Review

Nanocrystalline silver, gelatinases and the clinical implications

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Abstract

Nanocrystalline silver (NCS) has proven to be an important wound dressing particularly in chronic infected wounds. However, debate still rages around its use in the case of partially epithelialized wounds, particularly when these are non-infected. Much of the debate has revolved around seemingly contradictory research publications that blurred the use of NCS in these clinical situations, primarily based on reported cytotoxic effects of NCS on cell lines in vitro. MMPs, in particular MMP-9 (gelatinase) has been demonstrated to be pivotal in the progression from keratinocyte cleavage, to migration and re-epithelialisation. High levels promote increases in TNF-α; IL-8 and TGFβ, all associated with exaggerated ongoing inflammation and chronicity. Low levels impede the process of keratinocyte migration. Thus, as in so many clinical situations, a balance of MMP level is extremely important. NCS has been demonstrated to decrease these undesirable high levels of MMP-9 making it an ideal dressing for chronic infected wounds, acute inflamed wounds and burn wounds of all types which are associated with protracted raised MMP-9 levels. The converse applies too—NCS used in a situation of minimal inflammation may undesirably decrease the low levels of MMP-9 and adversely affect epithelialisation. NCS would be contra-indicated in conjunction with cell lines in vitro, cell cultured lines in vivo and integrated artificial matrices with added cell lines. Therapeutic decisions for different clinical situations may thus be made more predictably.

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1. Introduction

Wound healing involves a sequence of events aimed at repairing damaged tissue. The sequence begins at the time of wounding with the immediate formation of a provisional clot. This is then transformed into granulation tissue and a provisional matrix by the orchestration of cells, cytokines, growth factors and a complex series of messengers that initiate changes in the extracellular matrix (ECM) preparing the wound for its final stage of re-epithelialisation [1].

It is apparent that re-epithelialisation does not occur in isolation but follows a sequence of preparatory events initiated and maintained predominantly by matrix metalloproteinases (MMPs). This proteolytic breakdown of ECM begins with degradation of the provisional matrix, allowing angiogenesis, keratinocyte migration and re-epithelialisation to proceed [1–3]. MMPs are varied and serve different defined functions. Therapeutic interventions aimed at wound healing often directly or indirectly, intentionally or unintentionally affect MMP levels and alter the milieu of the wound bed, influencing ultimate healing of the wound. This balance of MMP levels has been shown to be critical to the process of healing—low MMP levels are associated with insufficient degradation of the provisional matrix and delayed re-epithelialisation and very high MMP levels associated with severe tissue degradation and chronicity of wound healing [3].

The expression of MMPs is not detected in vivo in normal intact skin [2]; however, after injury several MMPs are temporarily expressed during the wound healing process. [1,2,4] Therapeutic interventions may take the form of systemic medications, topical medications, dressings (passive or interactive) or simple actions such as wound debridement. Each intervention has the potential to change the critical balance of MMP/growth factor ratio influencing the ultimate healing cycle.

Nanocrystalline silver utilizes nanotechnology to release clusters of extremely small and highly reactive silver particles. The smaller the particles of silver, the greater the wound area that it reaches [5]. A unique property of nanocrystalline silver is that it dissolves to release Ag0 clusters and Ag+, whereas other silver sources release only Ag+ [5]. This difference in the dissolution properties of nanocrystalline silver dressings appears to alter the biological properties of the solution, including both antimicrobial and anti-inflammatory activity [10]. Nanocrystalline silver dressings have been demonstrated in vitro as effective antifungal agents [6], antibacterial agents [7], and antifungal agents for antibiotic resistant bacteria [8,10]. In vivo studies have shown that nanocrystalline silver is very effective at preventing infections [9] and healing wounds [10,11]. Nanocrystalline silver anti-inflammatory efficacy has also been demonstrated, both in vivo studies [12–15] and in clinical studies [16].

Controversy still abounds relating to cytotoxicity of the product to cell lines and more particularly its effect on re-epithelialisation in the clinical context. [11,12,15–21,49,50]. Differing opinions are proffered for its use in certain clinical situations based on the somewhat confusing research results. To date no unifying theory has been offered explaining the research results and marrying these in a clinical context.

While it is accepted that the nanocrystalline silver antimicrobial efficacy is responsible for a great deal of its success in chronic infected wounds, interest has only recently turned to the anti-inflammatory effect of this agent. More particularly, the unique effects on MMP-9 and to a lesser extent MMP-2 provide excellent explanations for the reported conflicting in vitro and in vivo effects of nanocrystalline silver. This review elaborates on the above offering a unifying theory for the effect of nanocrystalline silver on re-epithelialisation.

2. Matrix metalloproteinase (MMP) physiology

Keratinocyte migration and re-epithelialisation involves a tortuous path whereby these cells detach from the basement membrane and make their way to the surface of the wound traversing the fibrin clot initially and then the provisional matrix. To prepare their way through this obstacle ridden course, ECM degrading enzymes clear the path for the migrating keratinocytes.
These enzymes are divided into four main groups: serine proteases, cysteine proteases, aspartic proteases, and metalloproteinases, based on their catalytic sites. MMPs are a multigene family that belong to the superfamily of metalloproteinases. Altogether 23 human MMPs are known at present, classified as collagensases, gelatinases, stromelysins, membrane-type MMPs, and others. All members of the MMP family are structurally related [12]. MMPs are synthesized as inactivezymogens, with the prodomain masking the catalytic site. A conserved cysteine residue in the prodomain forms a "cysteine switch", which needs to be disrupted before removal of the propeptide domain and subsequent exposure of catalytic Zn\(^{2+}\) are possible. Once active, MMPs can be inhibited by either general endogenous inhibitors, such as 2-macroglobulin in plasma and tissue fluids, or by specific inhibitors, such as tissue inhibitors of metalloproteinases (TIMPs), which are anchored or secreted to the ECM. TIMPs inhibit active MMPs as well as proMMP activation [12].

Proteolysis in acute (healing) wounds is essential to enable migration of cells into the wound, to remodel granulation tissue, and to reform the architecture of normal dermis. However, excessive, inappropriate expression and activation of proteases occurs in chronic dermal ulcers. Also, the levels of endogenous MMP inhibitors TIMPs, are reduced [1–4,16,22].

Collagen is degraded and remodelled by a host of MMPs; MMP-8 (collagenase 2) and MMP-13 (collagenase 3), MMP-1 (collagenase-1), MMP-2 (type IV collagenase, gelatinase A), and MMP-9 (type IV collagenase, gelatinase B). MMP-1 is expressed by the wound edge keratinocytes in both acute and chronic wounds, and its expression is rapidly shut off after the re-epithelialization phase [36]. In contrast MMP-9 is persistently elevated in chronic wounds. Increased MMP-9 is seen in decubitus ulcers, venous stasis ulcers and non-healed burn wounds [22,26,27]. Not only does MMP-9 persist in non-healing wounds, it does so at five times levels seen in healing wounds [22,36]. As these wounds heal, MMP-9 disappears [22,33,34,48].

Tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) induced MMP-9 activation by human skin is mediated by a down regulation of tissue inhibitor of TIMP, which is absent from chronic ulcer beds. As patients age, they also tend to produce less TIMP-1. The delay in wound healing with age is thus associated with a decrease in production of MMP-9 inhibitor [22]. MMP-9 is not expressed in normal, uninjured skin [22].

To summarise, it is clear that MMP-9 activity is necessary for the normal progression of wound healing. MMP-9 inhibition is consistently associated with delays in keratinocyte motility in vitro, delayed epithelialisation in vivo, and delayed angiogenesis. Thus a certain amount of MMP-9 is necessary. Keratinocyte migration, re-epithelialisation, and angiogenesis are dependent on MMP-9, but in excess, it may delay wound healing [22]. Agren et al. [37] demonstrated in healthy human volunteers that pharmacologic inhibition of MMP led to delayed re-epithelialisation of suction blisters. In a series of healthy patients Ashcroft et al. [38] created 4 mm punch biopsy excisional wounds and observed their healing. Some MMP-9 appears necessary for healing but excess levels of MMP-9 inhibit angiogenesis.

Thus there are conflicting effects of MMP-9—it is essential for keratinocyte separation from the basement membrane, and subsequent migration into the wound. At the same time excess activation may inhibit the deposition of new basement membrane, and the reattachment of keratinocytes that is essential for migration [22]. Levels of activated MMP-9 must allow membrane digestion that is required for mobility, but levels of MMP-9 was found in patients, regardless of the treatment regime [27]. Additionally, Wysocki et al. found that as healing proceeds, the levels of MMP-9 decrease in chronic wound fluid and reach those found in acute wounds [26].
not enough to inhibit deposition. Reis et al. [22] speculate that the excess MMP-9 found in the centre of burn wounds, or in the chronic wound milieu, prevents reconstitution of the normal dermal and epidermal structures. According to these authors, the complicated interplay between the matrix attachment molecules, cytokines, inhibitors and activators in the wound environment becomes disjointed and unbalanced in the wound that fails to heal. The therapeutic implications of this are very exciting [22].

3. Nanocrystalline silver research publications

There is no major controversy concerning the antimicrobial efficacy of nanocrystalline and its effect on wound bioburden [40–46]. Interestingly NCS appears to be effective not only against commonly described bacterial pathogens (Staphylococcus; Pseudomonas; E. coli; Klebsiella), but also uniquely effective against Candida albicans too [49]. Research publications related to antimicrobial effects are therefore not included below. As detailed above, the controversy relating to nanocrystalline silver revolves around cytotoxicity and more particularly re-epithelialisation of the partial thickness wound in the clinical context.

3.1. Cytotoxicity and donor site re-epithelialisation

3.1.1. A comparative study of the cytotoxicity of silver-based dressings in monolayer cell, tissue explant, and animal models [21]

“We examined five commercially available silver-based dressings (Acticoat, Aquacel Ag, Contreet Foam, PolyMem Silver, Urgotul SSD). We assessed their cytotoxicity in a monolayer cell culture, a tissue explant culture model, and a mouse excisional wound model. The results showed that Acticoat, Aquacel Ag, and Contreet Foam, when pretreated with specific solutes, were likely to produce the most significant cytotoxic effects on both cultured keratinocytes and fibroblasts, while PolyMem Silver and Urgotul SSD demonstrated the least cytotoxicity. In the tissue explant culture model, in which the epidermal cell proliferation was evaluated, all silver dressings resulted in a significant delay of re-epithelialization. In the mouse excisional wound model, Acticoat and Contreet Foam indicated a strong inhibition of wound re-epithelialization on the postwounding-day 7. These findings may, in part, explain the clinical observations of delayed wound healing or inhibition of wound epithelialization after the use of certain topical silver dressings. Caution should be exercised in using silver-based dressings in clean superficial wounds such as donor sites and superficial burns and also when cultured cells are being applied to wounds”.

3.1.2. In vitro cytotoxicity of silver: implication for clinical wound care [17]

“In this study, we look at the cytotoxic effects of silver on keratinocytes and fibroblasts. We have assessed the viability of monolayer cultures using the MTT and BrdU assays. The composition of the culture medium and also the culture technique were modified to assess the effects of culture ‘environment’ on the susceptibility of the cells to the toxic action of silver. Further in vitro, experiments were performed using tissue culture models to allow cellular behavior in three-dimensional planes which more closely simulated in vivo behavior. The silver source was both silver released from silver nitrate solution but also nanocrystalline silver released from a commercially available dressing. The results show that silver is highly toxic to both keratinocytes and fibroblasts in monolayer culture. These results suggest that consideration of the cytotoxic effects of silver and silver-based products should be taken when deciding on dressings for specific wound care strategies. This is important when using keratinocyte culture, in situ, which is playing an increasing role in contemporary wound and burn care.”

3.1.3. An in vitro evaluation of the cell toxicity of honey and silver dressings [50]

Objective: To establish whether honey and silver-impregnated dressings used by wound healing practitioners are cytotoxic in vitro to human skin keratinocytes and dermal fibroblasts.

Method: Human keratinocyte and fibroblast tissue cultures were established in vitro. Untreated cultures served as controls (group I). Small dressing implants of monoforal, medicinal honey (l-mesitran) (group 2) and nanocrystalline silver (Acticoat) (group 3) were placed in test wells and cocultured with each of the two cell lines. Morphological changes, including cell toxicity, were assessed using inverted microscopy, trypan blue staining and the rosy and Clauss cell toxicity scoring system.

Results: Untreated cultures consisting of both keratinocytes and fibroblasts (group 1) were established in 90% of all cases. In group 2, cultures with honey-impregnated implants, cell proliferation remained present at 2 and 4 months. Cell viability remained intact and cell toxicity was not evident at 4 months after continuous tissue culture. In group 3, marked toxicity was observed with high non-viability staining and cell-scoring counts compared with groups 1 and 2 (P < 0.05). This demonstrates that the silver interfered with epidermal cell proliferation and migration, implying that it contains cytotoxic material.

Conclusion: The honey-based product showed excellent cytocompatibility with tissue cell cultures compared with the silver dressing, which demonstrated consistent culture and cell toxicity. Further studies are needed to assess if these comparative in vitro findings should influence a clinician’s choice of wound dressing.


Objective: To compare rates of healing of donor sites in pigs between those dressed with silver-coated dressings and
those dressed with petrolatum-impregnated absorbent gauze.

Design: Open study with each animal acting as its own control.

Setting: University research facility, Canada.

Animals: 6 young specific-pathogen-free domestic pigs.

Interventions: A total of 72 wounds about 1 cm 2 cm 0.4 mm were made in rows of eight on each pig with a dermatome. They were divided into three groups of 24, and dressed with petrolatum gauze, or silver-coated dressings moistened with sterile water either once only or daily for 10 days. All dressings were secured in place with an elastic bandage.

Main outcome measures: Erythema, infection, epidermal migration, and healing.

Results: Wounds dressed with moistened silver-coated dressings re-epithelialised significantly more quickly. This resulted in complete re-epithelialisation within 70% of the time taken by those wounds dressed with petrolatum gauze.

Conclusion: Silver-coated dressings provide a moist environment for the healing wound combined with an effective antimicrobial agent, and this significantly accelerates healing compared with wounds dressed with traditional petrolatum gauze dressings.

3.2 Inflammation

3.2.1. Anti-inflammatory activity of nanocrystalline silver in a porcine contact dermatitis model [15]

‘The anti-inflammatory activity of nanocrystalline silver was examined using a porcine model of contact dermatitis. Inflammation was induced with dinitrochlorobenzene and then treated daily with nanocrystalline silver dressings, 0.5% silver nitrate, or saline. Erythema, edema, and histological data showed that nanocrystalline silver-treated pigs had near-normal skin after 72 h, while other treatment groups remained inflamed. The decreased inflammation in the nanocrystalline silver-treated group was associated with increased inflammatory cell apoptosis, a decreased expression of pro-inflammatory cytokines, and decreased gelatinase activity. Silver nitrate treatments induced apoptosis in all cell types, including keratinocytes, resulting in delayed wound healing. These results demonstrate that nanocrystalline silver had a direct anti-inflammatory effect in the porcine contact dermatitis model that improved the overall outcome of the healing process. These data offer support that a species of silver (e.g., Ag0) that is uniquely associated with nanocrystalline silver may be responsible for the anti-inflammatory activity and improvement in healing.’

3.2.2. Topical nanocrystalline silver cream suppresses inflammatory cytokines and induces apoptosis of inflammatory cells in a murine model of allergic contact Dermatitis [14]

‘Nanocrystalline silver has both antimicrobial and anti-inflammatory properties. However, the exact mechanisms underlying these activities are not known.

Objectives: The objectives of this study were to assess the anti-inflammatory effects of nanocrystalline silver using a murine model of allergic contact dermatitis, compare the effects with those of tacrolimus and a high potency steroid, and to relate the effects to modulation of pro-inflammatory cytokines and apoptosis of inflammatory cells.

Methods: Dermatitis was induced on the ears of BALB/c mice using dinitrofluorobenzene. Topical treatment, including vehicles, 1% nanocrystalline silver cream, tacrolimus ointment and a high potency steroid, was applied once a day for 4 days. Ear swelling was measured and the erythema was evaluated daily. After 4 days of treatment the mice were killed and samples from the ears were collected for histological and immunohistochemical examination.

Results: Significant reductions of ear swelling, erythema and histopathological inflammation in mice ears were observed after 4 days of treatment with 1% nanocrystalline silver cream, tacrolimus ointment or a high potency steroid with no significant difference among them. TUNEL staining demonstrated a significant increase in the numbers of apoptotic cells in material from the group treated with nanocrystalline silver when compared with that from groups treated with vehicle, tacrolimus or steroid.

Conclusions: This study demonstrates that nanocrystalline silver inhibits allergic contact dermatitis in mice, similar to steroid and tacrolimus. Nanocrystalline silver suppresses the expression of TNF-a and IL-12 and induces apoptosis of inflammatory cells; mechanisms by which nanocrystalline silver may exert its anti-inflammatory effects.’

3.2.3. Early healing events in a porcine model of contaminated wounds: effects of nanocrystalline silver on matrix metalloproteinases, cell apoptosis, and healing [12]

“A porcine model of wound healing was employed to examine the impact of nanocrystalline silver-coated dressings on specific wound healing events. Full-thickness wounds were created on the backs of pigs, contaminated with an experimental inoculum containing Pseudomonas aeruginosa, Fusobacterium sp., and coagulase-negative staphylococci, and covered with dressing products either containing silver or not. Nanocrystalline silver-coated dressings promoted rapid wound healing, particularly during the first several days post-injury. Healing was characterized by rapid development of well vascularized granulation tissue that supported tissue grafting 4 days post-injury, unlike control dressed wounds. The proteolytic environment of wounds treated with nanocrystalline silver
was characterized by reduced levels of matrix metalloproteinases. Matrix metalloproteinases have been shown to be present in chronic ulcers at abnormally high levels, as compared with acute wounds, and may contribute to the non-healing nature of these wounds. Cellular apoptosis occurred at a higher frequency in the nanocrystalline silver-treated wounds than in wounds dressed with other products. The results suggest that nanocrystalline silver may play a role in altering or compressing the inflammatory events in wounds and facilitating the early phases of wound healing. These benefits are associated with reduced local matrix metalloproteinase levels and enhanced cellular apoptosis.”

3.3. Clinical studies

3.3.1. The role of Acticoat™ with nanocrystalline silver in the management of burns [39]

“Silver is an effective antimicrobial agent, but older silver-containing formulations are rapidly inactivated by the wound environment, requiring frequent replenishment. These older formulations may also be pro-inflammatory and may delay healing. Acticoat™ (Smith & Nephew, Hull, UK) is a relatively new form of silver antimicrobial barrier dressing which helps avoid the problems of earlier agents. It has rapid and sustained bactericidal activity, and because of this may reduce inflammation and promote healing. Despite extensive testing and clinical experience, no evidence has emerged of resistance or cytotoxicity to nanocrystalline silver. This article collects together a number of presentations that were given at the 2003 European Burns Association Meeting on the use of ActicoatTM in the management of burns.”

3.3.2. The rate of re-epithelialization across meshed skin grafts is increased with exposure to silver [20]

“The objective in this study was to determine whether exposure to pure silver increases the rate of re-epithelialization across a partial thickness wound. A meshed skin graft, placed on an excised burn wound was used as a healing model. Methods: The rate of meshed skin graft epithelial closure on an exposed burn using a moist healing environment was shown. A moistened silver delivery system (Acticoat) was compared with a standard xeroform and eight ply gauze dressing continually moistened with a 0.01% neomycin and polymyxin solution (NP). Twenty burn patients with deep burns of over 15% of TBS were excised and grafted with 2:1 meshed grafts. One graft area was treated with the antibiotic solution and another with the silver delivery. The meshed graft was performed within 3 days of injury. Results: No infections were noted and quantitative swab cultures gave less than 102 bacteria in all cases at wound closure. At day 7, re-epithelialization was complete with silver and 55% closed with NP solution. Wound closure was complete in the NP solution group at day 10. Silver increased re-epithelialization rate by over 40%, a significant increase. Graft take was over 95% in both groups. Conclusion: Silver released in a moist wound surface environment significantly increases the rate of re-epithelialization compared to a standard antibiotic solution.”

3.3.3. The use of silver-coated dressings on donor site wounds: a prospective, controlled matched pair study [18]

“Acticoat, a new silver-coated dressing, produces a moist healing environment along with the sustained release of ionic silver for improved microbial control. These properties suggest that Acticoat might be a useful donor site dressing. However, there are no human studies which assess Acticoat for this use. The purpose of this study was to compare the healing of human skin graft donor sites dressed with Acticoat, to the healing of those dressed with Allevyn, an occlusive moist healing environment material, which is our standard donor site dressing. In burn patients who had undergone burn excision and grafting, identical side-by-side split thickness donor site wound pairs were dressed with Allevyn and Acticoat. Re-epithelialization was directly assessed daily by a single observer from post-operative day 6 onward, and by four independent observers who rated the extent of re-epithelialization by viewing standardized digital images of the wounds that had been obtained on post-operative days 6, 8, 10, and 12. Donor sites were swabbed for bacterial culture on days 3, 6, and 9. Subsequently, each study donor site scar was rated by a blinded observer using the Vancouver Scar Scale at 1, 2, and 3 months. Sixteen paired sites in 15 patients (3 female, 12 male) were studied. Donor sites dressed with Allevyn were >90% re-epithelialized at a mean of 9.1 ± 1.6 days while donor sites dressed with Acticoat required a mean of 14.5 ± 6.7 days to achieve >90% re-epithelialization. The Allevyn sites had significantly greater estimated re-epithelialization at days 6, 8, 10 and 12 than the Acticoat sites based on the observations of the digital images. There were no significant differences in the incidence of positive bacterial cultures with either dressing at days 3, 6, and 9. Donor sites dressed with Acticoat had significantly worse scars at 1 and 2 months but this difference resolved by 3 months. Our findings do not support the use of Acticoat as a skin graft donor site dressing.”

4. Summary of publications—identifying the controversies

The publications presented above are not meant to be exhaustive or complete. They do however, present a picture representative of most publications in the ascribed subsections.

4.1. Cytotoxicity and donor site re-epithelialisation [11,17,21,49]

In vitro publications indicate that cultured cell lines – keratinocytes and fibroblasts – manifest cytotoxic effects from nanocrystalline silver. Authors extrapolate that caution should be observed when treating partially epithelialized...
wounds, and in particular if cultured cell line are used therapeutically.

In contrast swine donor sites appeared to epithelialize faster than petrolatum gauze dressings when nanocrystalline silver was used.

4.2. Inflammation [12,14,15,25]

Publications appear to be unanimous in their findings related to inflammation and nanocrystalline silver (NCS). NCS decreases MMPs, inflammatory mediators and results in dermal inflammatory cell apoptosis.

4.3. Clinical studies [39,20,18]

Despite extensive testing and clinical experience, no evidence has emerged of resistance or cytotoxicity to nanocrystalline silver. Interestingly, meshed skin grafts (in burn patients) appeared to epithelialize faster and more efficiently with NCS dressings than with standard dressings, but when donor site healing was compared with Allevyn dressings, NCS appeared to delay epithelialisation.

It is clear that NCS is effective at decreasing the bioburden of the wound. This in turn should decrease the inflammatory mediators, decrease MMP levels, increase TIMP levels and return many chronic wounds to an acute-like state thus initiating healing. In the acute situation however, cracks begin to appear in the accepted mechanism of action. Cultured epithelial growth appears to be retarded by NCS, clean partial thickness donor sites have varying responses to NCS and there is little direction for usage of NCS in partially epithelialized non-infected wounds. Many authors have cautioned against its use in these wounds, yet meshed skin grafts in burn patients did very well with NCS. Is there a unifying theory for these findings?

5. Unifying theory of NCS

Before rigidly accepting all research publication findings and basing a hypothesis on these findings, it is essential to recognise shortcomings in methodology and research practices that may impact on results of any study. Thus the frequency of dressing changes, the saturation of dressings with fluid, the inability to handle copious exudate may all have affected the efficacy of NCS, the release of silver ions and the potential toxicity of the dressing. Also comparisons to petrolatum gauze dressings which can initiate severe inflammatory reactions are also not ideal when comparing epithelialisation in clean wounds. However, basic patterns that have emerged appear to be in keeping with the appearance of new basic scientific studies.

Acticoat™ (Smith & Nephew, UK) NCS consists of an absorbent rayon-polyester core sandwiched between two layers of silver-coated high-density polyethylene. Acticoat™ releases as much as 30 times less silver than other topical silver products such as silver nitrate and silver sulfadiazine, but more of the silver released is effective as it is not bound by halide, and it is released over a longer period of time [39]. Silver is rapidly released from the nanocrystalline form when it is exposed to water. The dissolution reaches a steady state at which point the silver concentration in solution is between 70 and 100 ppm [47]. In vitro tests have confirmed that the Acticoat 7™ dressing (an extended release version of the Acticoat™ dressing) can sustain antimicrobial levels of silver for at least 7 days [47]. Sustained release of silver is important in reducing bacterial burden but is also highly significant in terms of decreasing mechanical trauma.

The bimodal expression of MMP-9 has been described in detail above. When inhibited at low levels in acute settings or in clean wounds with low levels of MMP-9, keratinocyte separation and migration is affected, and epithelialisation is delayed [35]. MMP-9 is not expressed in quiescent human skin keratinocytes [33,34]. Conversely over expression of MMP-9 is associated with chronic wounds and non-healing [12,25–30].

Over expression of MMPs including MMP-2 and MMP-9 contributes to tissue injury and inflammation. Therefore, MMP inhibition has been suggested as a therapeutic approach to controlling inflammation [15]. Studies have convincingly demonstrated decreased gelatinase (MMP-9) expression and activity after NCS treatment [12,15]. In nanocrystalline silver-treated animals, inflammatory cell apoptosis at a dermal level is observed. This in turn leads to decreased levels of decreased TNF-α, IL-8 (another major mediator of inflammatory responses) and TGF-β.

The level of inflammatory mediators, proteases and tissue inhibitors of proteases are extremely important in the pathogenesis, tissue remodelling and regeneration in burn injuries. In a study of severely burnt children, Dasu et al. examined serum samples from 12 children (mean age 7.9 ± 2.5 years) with >40% total body surface area burns were obtained within 0.5 h, 3, 7, and 21 days after injury [4]. Pro-MMP-1 levels in the serum were significantly elevated by the seventh day after burn. Of major interest is that MMP-9 levels showed significant increases by day 3 and 21 compared to normal, respectively. Thus a sustained elevation of MMP-9 levels was apparent in burn cases as opposed to the other MMP types [4].

Therapeutic implications of these factors can be summarised as follows:

1. NCS is effective at lowering levels of MMP-9, an extremely important gelatinase that contributes to epithelialisation in low levels and impedes the process at high levels.
2. High levels of MMP-9 have a knock-on effect on TNF-α, IL-8 and TGF-β, all important inflammatory mediators that prolong the healing process at exaggerated levels.
3. High sustained levels of MMP-9 have been demonstrated in burn cases, where ongoing inflammation is likely.
4. The inhibiting effect of NCS on MMP-9 in all inflammatory-prone processes described above is beneficial and therapeutic.
5. Cultured cells and quiescent cell lines exhibit low levels of MMP-9.
6. Further lowering of MMP-9 in these non-inflamed situations appears to inhibit keratinocyte separation, migration and epithelialisation.

On re-examination of the research publications, possible explanations now become apparent. Cytotoxicity and/or delayed epithelialisation is likely in cases where NCS is used
in quiescent, clean situations with a background of little inflammation-cell cultures, donor sites, clean non-infamed wounds. It is likely that NCS in these situations decrease MMP-9 levels to the extent that epithelialisation is negatively affected.

In the case of burns, in particular the reported series where meshed skin grafts epithelialized favourably [20], the sustained high levels of MMP-9 levels reported in severe burn cases from 3 days to 21 days after-injury and beyond would explain the positive response to healing seen with NCS in these cases.

6. Clinical implications

When extrapolating these findings to the clinical situation it is apparent that NCS is well suited to any situation where MMP-9 levels are expected to be raised. It is also apparent that the reported in vitro cytotoxicity of NCS is overshadowed and negated in many clinical situations where protracted exaggerated inflammation (as in significant burn injuries) is effectively managed by NCS dressings. This translates to the following clinical indications for usage of NCS:

1. Chronic wounds of all types, infected and non-infected.
2. Burn wounds in the acute and sub-acute stages within the first 3–4 weeks of injury (with or without skin grafting, preferably with meshed skin grafting).
3. Most infected wounds with any background pathology as MMP-9 levels are anticipated to be high in all cases.
4. In conjunction with biologic dressings that involve ground substance matrix components (rather than cultured cell lines). Examples include Integra (Life Sciences Corporation, NJ, USA); AlloDerm (Life Cell, NJ, USA); Biobrane (Smith & Nephew, London, UK); Pelnac (Gunze Ltd., Japan).

Clinical situations where NCS may be found to be problematic:

1. Clean, uninfected, non-infamed, non-burn wounds such as routine donor sites.
2. Cultured epidermal grafts (Epicel, Genzyme Corp, USA) and cell cultured biologic dressings where keratinocyte and/or fibroblast cell lines are incorporated (Apligraf, Organogenesis, USA; Dermagraft, Advanced Biohealing, USA).

7. Conclusion

NCS has proven to be an important wound dressing particularly in chronic infected wounds. However, debate still rages around its use in the case of partially epithelialized wounds, particularly when these are non-infected. Much of the debate has revolved around seemingly contradictory research publications that blurred the use of NCS in these clinical situations, primarily based on reported cytotoxic effects of NCS on cell lines in vitro. MMPs, in particular MMP-9 (gelatinase) has been demonstrated to be pivotal in the progression from keratinocyte cleavage, to migration and re-epithelialisation. High levels promote increases in TNF-α; IL-8 and TGFβ, all associated with exaggerated ongoing inflammation and chronicity. Low levels impede the process of keratinocyte migration. Thus, as in so many clinical situations, a balance of MMP level is extremely important. NCS has been demonstrated to decrease these undesirable high levels of MMP-9 making it an ideal dressing for chronic infected wounds, acute inflamed wounds and burn wounds of all types which are associated with protracted raised MMP-9 levels. The converse applies too—NCS used in a situation of minimal inflammation may undesirably decrease the low levels of MMP-9 and adversely affect epithelialisation. NCS would be contra-indicated in conjunction with cell lines in vitro, cell cultured lines in vivo and integrated artificial matrices with added cell lines. Balancing and controlling levels of gelatinases appear to be important in the clinical setting. Therapeutic decisions for different clinical situations may thus be made more predictably.

Conflict of interest statement

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