5 × 1.5 cm dimensions and were weighed:
– Acticoat® 7: (0.044 ± 0.005) gram;
– Biatain®-Ag: (0.146 ± 0.010) gram;
– Comfeel®-Ag: (0.471 ± 0.040) gram;
– Seasorb®-Ag: (0.044 ± 0.005) gram.
The dressings were placed on the transwell inserts and overlaid with 1.2 mL of growth medium. Cells grown in the presence of transwell inserts alone were used as a control. The experimental set-up ensured no direct contact between cells and dressings. Because of this, consequent cytotoxicity can only be the result of diffusion from the dressings under investigation *(figure 1A)*.
Cells with dressings were incubated at 37 °C/5% CO_2 for 4 hours, after which the cells were analysed visually. For cell death analysis the cells were incubated for 2 hours in the same experimental set-up as above. The type of cell death programme was analysed using the Apoptosis Detection Kit (Sigma, Bornem, Belgium) according to the manufacturer's instructions. In brief, cells were detached from the wells by a short trypsin/EDTA incubation and pooled with the cells from the supernatants. Next, cells were washed twice in PBS and the cell pellet was re-suspended in AnnexinV binding buffer and propidium iodide. Labelled cells were analysed by flow cytometry on a FACScan (Becton Dickinson) at gates FL1/FL3. As a positive control, cells were incubated in the presence of 5 mM hydrogen peroxide.

Bioactivity of silver from different dressings

Gram-positive *S. aureus* and Gram-negative *E. coli* cells were dispersed in liquid broth (TS) to obtain an optical density of 600 nm (OD_{600}) of 0.1; *C. albicans* cells were re-suspended to obtain an OD_{600} of 0.5.
Dressings were dispersed in liquid TS medium at 40 mg/mL for 24 h at 37 °C/230 rpm. Afterwards, 1/2 serial dilutions of the 'extracts' were made in microbial growth media. One hundred microlitres of the freshly made dilutions were poured into 96 well plates and 2 µL of the microbial suspensions were added to the mixture. Wells with only liquid growth medium served as a positive control for growth. Micro-organisms were allowed to grow for 24 h at 37 °C. Growth was measured spectrophotometrically at 600 nm (MRX II, Dynex Technologies, US) and MIC_{50} values were calculated.

Zone of inhibition

S aureus, *E coli* and *C albicans* were susupended in sodium chloride-peptone (OD_{600} = 1) and plated on agar plates. Dressings were divided into pieces of 1 cm × 1 cm and placed directly on top of the agar plates. Plates were incubated for 24 hours at 37 °C. After incubation the zone of inhibition surrounding the dressings was measured.

Statistical analysis

The results are the mean and standard deviation of at least 3 independent experiments. The data were analysed by the Student t-test.

Results

Influence of active silver on keratinocytes

To verify the influence of active silver from dressings on cells *in vitro*, we visually analysed the cells after 2 h incubation in the presence of various dressings. Incubation was as described in Materials and Methods *(figure 1A)*. After 4h of incubation, a short time relative to the indented contact time on wounds, visual inspection of the cells clearly showed that for all but one dressing (Biatain®-Ag) cells were rounding up or deforming, indicative of a cell being in a stress situation *(figure 1B)*. Although the Biatain®-Ag dressing least influenced cell morphology, it should be noted that this dressing strongly absorbed the growth medium throughout the experiment. MTT analysis confirmed our visual observations in that the silver dressings significantly influence cell survival compared to control cultures ($p < 0.01$). Cell loss in Biatain®-Ag treated cultures was significantly less severe ($p < 0.03$) compared to the other dressings *(figure 1C)*.

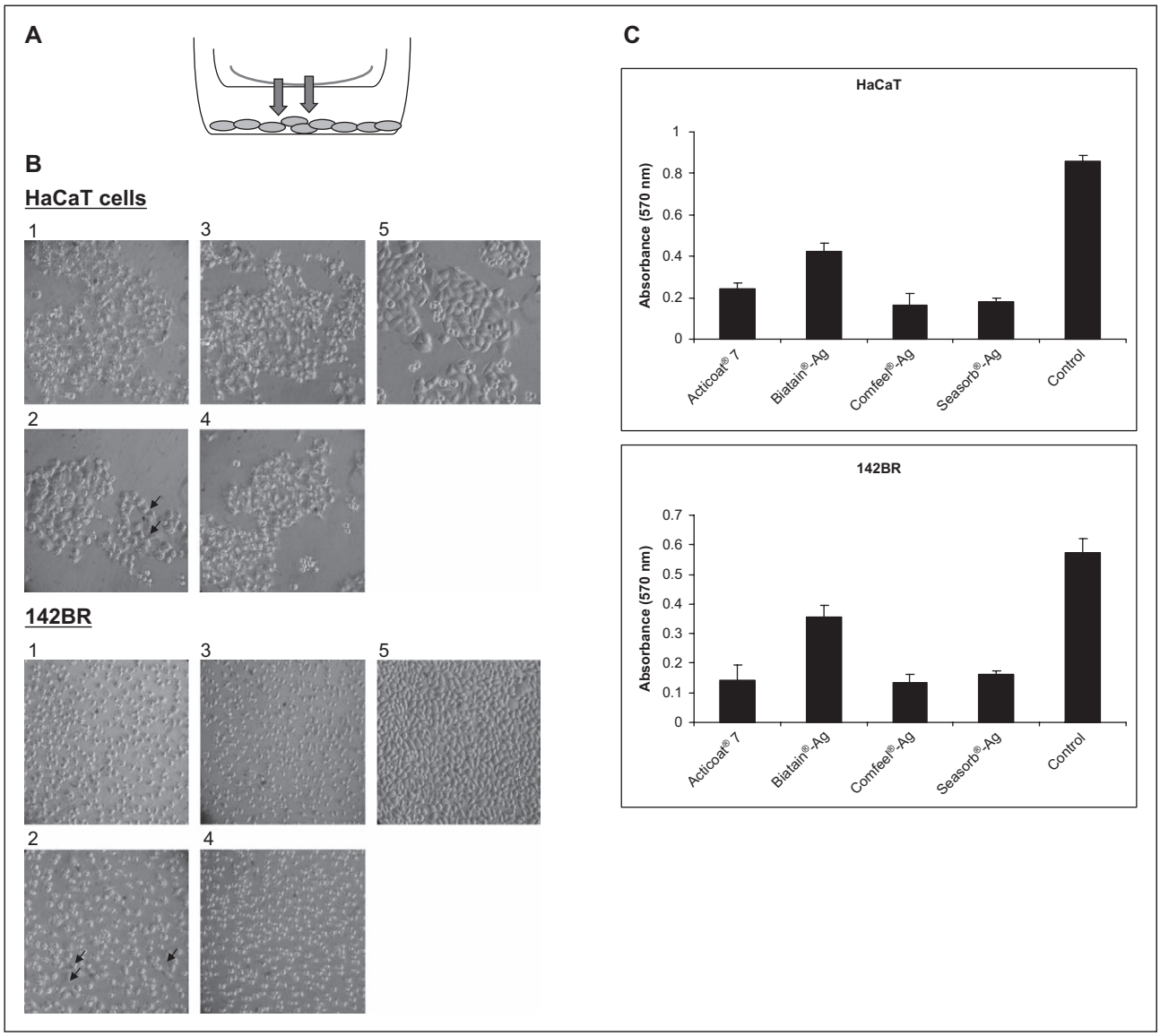

Figure 1. Silver dressings influence cell survival by diffusion. **A**) Graphic illustration of the experimental set-up. Dressings were applied on well inserts, **B**) Pictures of cells underneath the insert were taken 4h after the start of the experiment; arrows indicate cells with minimal change in morphology; 1: Acticoat® 7, 2: Biatain®-Ag; Comfeel®-Ag; 4: Seasorb®-Ag, 5: control. **C**) Cytotoxicity measured by MTT analysis.

Both types of analysis demonstrate that all tested silver dressings release their active silver complexes which then diffuse into the surrounding medium thus influencing cell morphology and survival.

Analysis of cell death programme induced by the various dressings

Because of the fast onset of cell death observed, we decided to investigate in more detail the cell death process induced by the different dressings. As programmed cell death is typically induced within a few hours of the challenge [12], we decided to perform flow cytometric analysis after 2 h incubation of cells in an experimental set-up analogous to *figure 1A*. After incubation, cells were double stained with AnnexinV-FITC, a marker for early apoptosis (phosphatidylserine exposure to the outer cell surface) and propidium iodide, a marker for necrosis (cell membrane damage). In the cytometric analysis plot *(figure 2)*, the four quadrants are indicative of different types of cell death: 1 for necrosis, 2 for late apoptosis/necrosis, 3 for living cells and 4 for early apoptosis.

A marked difference in response was seen for the two cell lines *(table 1)*.

For the keratinocyte HaCaT cells *(figure 2A)*, a statistical difference in cell survival was seen for all dressings. Seasorb®-Ag roughly showed equal amounts of cells in early apoptosis and late apoptosis/necrosis, whereas in that same time frame Acticoat® 7 and Biatain®-Ag cultures were mostly in early phase apoptosis. Comfeel®-Ag challenged cells were in late phase apoptosis or necrosis. Incubation with the pro-oxidant hydrogen peroxide (5 mM) resulted in a high number of cells in early apoptosis, although a significant number of cells were also in late apoptosis (quadrant 2) and necrosis (quadrant 1 and 2). In

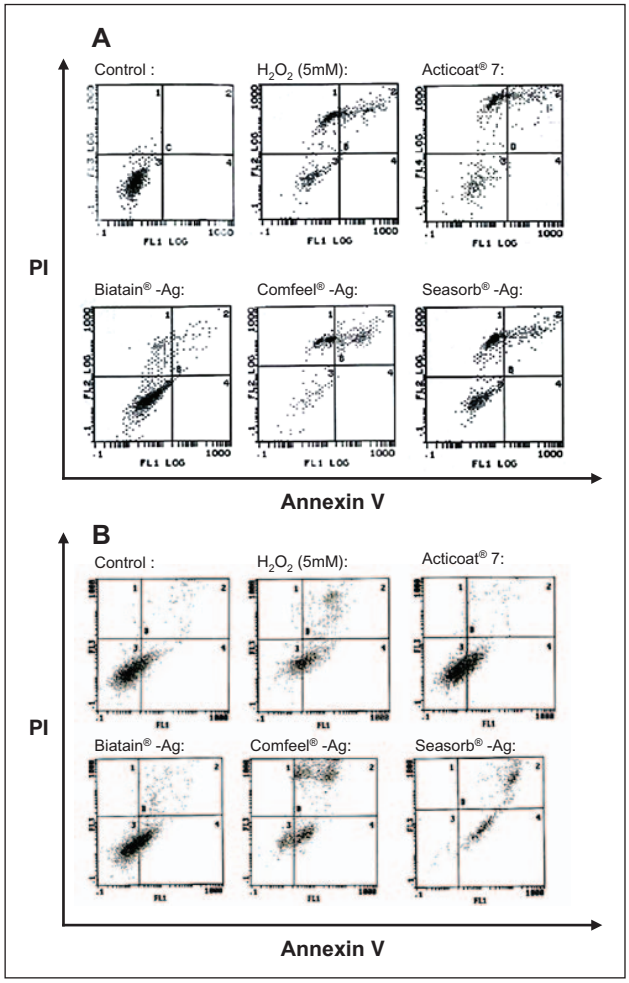

Figure 2. Flow cytometric analysis of Fibroblast 142BR **(A)** HaCaT keratinocytes **(B)** grown in the presence of silver dressings in transwell inserts for 2 h (see materials and methods). Quadrant 1: necrotic; Quadrant 2: late apoptotic or necrotic Quadrant 3: living; Quadrant 4: early apoptotic.

this experimental set-up, Biatain®-Ag was the least cytotoxic when compared with the other products. More keratinocytes were dying in the control set-up compared with fibroblasts.

Fibroblast 142BR cells overall *(figure 2B*, *table 1)* showed higher survival rates than HaCaT cells. Cell survival was significantly reduced by the silver dressings compared with control cells, with most surviving cells found in Biatain®-Ag treated cultures and least in Comfeel®-Ag treated cultures. A small but significant number of cells challenged with Biatain®-Ag or hydrogen peroxide were in early apoptosis. All silver dressing treated cultures showed significant numbers of cells in necrosis and late apoptosis or necrosis (quadrant 1 and quadrant 2 respectively).

Anti-microbial activity of the silver dressings on agar plates: zone of inhibition

In order to compare the anti-microbial activity of the four silver dressings by diffusion, *S. aureus*, *E. coli* and *C. albicans* suspensions on agar plates were challenged with the different silver dressings on plate. *Figure 3* and *table 2* clearly show that the susceptibility of the micro-organisms to the dressings is very different. Whereas *E. coli* was susceptible to Biatain®-Ag, *S. aureus* and *C. albicans* were to a much lesser extent. *E. coli* was also most susceptible to Comfeel®-Ag, but was not towards Acticoat® 7. The latter dressing was active against all three micro-organisms.

Biological effect by dilution

As every dressing has its own density, this results in varying amounts of silver being released per surface area and so we decided to suspend a fixed amount of the silver dressings in growth medium for 24 hours and make serial dilutions of the "extract". Next, a fixed number of micro-organisms were challenged with the dilutions for 24h and growth was assessed spectophotometrically.

From *table 3* it is clear that for the three micro-organisms tested, the smallest MIC_{50} values were obtained for the Acticoat® 7 dressing compared with the other dressings. These differences between Acticoat® 7 and the other dressings were statistically different for all organisms ($p < 0.05$) other than Seasorb®-Ag on *E. coli*, where the statistical difference was $p = 0.08$. For *S. aureus*, a statistical difference was seen for Biatain®-Ag and Seasorb®-Ag ($p < 0.01$) but not for Biatain®-Ag versus Comfeel®-Ag or Comfeel®-Ag versus Seasorb®-Ag. For *C. albicans*, a statistical difference was seen between Comfeel®-Ag and Seasorb®-Ag ($p < 0.02$) but not for Biatain®-Ag versus Comfeel®-Ag or Biatain®-Ag and Seasorb®-Ag. For *E. coli*, no statistical difference was noticed between Biatain®-Ag, Comfeel®-Ag and Seasorb®-Ag.

Conclusion

As reported by Atiey *et al*. [3], no two silver dressings are alike. This is also true of the form in which the silver is incorporated in the various dressings. Silver can be present in a variety of forms: It can be either metallic (Ag^0) or ionic

Table 1. Flow cytometric analysis of HaCaT keratinocytes and Fibroblast 142BR. Values are means of three experiments expressed as percentage; numbers between brackets represent standard deviations. * $p < 0.05$; ** $p < 0.03$

	Necrotic		Late apoptotic or necrotic		Living		Early apoptotic	
	HaCaT	142BR	HaCaT	142BR	HaCaT	142BR	HaCaT	142BR
Control	0.40 (0.15)	1.62 (0.37)	4.86 (1.00)	0.02 (0.02)	83.37 (6.47)	98.33 (0.38)	11.36 (7.50)	0.05 (0.07)
H2O2	1.62 (0.33)**	9.41 (1.28)**	27.27 (6.96)*	42.22 (1.67)**	26.00 (10.62)**	43.63 (5.92)**	45.13 (4.50)*	1.39 (0.48)*
Acticoat® 7	0.75 (0.51)	16.58 (1.33)**	11.71 (1.14)**	19.07 (4.92)**	39.97 (8.78)**	63.73 (4.63)**	47.57 (8.22)*	0.61 (0.52)
Biatain®-Ag	0.38 (0.29)	4.47 (0.37)**	5.6 (0.89)	3.61 (0.16)**	58.47 (5.47)**	89.27 (0.67)**	35.53 (5.67)**	1.99 (0.73)*
Comfeel®-Ag	1.23 (0.82)	36.87 (4.78)**	58.50 (10.92)*	34.04 (13.18)**	16.42 (9.17)**	32.27 (4.82)**	23.97 (4.18)	0.28 (0.24)
Seasorb®-Ag	0.08 (0.07)	28.99 (3.52)**	51.27 (6.81)**	13.71 (0.26)**	2.99 (1.33)**	56.80 (3.16)**	45.67 (7.53)*	0.50 (0.33)

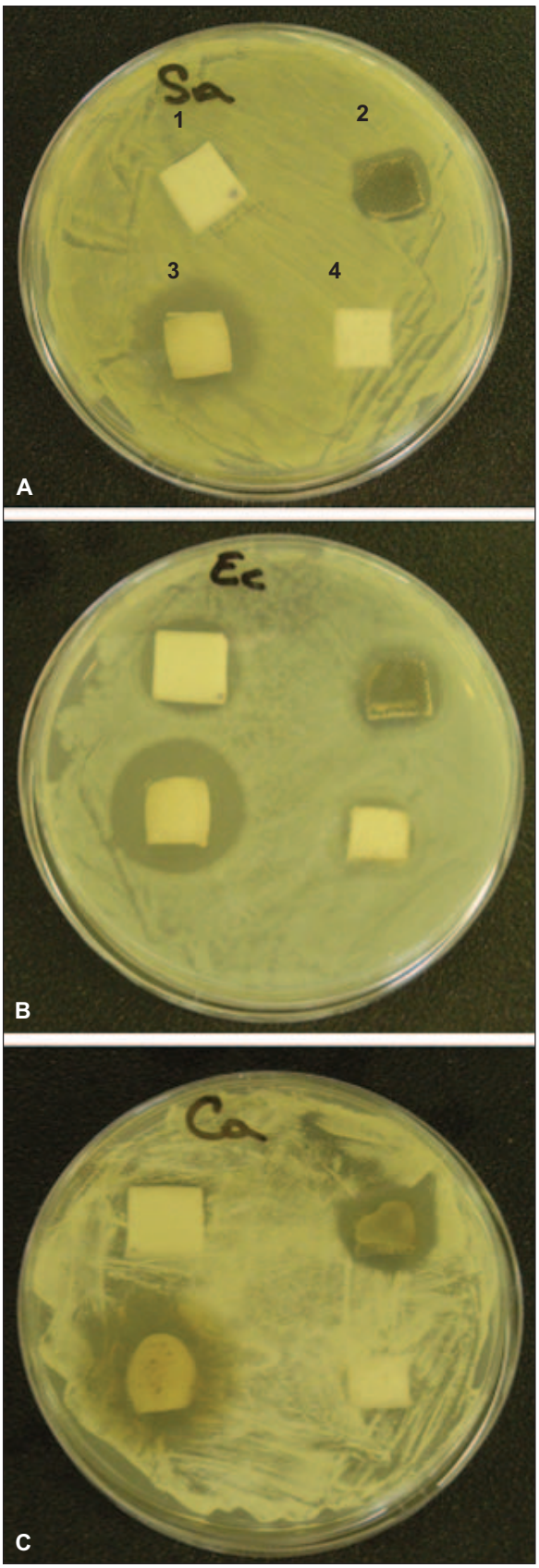

Figure 3. Zone of inhibition for *S. aureus* **(A)**, *E. coli* **(B)** and *C. albicans* **(C)** with indicated dressings. 1: Biatain®-Ag; 2: Acitcoat® 7; 3: Comfeel®-Ag; 4: Seasorb®-Ag.

Table 2. Zone of inhibition measurements in mm. Results are expressed as mean + SD of three independent experiments

	Staphylococcus aureus	*Candida albicans*	*Escherichia coli*
Acticoat® 7	2.10 (0.10)	3.67 (0.29)	1.83 (0.76)
Biatain®-Ag	0.05 (0.07)	0.05 (0.07)	2.25 (0.35)
Comfeel®-Ag	2.83 (1.04)	3.90 (0.10)	6.00 (0.50)
Seasorb®-Ag	0 (0.0)	0 (0.0)	0.83 (0.58)

(Ag^+). In the latter case, many silver salts are insoluble and hence precipitate from water solutions. Nanocrystalline silver consists of metallic silver (Ag^0) with altered grain boundaries and it has been suggested that this is a third form of silver [13]. These different forms or states of silver therefore make it difficult to compare the cytotoxicity/bactericidicity of the silver dressings on the market. In order to circumvent the problem, it seemed justified that we compare the *relative* cytotoxicity/bactericidicity of the different dressings in a well-defined water solution (growth medium), irrespective of the absolute amount of silver in the dressings. We further hypothesized that this would mirror more closely the situation in wound conditions as compared with absolute amounts of the metal.

In a first experimental set-up, we placed silver dressings on a Transwell insert with cells growing beneath it. In such a set-up, emerging cytotoxicity can only be the result of diffusion from silver from the dressings. It was observed that both keratinocyte cells (HaCaT) as well as fibroblast cells (142BR) were sensitive to all silver dressings. However, more surviving fibroblasts were observed compared to keratinocytes. This difference in sensitivity is probably the result of the different metabolisms and oxidative sensitivities of the two cell types. Interestingly, Biatain®-Ag had the least influence on both cell types, although this could be attributed to the high absorption of the dressing resulting in a delayed equilibrium concentration of silver in the growth medium. Concurrent with this hypothesis, we clearly observed cell stress when leaving the cells for 24 h in contact with this dressing (data not shown).

We hypothesize that the amount of cells in early apoptosis compared to late apoptosis or necrosis is dependent on the amount of biologically available silver in the medium. Indeed we observed for Biatain®-Ag, where high absorption of growth medium was noticed, the highest ratio of cells in early apoptosis after 2 h incubation. Comfeel®-Ag on the other hand, demonstrating little absorption of growth medium, resulted in the highest number of cells in late apoptosis or necrosis after 2 h incubation. Interestingly, this

Table 3. MIC_{50} values for dressings extracted for 24 h in bacterial growth media (see materials and methods). Values are means (± SD) of at least three independent experiments expressed as mg/mL in bacterial growth medium

	Staphylococcus aureus	*Candida albicans*	*Escherichia coli*
Acticoat® 7	7.6 (1.8)	7.9 (1.6)	8.1 (1.3)
Biatain®-Ag	29.9 (1.0)	28.5 (6.4)	26.7 (1.9)
Comfeel®-Ag	23.7 (7.8)	37.3 (0.8)	25.9 (2.2)
Seasorb®-Ag	21.9 (0.2)	20.8 (3.1)	23.7 (8.6)

dressing was the heaviest per surface unit, which could well result in a high silver release at equilibrium state. This dressing also showed the largest zones of inhibition on agar plates.

A similar observation was made by Burd *et al.* [14] when they demonstrated cytotoxicity for different silver dressings. This group, as well as Brett [15] and Walker and colleagues [16], clearly established that silver release is strongly dependent on the incubation conditions of the dressings. This could explain the great discrepancy in our observations for Seasorb® Ag in the high mortality of HaCaT cells, yet absence of zones of inhibition, but MIC_{50} values comparable to other dressings.

In our two experimental set-ups for the antimicrobial activity of the dressings, no clear correlation could be found. Some dressings showed poor zones of inhibition, but clearly had anti-microbial effects in the 24 h extraction procedure. This, however, should not be a surprise. In the extraction procedure, complete equilibrium between the silver in the dressing and the surrounding medium should have been established after 24 h, as for Burd *et al.* [14] reported a pre-incubation of only 10 minutes in different media. In the case of diffusion on agar plates, however, only at the place in direct contact between the plates and the silver dressing, was there a release of silver. Concurrent with this hypothesis, Comfeel®-Ag, on average 5-10 times heavier per square unit, was the most potent of all dressings in this experimental set-up. An interesting observation was made for Acticoat® 7. It performed well in both antimicrobial set-ups. This could be related to its reported nanocrystalline structure [13, 17, 18] which might be less susceptible to inhibition by organic materials present in the bacterial growth media.

In conclusion, our experiments clearly show that the antimicrobial activity of silver dressings is accompanied by cellular cytotoxicity. This is in line with the conclusion reported by Atiyah and colleagues [3] who stated that silver-based products cannot discriminate between healthy cells and pathogenic bacteria. As our experimental data were performed on time scales which are relatively short compared with *in vivo* situations, it therefore seems that silver dressings should be used only on critically contaminated wounds rather than used *de facto*. A delay in reepithelialisation could result from cytotoxicty of the newly formed, sensitive, keratinocytes, as has been shown by Burd *et al.* [14] in *in-vivo* situations. The search for antimicrobial dressings with good antimicrobial activity but with minimal toxicity towards eukaryotic cells should therefore continue. ■

Acknowledgement. *We wish to thank Dr P. Ponsaerts of the University of Antwerp for his assistance in the flow cytometry analysis. Financial support: none. Conflict of interest: none.*

References

1. Robson MC. Wound infection. A failure of wound healing caused by an imbalance of bacteria. *Surg Clin North Am* 1997; *77*(3): 637-50.
2. Robson MC. Infection in the surgical patient: an imbalance in the normal equilibrium. *Clin Plast Surg* 1979; 6(4): 493-503.
3. Atiyeh BS, Costagliola M, Hayek SN, Dibo SA. Effect of silver on burn wound infection control and healing: review of the literature. *Burns* 2007; 33(2): 139-48.
4. Warriner R, Burrell R. Infection and the chronic wound: a focus on silver. *Adv Skin Wound Care* 2005; 18(Suppl 1): 2-12.
5. Moyer CA, Brentano L, Gravens DL, Margraf HW, Monafo WW. Treatment of large human burns with 0.5% silver nitrate solution. *Arch Surg* 1965; 90: 812-67.
6. Stanford W, Rappole BW, Fox Jr. CL. Clinical experience with silver sulfadiazine, a new topical agent for control of pseudomonas infections in burns. *J Trauma* 1969; 9(5): 377-88.
7. Klasen HJ. A historical review of the use of silver in the treatment of burns. II. Renewed interest for silver. *Burns* 2000; 26(2): 131-8.
8. Bader KF. Organ deposition of silver following silver nitrate therapy of burns. *Plast Reconstr Surg* 1966; 37(6): 550-1.
9. Hussain SM, Hess KL, Gearhart JM, Geiss KT, Schlager JJ. In vitro toxicity of nanoparticles in BRL 3A rat liver cells. *Toxicol In Vitro* 2005; 19(7): 975-83.
10. Poon VK, Burd A. In vitro cytotoxity of silver: implication for clinical wound care. *Burns* 2004; 30(2): 140-7.
11. Lam PK, Chan ES, Ho WS, Liew CT. In vitro cytotoxicity testing of a nanocrystalline silver dressing (Acticoat) on cultured keratinocytes. *Br J Biomed Sci* 2004; 61(3): 125-7.
12. Teraki Y, Shiohara T. Apoptosis and the skin. *Eur J Dermatol* 1999; 9(5): 413-25.
13. Birringer R. Nanocrystalline materials. *Mater Sci Eng* 1989; A117: 33-43.
14. Burd A, Kwok CH, Hung SC, Chan HS, Gu H, Lam WK, Huang L. A comparative study of the cytotoxicity of silver-based dressings in monolayer cell, tissue explant, and animal models. *Wound Repair Regen* 2007; 15(1): 94-104.
15. Brett DW. A discussion of silver as an antimicrobial agent: alleviating the confusion. *Ostomy Wound Manage* 2006; 52(1): 34-41.
16. Walker M, Cochrane CA, Bowler PG, Parsons D, Bradshaw P. Silver deposition and tissue staining associated with wound dressings containing silver. *Ostomy Wound Manage* 2006; 52(1): 46-50.
17. Djoki SS, Burrell RE, Field DJ. An electrochemical analysis of thin silver films produced by reactive sputtering. *J Electrochem Soc* 2001; 148(3): 191-6.
18. Taylor PL, Omotoso P, Wiskel JB, Mitlin D, Burrell RE. Impact of heat on nanocrystalline silver dressings. Part II. Physical properties. *Biomaterials* 2005; 26(35): 7230-40.