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Review

Effect of silver on burn wound infection control and healing: Review of the literature

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ABSTRACT

Silver compounds have been exploited for their medicinal properties for centuries. At present, silver is reemerging as a viable treatment option for infections encountered in burns, open wounds, and chronic ulcers. The gold standard in topical burn treatment is silver sulfadiazine (Ag-SD), a useful antibacterial agent for burn wound treatment. Recent findings, however, indicate that the compound delays the wound-healing process and that silver may have serious cytotoxic activity on various host cells. The present review aims at examining all available evidence about effects, often contradictory, of silver on wound infection control and on wound healing trying to determine the practical therapeutic balance between antimicrobial activity and cellular toxicity. The ultimate goal remains the choice of a product with a superior profile of infection control over host cell cytotoxicity.

Contents

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1. Introduction

The final aim of burn management and therapy is wound healing and epithelization as soon as possible in order to prevent infection and to reduce functional and aesthetic after effects [1]. The use of topical chemotherapy has been fundamental in that regard and has helped to improve the survival of patients with major burns and to minimize the incidence of burn wound sepsis, a leading cause of mortality and morbidity in these patients [2]. One of the strategies that is gaining renewed attention for combating the threat of bacterial infection and preventing wound sepsis, is the use of noble metal antimicrobials the most prevalent of which is silver [3]. For centuries silver has been known to have bactericidal properties. As early as 1000 B.C., the antimicrobial properties of silver in rendering water potable were appreciated [4,5]. Silver compounds have been exploited for their medicinal properties for centuries as well [6]. They were popular remedies for tetanus and rheumatism in the 19th century and for colds and gonorrhea before the advent of antibiotics in the early part of the 20th century [7]. A detailed historical review about the early usage of silver to treat various conditions has been recently published [8]. Interest in silver salts or silver salt solutions in the treatment of burn patients, however, completely disappeared around the Second World War [9]. It took many years for interest in silver (nitrate) to revive, under the stimulus of a publication by Moyer et al. [10]. At present, silver has reemerged as a viable treatment option for infections encountered in burns, open wounds, and chronic ulcers.

Several products have incorporated silver for use as a topical antibacterial agent, such as silver nitrate, silver sulphadiazine (SSD) (Flammazine™, Smith & Nephew Healthcare Limited, Hull, Canada) [11], silver sulphadiazine/chlorhexidine (Silverex®, Motiff Laboratories Pvt. Ltd. Kare Health specialties, Verna, Goa), SSD with cerium nitrate (Flammacerium®, Solvay, Brussels, Belgium), and silver sulphadiazine-impregnated lipidocolloid wound dressing Urgotul SSD® (Laboratories Urgo, Chenove, France) [5,11–13]. In contrast to these silver agents, newly developed products such as Acticoat™ (Westaim Biomedical Inc., Fort Saskatchewan, Alberta, Canada) and Silverlon® (Argentum Medical, L.L.C., Lakemont, Georgia) have a more controlled and prolonged release of nanocrystalline silver to the wound area. This mode of silver delivery allows the dressings to be changed with less frequency, thereby reducing risk of nosocomial infection, cost of care, further tissue damage and patient discomfort [4,14–16].

The gold standard in topical burn treatment is silver sulfadiazine (Ag-SD), a useful antibacterial agent for burn wound treatment. Recent findings, however, indicate that the compound delays the wound-healing process [17] and that silver may have serious cytotoxic activity on various host cells [2,17–22]. On the other hand, the beneficial effects of silver on wound biology due to its potent antimicrobial activity have been overlooked in general until recently. The literature is becoming replete with clinical trials purporting to show the benefits of silver therapeutics and silver-release dressings on wound repair and regeneration through its antimicrobial efficacy. Little is published, however, to show how the released silver ion influences the wound bed, or to what extent it is metabolized or deposited in the tissue. Moreover, results of the extensive literature review we conducted failed to reveal any clinical studies regarding the risks and probabilities of wounds in general to become infected, about the effect of silver dressings on already infected wounds, nor about studies comparing the effect of silver or other antiseptic dressings on prevention of wound infection.

Irrespective of the source of silver, whether released from solutions, creams and ointments or nanocrystalline silver released from commercially available new dressings, silver is highly toxic to both keratinocytes and fibroblasts [23]. Fibroblasts appear to be more sensitive to silver than keratinocytes. 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 particularly important when using keratinocyte culture, in situ, which is playing an increasing role in contemporary wound and burn care [23,24]. Moreover, certain recent clinical studies in major burn centers have demonstrated the emergence of bacterial resistant strains mainly *Escherichia coli*, to silver as well as to many antibiotics following the prolonged usage of silver based dressings. The present review aims at examining all available evidence about effects, often contradictory, of silver on wound infection control and on wound healing trying to determine the practical therapeutic balance between antimicrobial activity and cellular toxicity.

2. Silver products and delivery modalities

Elemental silver requires ionization for antimicrobial efficacy [25]. Silver ion is a highly reactive species, readily binding to negatively charged proteins, RNA, DNA, chloride ions, and so on. This property lies at the heart of its antibacterial mechanism but also complicates delivery to the wound bed, because it is readily bound to proteins within the complex wound fluid [26]. Different silver delivery systems exist, including those that deliver silver from ionic compounds, such as silver calcium phosphate and silver chloride, and those that deliver silver from metallic compounds, such as nanocrystalline silver [27,28]. However, the difficulties with many current topical silver antimicrobials lie in their low silver release levels, the limited number of silver species released, the lack of penetration, the rapid consumption of silver ions, and the presence of nitrate or cream bases that are pro-inflammatory negatively affecting wound healing. Other issues include staining, electrolyte imbalance, and patient discomfort. Over the past few years, there has been a rapid increase in the number of silver dressings made available to physicians to address these issues [27,29]. Various available silver products may be summarized as follows:

2.1. *Colloidal silver solutions—electrically charged*

This is the most common delivery system prior to 1960. Charged pure silver particles (3–5 ppm) are held in suspension by small electric currents. Positive ions repel each other thus remain in solution even when applied topically to a wound.

2.2. Silver proteins

Consist of silver complexed to small proteins in order to improve stability in solution. These however proved to possess much less antibacterial action than pure ionic silver and were rapidly replaced by silver salts in the 1960s.

2.3. Silver salts

Delivery system becomes more stable when positively charged silver ion is complexed to negatively charged ions (AgCl, $AgNO_3$, $AgSO_4$). 0.5% Silver nitrate is the standard and most popular silver salt solution used for topical burn wound therapy. Concentrations exceeding 1% silver nitrate are toxic to the tissues. Ionic silver solutions are highly bactericidal, with no reported resistance and have a beneficial effect in decreasing wound surface inflammation. The solutions, however, are unstable and when exposed to light produce typical black stains therefore extremely unpractical. On the other hand, nitrate is toxic to wounds and to cells and appears to decrease healing offsetting to some degree the beneficial antibacterial effect of silver. Moreover, the reduction of nitrate to nitrite causes oxidant induced cell damage. This effect is most likely the reason for the impaired re-epithelialization reported with its use in partial thickness burns or donor sites. Bacterial resistance to $AgNO_3$ has been described.

2.4. Silver compounds—silver sulfadiazine

Silver sulfadiazine (Flammazine®, Silvadene®) was introduced by Fox [30] in 1970s as an antibacterial agent for topical treatment of burns and wounds. Silver is complexed to propyleneglycol, stearyl alcohol, and isopropyl myrislate and mixed with the antibiotic Sulfadiazine producing a combined formulation made from silver nitrate and sodium sulphadiazine by substituting a silver atom for a hydrogen atom in the sulphadiazine molecule and combining the inhibitory action of the silver with the antibacterial effect of sulphadiazine [9,31]. This silver complex acts on the bacterial wall in contradistinction to the silver ions which act on the bacterial energy system. All kinds of combinations of sulpha drugs with silver were tested in vitro, but silver sulphadiazine appeared to be the most effective [32]. A possible explanation of this effectiveness could be the relatively strong bonding of silver sulphadiazine to DNA [9] which differs from that of silver nitrate or other silver salts [9,33]. Bacterial resistance to these products does develop. Impaired re-epithelialization has been described. Observed bone marrow toxicity with silver sulfadiazine is primarily due to the propylene glycol component.

2.5. Sustained silver releasing systems—nanocrystalline silver

Various silver-based dressings have been introduced in the past few years and have become the latest and greatest "innovation" in wound care products. The "innovation" involved in these new wound care products is the simple fact that silver itself is incorporated within the dressing rather than being applied as a separate salt, compound, or solution. The basic issues in choosing a silver-containing dressing can be broadly conceptualized in terms of: (1) the characteristics of the "carrier" dressing and (2) the delivery of silver by the dressing to the wound. Keeping these basic issues in mind can help make sense of some of the media marketing blitz accompanying these products [26]. The following list of available silver dressings is not intended to be exhaustive, as the list is growing rapidly. Rather, it should be seen as illustrating various carrier dressing materials used in conjunction with various silver delivery "reservoirs" [26].

- Acticoat-7 (Smith & Nephew, Hull, United Kingdom) dressing consists of three layers of polyethylene mesh coated with nanocrystalline (<20 nm diameter) silver and two layers of rayon polyester. The nanocrystalline silver provides an initial large bolus of silver to the wound followed by a sustained release.
- Actisorb Silver 220 (Johnson & Johnson, New Brunswick, N.J.) is an activated charcoal dressing to which silver is bound. Actisorb works by adsorbing bacteria onto the charcoal component, where they are killed by silver. The "odor-eating" nature of the charcoal is used as a marketing focus.
- Aquacel-Ag hydrofiber (Convatec, Skillman, N.J.; 70:30 sodium: silver carboxymethylcellulose hydrofiber) is an absorptive dressing. Silver ion is displaced from the carboxymethylcellulose carrier as it is hydrated, thereby achieving a gradual, sustained slow release.
- Arglaes (Medline, Mundelein, Ill.) is silver-impregnated polymer film. The silver reservoir is $Ag/CaPo_4$, formed as glasses co-extruded in a polymer matrix.
- Contreet-H (Coloplast, Marietta, Ga.) is a dense hydrocolloid dressing that has silver bound to the hydrocolloid.
- SilvaSorb (Medline) is a polyacrylate matrix with a silver halide reservoir.
- Silverlon (Argentum LLC, Willowbrook, Ill.) is a polymeric fabric coated with metallic silver by autocatalytic electroless chemical plating. A marketing focus is the three-dimensional fabric, which has a large surface area and is flexible.

3. Silver products efficacy

Very few randomized prospective studies on the use of silver have been published [26] however, the role and the mechanism of action of silver ions in vivo continue to provide a steady contribution to the surgical literature [23]. For silver to be biologically active, it must be in a soluble form such as Ag^+ or Ag^0 clusters [34,35] and any silver dressing efficacy is determined by total available soluble silver, not total silver in the dressing [36]. Ag^0 is the metallic or uncharged form of silver found in crystalline, including nanocrystalline, silver structures. In solution, it exists in a sub-crystalline form, less than eight atoms in size. Ag^+ is the familiar ionic form present in silver nitrate, silver sulfadiazine and other ionic silver compounds [34]. In wound management, silver quantities should be sufficient to provide sustained bactericidal action [34]. Since there is no point in having a long duration of activity if the low concentration may result in the development of resistance, maintaining an adequate concentration of silver in a dressing over time has been a challenge. Metallic-coated dressings release silver over a long period but provide a low

concentration of silver in the wound bed. Silver nitrate has a high concentration of silver but no residual activity necessitating very frequent applications up to 12 times a day. Silver sulfadiazine, on the other hand, provides an adequate concentration of silver but has limited residual activity. However, it is a significant improvement over silver nitrate because it needs to be applied only twice a day. Galvanic action has a long duration of release but a low concentration of silver. Silver carbomethylcellulose releases a low concentration of silver and has no residual activity. Silver calcium phosphate and silver chloride release silver over a long period but not at high enough concentrations [27,37].

Silver release at concentrations up to 3200 ppm is observed following application of silver nitrate or silver sulfadiazine (release from silver sulfadiazine is much slower than that from silver nitrate). The large immediate concentration of silver ions released after silver nitrate application becomes chemically consumed and rapidly inactivated through the formation of chemical complexes by chloride within two hours. This can be compensated by frequent replacement necessitating several daily dressing changes. In burn units silver sulfadiazine is commonly applied twice a day and silver nitrate up to 12 times a day. Frequent dressings, however, create problems for healthcare professionals and patients, and result in large excesses of silver being delivered to the wound. [34]. On the other hand, the nature of the solute affects also the biological activity of silver. In phosphate-buffered saline, silver can be active in concentrations as low as 0.05 ppm, but organic matter significantly diminishes the efficacy of silver. Nutrient broth decreases the efficacy of silver by a factor of at least 80 compared with pure water [38], and serum decreases the activity by a factor of more than 250 [39]. In complex organic biological fluids, concentrations >50 ppm [40] and as high as 60.5 ppm [41] are needed. For a long while it was thought that the antimicrobial action of silver nitrate was due to the formation of silver chloride. However, it was later demonstrated that constant replenishment of silver ions is responsible for the antibacterial activity and that chloride ions actually deactivated the silver ions [38].

Perhaps the most unique form of silver developed for wound dressings is nanocrystalline silver, which differs in both physical and chemical properties from micro- or macrocrystalline silver and from silver salts [36]. This is, in part, related to the increase in grain boundary atoms as a percentage of the total atoms in the material, which is due to the small crystal size. These grain boundaries, according to Birringer [42], may represent a third state of solid matter. New silver-impregnated dressings such as Acticoat™ were designed to overcome limitations encountered with application of silver nitrate and silver sulfadiazine, in particular the necessity for frequent applications and the rapid inactivation of silver. Nanocrystalline silver products provide the Ag^0 form of silver which is far less rapidly deactivated by chloride or organic matter than the ionic form [34]. In these dressings, as silver is consumed by interaction with target cells or inactivated by protein and anion complexes in wound fluid, additional silver is released, thus producing a sustained, steady supply of active silver [34].

Nanocrystalline silver is a unique structure of silver that was developed for use in wound dressings [43]. it is a metastable, high-energy form of elemental silver prepared by physical vapor deposition reactive sputtering producing crystals of oxidized silver (Ag_2O and Ag_2CO_3) and metallic silver [6,34,44]. Normal silver placed in water will not dissolve, but nanocrystalline silver dissolves to provide a concentration in solution of around 70 ppm releasing both Ag^+ and Ag^0 whereas other silver sources release only Ag^+ [6,36,43]. 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 [36]. As the ions in solution at the dressing wound bed interface are depleted, the equilibrium shifts and more Ag^+ and Ag^0 ions are released [34]. It is however clear from the literature that nanocrystalline materials may be thermally unstable [36].

4. Silver and wound infection

The use of topical chemotherapy is fundamental to prevent infections in deep and superficial burns or extensive intermediary burns [1]. Increasingly, antibiotics, due to widespread indiscriminate prescription, are becoming less effective as pathogens are becoming more resistant to their action. Silver may be a useful prophylactic or therapeutic agent for the prevention of wound colonization by organisms that impede healing, including antibiotic-resistant bacteria [35]. It has been a choice antibacterial for use in wound dressings and therapeutics on account of its acknowledged low toxicity [45]. It is a well-known bactericidal agent routinely used in clinical settings [35] and the antimicrobial activity of silver ion is well defined. Silver is a broad-spectrum antimicrobial agent that controls yeast, mold, and bacteria, including methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE), whenever provided at an appropriate concentration [28,46–49]. As a metal, silver is relatively inert and is poorly absorbed by mammalian or bacterial cells. However, in the presence of wound fluids or other secretions, it readily ionizes and becomes highly reactive in binding to proteins and cell membranes [45]. Similar to other heavy metals, silver is toxic to microorganisms by poisoning respiratory enzymes and components of the microbial electron transport system as well as impairing some DNA function [35,50,51]. The inhibitory action of silver can be attributed to its strong interaction with thiol groups present in cell respiratory enzymes in the bacterial cell. Additionally, silver has been shown to interact with structural proteins and preferentially bind with DNA bases to inhibit replication [4,5]. In vitro studies provided evidence that the bactericidal effect of silver is attributable largely to the binding of the silver ion to free sulphydryl groups in the bacterium or on its surface leading to inactivation of the enzyme phosphomannose isomerase [45]. More substantive information on the bactericidal action of silver relates to its accumulation in the bacterial cells and its opportunity to interact with the cytosolic proteins, mitochondrial enzymes and nuclear DNA or RNA synthesis [45].

Referring to the ability of sensitive bacteria to absorb and concentrate Ag^+ from dilute solutions, early pharmacologists coined the term *oligodynamic* [45]. When absorbed by bacteria

or yeast cells, the silver ion (Ag^+) is lethal in sensitive strains. The biocidal effects of silver, however, are complex and different organisms respond to silver to varying extents. Evidence from the development of silver-copper filters in the sterilization of hospital water systems suggests that silver is accumulated preferentially in sensitive bacterial strains and that concentrations of 105–107 ions per cell are lethal [45]. The speed of action is almost instantaneous once the silver reaches the microorganism. This lethal effect is due not only to the amount of silver ion present, but likely also to the presence of other silver radicals generated by a silver releasing product or agent. It was suggested that the lethal concentration of ion in a cell was equivalent to the number of bacterial cell enzymes present [45].

Microbial resistance to silver itself has not been reported. However, clinically, silver resistant strains of bacteria are a continuing problem in wound care despite many claims in the literature to the contrary. In fact, resistance to silver is rare, but not unknown. There are two forms of resistance: silver can be bound by cells in the form of an intracellular complex; and it can also be excreted from microorganisms using cellular efflux systems [34]. Accumulating evidence indicates that the bactericidal activity of silver is directly related to the amount of silver accumulating within the bacterial cell and its ability to denature or otherwise impair physiological processes [45]. It was demonstrated in a laboratory study that resistance was induced using low concentrations of silver [52]. Bactericidal levels of silver do not produce resistance, however, minimum inhibitory concentration (MIC) (2–4 mg Ag^+/l) and sub-MIC levels can result in the development of resistance. Resistant cells appear to develop reduced permeability to silver combined with an upgraded active efflux mechanism to pump silver out of the cell. It is therefore clear that non-controlled use of silver in sublethal levels may result in bacteria developing resistance in the way that antibiotic and resistant bacteria have emerged [10,34].

It is worth noting that substances in the medium (or in the wound bed) that chelate free silver ion or precipitate it as an insoluble salt, inhibit bacteriostasis. Thus sodium chloride (as found in wound exudates) inhibits the antibacterial action of silver nitrate by precipitating the silver as insoluble silver chloride. On the other hand, EDTA or EGTA, enhance the biocidal effect of silver nitrate, possibly through chelating silver binding substances [45].

The concurrent emergence of resistance to antibiotics and noble metals, particularly silver, in clinical isolates is rare [53,54]. In addition, the literature does not provide evidence for the clinical isolation of bacterial strains with documented cross-resistance between silver and antibiotics [41,55,56]. Silver, particularly in the nanocrystalline form, appears to be an effective means of prophylaxis given its rapid and broad-spectrum efficacy. Nanocrystalline silver dressings have been demonstrated in vitro as effective antifungal agents [29], antibacterial agents [57], and antibacterial agents for antibiotic resistant bacteria [35]. These characteristics suggest that the use of nanocrystalline silver dressings may decrease the incidence of infections that delay wound healing when they occur [29].

5. Silver and wound healing

Prior reported effects of silver (nitrate) on burn wounds were based primarily on clinical studies and observations. The toxicity of silver ions per se has not been an issue in burn care that has received much attention [23]. Extensive treatment of acute burn wounds with silver sulfadiazine (SSD), however, has recently raised concern about potential silver toxicity [25]. Laboratory studies confirm that both keratinocytes and fibroblasts are susceptible to lethal damage when exposed to concentrations of silver which are lethal for bacteria and that silver-based products cannot discriminate between healthy cells involved in wound healing and pathogenic bacteria [23].

Typically, the wound repair process involves steps that include inflammation around the site of injury, angiogenesis and the development of granulation tissue, repair of the connective tissue and epithelium, and ultimately remodeling that leads to a healed wound. However, the progression from an injured site to a healed wound is potentially slowed or arrested by a number of different events and conditions. One event that impedes wound healing is colonization of the wound bed by microorganisms [3,58]. In addition to the production of a variety of toxins and proteases, the presence of microorganisms in a wound bed may also lead to a prolonged inflammatory response. The host inflammatory response is remarkably effective at eliminating the invading microbial population, but that same process, over time, may also damage the surrounding tissues [3].

The use of antimicrobial prophylaxis is important in reducing the wound's microbial load. Once a wound becomes infected, healing is delayed [59,60]. Increased bacterial burden on the surface and in wounded tissue increases the metabolic requirements of the wound and of the host's response to that heavy bacterial load. Bacteria produce endotoxins, exotoxins, proteases, and local tissue injury. The presence of a bacterial burden in a wound stimulates a proinflammatory environment; the presence of bacteria induces also migration of monocytes, macrophages, and leukocytes, all of which initially act in an appropriate fashion but later produce a response that is exaggerated and deleterious. This is evidenced by the fact that wounds associated with a heavy bacterial burden often show healing failure [27].

Bioburden may be defined as the metabolic load imposed by bacteria in the wound bed. Bacteria will compete with normal cells for available oxygen and nutrients. In addition, bacteria and bacterial products, such as endotoxins and metalloproteinases, can cause disturbances in all phases of wound healing [27] prolonging the debilitation of the patient by slowing wound healing and increasing health care costs for the patient [61,62]. Increased bacterial burden in a wound also affects tissue oxygen availability. Leukocytes are needed in the wound bed to kill phagocytic bacteria-by mechanisms that involve an oxydated burst and the consumption of significant amounts of molecular oxygen. In severely underperfused wounds, increased oxygen consumption by inflammatory cells can act as a sump, ''stealing'' oxygen required for basic wound metabolism. In addition, the white blood cells' inflammatory response needed to kill bacteria increases the release of damaging oxygen free radicals. The increased

production of enzymes and the release of toxins can also facilitate an induced cellular failure [27].

Studies support the concept of eradicating infection to help wound healing. From a pathophysiologic standpoint, treating an infection reduces the wound's bacterial burden, which has favorable effects on the dynamics of oxygen delivery and utilization within the wounds. This favorably impacts cellular metabolism. Treating infection also diminishes the chronic inflammatory response, which is primarily degradative. Finally, treating infection adjusts the tissue's capacity to respond to cell signaling and to develop sustained growth [27]. Silver-based wound dressings are often used to prepare the wound for healing and from that perspective silver products may have a definite positive effect on wound healing and may be used to maintain a microbe-free, moist wound healing environment [27].

Besides its antimicrobial activity, silver was proven to have other beneficial effects on the wound bed [27]. A number of the biochemical effects of silver on the wound have been documented. However, only recently with the new concepts on wound healing and healing impairment, can a mechanism of action be presented. The major focus of wound healing has been on the relationship between tissue destruction by a group of collagenase enzymes known as metalloproteinases (MMP) and tissue synthesis which is stimulated by growth factors. It is well recognized that matrix metalloproteinases are needed to heal a wound, but excess levels degrade fibronectin and peptide growth factors. This effect is exacerbated further by diminished levels of tissue inhibitors of metalloproteinase (TIMPs) [27]. Silver-based technologies in particular provide added benefits by down-regulating MMPs to levels that facilitate wound healing [27]. The results of several studies suggest that nanocrystalline silver specifically 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 [46,63]. Wright et al. [46] noted reduced levels of matrix metalloproteinases and a higher frequency of apoptosis in a porcine model of contaminated wounds treated with nanocrystalline silver confirming that silver alters the inflammatory events in the wound. Paddock et al. [47] found an inhibitory effect on certain proinflammatory cytokines (tumor necrosis factor-alpha) as well.

In one study, zinc metabolism was up regulated, implying increased epithelialization [64,65]. Evidence was provided through immunocytochemical evaluation of key metal-binding metallothioneins, to show that silver induced these proteins and enhanced the local concentrations of zinc and copper. Both metals are essential micronutrients involved in epithelial cell proliferation. Increased zinc leading to enhanced production of RNA and DNA-synthetases, matrix metalloproteinases and other essential enzymes in the wound bed are held to contribute to the improved healing observed [45]. In other contradicting reports, it was shown that silver decreases surface zinc which could decrease excess MMP activity and in that regard may increase healing rate since MMPs action clearly incriminated in delaying healing is dependent on the availability of free zinc. In addition, silver oxidizes and binds to sulfur bonds that are necessary for MMP activity. Interestingly, increased calcium levels have also been observed in experimental wounds treated with silver. The implications of this are unclear at the moment, but we do know that calcium is an essential component of haemostasis as Factor IV, and that increases in calcium in the wound margin are a normal feature of healing in acute skin wounds [45].

The potent anti-inflammatory properties of silver ion on a wound have been recognized for centuries and have been demonstrated histologically. Most of the reports, however, are purely descriptive in nature identifying the decrease in erythema and increased healing. It must be stressed however that not all silver is anti-inflammatory. The anti-inflammatory properties depend on the delivery vehicle, the available concentration and species of silver, and the duration of release [27,28,46,47]. Increased inflammation observed with silver sulfadiazine is caused by the water soluble cream base itself. This surface inflammation increases neutrophil exudate and increases protease activity on the wound surface, which may be useful to break down surface dead tissue but is deleterious to a viable healing wound bed. On the other hand, MMP levels in wounds treated with silver nitrate for example skyrocket, indicating an exaggerated inflammatory response [27]. Nanocrystalline silver dressing, on the other hand, modulates the inflammatory process at or above the level of TNF-[alpha] expression, thus generating an anti-inflammatory effect [47]. It also induces apoptosis, which is an anti-inflammatory process in the sense that it prevents cells from undergoing necrosis, which is a highly inflammatory [27,46].

Despite its beneficial effects, some adverse effects of silver products on wound healing have also been described. Delayed wound healing is often observed clinically following the use of silver-containing topical antimicrobial agents [23,66]. Clinical trials undertaken to look at the effect of silver sulphadiazine on the rate of healing of burn wounds comparing silver sulphadiazine to vaselinated tulle gras indicated that there is a clear delay in the healing process of the silver sulphadiazine treated wounds [23]. The same comparison effected on STSG donor sites did not demonstrate any difference in healing rate [67] suggesting that the observed delay in burn wound healing may not be due to re-epithelialization [23]. Delay in eschar separation associated with silver sulphadiazine treatment of deep burns is due to the low bacterial load of the burn wounds. Necrotic tissues are not quickly sloughed because silver sulphadiazine delays or prevents colonization by microorganisms. Prolonged conservative treatment with silver sulfadiazine, especially in the early years even longer than three weeks, usually results in healing with hypertrophic or atrophic scars [9,68]. Apart from the possibility that the sloughing of dead tissue in partial thickness burns is retarded, silver sulphadiazine ointment might also slow down the proper healing mechanisms of the wound [69].

Numerous adverse reactions and side effects have also been reported together with increasing resistance to silver sulphadiazine [25]. In addition to adverse effects of sulphonamides, prolonged topical application of silver sulfadiazine cream can induce argyria [70] even though it has never been

reported yet as a result of topical application [34] except locally. Direct silver-induced renal toxicity has also been reported and confirmed by high concentration of silver in blood and urine. Kidney function improved on withdrawal of the topical cream [70]. Leukopenia has been documented as well following prolonged silver sulfadiazine application and could be secondary to medullar toxicity [70]. In vitro studies showed that silver sulfadiazine is cytotoxic [71] but that cytotoxicity can be reduced by controlling the delivery of the active agent [72]. Even though other in vivo studies have found no evidence for cytotoxicity [73] and despite the fact that after decades of use, the evidence for cytotoxicity is not clear and silver sulfadiazine remains the main topical product used in burn units [34,74], various observed toxic effects confirm that this topical cream should not be used for long periods on extensive wounds [70].

Bacterial colonization of wounds may delay wound healing. Modern silver-containing dressings are antimicrobial, yet cellular toxicity is a serious side-effect [75]. Though it has been reported traditionally that silver has a low mammalian cell toxicity [28,46,47,76], silver ion does have direct cytotoxic effects on various mammalian cells. Cytotoxicity of cement loaded with silver salts made this kind of silver unsuitable for clinical use in the past [77]. Silver nitrate in vitro has been shown to have a negative impact on fibroblasts [78], hepatocytes [79] and lymphocytes. Studies on anodically generated silver ions, however, did not demonstrate any cytotoxic effect on mammalian cells in culture and no tissue toxicity could be determined by clinical evaluations [80,81].

Even though it was claimed in earlier reports that nano-silver was free of in vitro cytotoxicity and showed high effectiveness against multi-resistant bacteria [77] it was later reported that high concentrations of nano-silver base inorganic antibacterial agents had cytotoxic effects on rat fibroblasts. Cytotoxicity was directly proportional to the silver concentration. Low silver ion release rate may prevent interference with wound-healing mechanisms [75]. No cytotoxic effects were observed at or below the concentration of 25 g/l [21]. In another study to evaluate the acute toxic effects of metal/metal oxide nanoparticles on in vitro rat liver derived cell line (BRL 3A), mitochondrial function decreased significantly in cells exposed to Ag nanoparticles at 5–50 μg/ml concluding that the Ag was highly toxic. Due to this demonstrated toxicity of silver, further study conducted with reference to its oxidative stress exhibited significant depletion of glutathione (GSH), reduced mitochondrial membrane potential, and increased reactive oxygen species (ROS), which suggested that cytotoxicity of Ag (15, 100 nm) in liver cells is likely to be mediated through oxidative stress [18]. The effects of different types of nanoparticles on gametogenesis were evaluated in another study by light microscopy, and by cell proliferation and standard cytotoxicity assays. Results demonstrated a concentration-dependent toxicity for all types of particles tested, whereas the corresponding soluble salts had no significant effect. Silver nanoparticles were the most toxic [19]. More clinically oriented studies testing the effect of Acticoat Burn Dressing (Acticoat; Westaim Biomedical, Exeter, NH), a silver-coated barrier dressing, on cultured skin substitutes (CSS) showed that exposure in vitro of CSS to Acticoat was cytotoxic within 1 day. However, 1 week of exposure in vivo did not injure CSS or inhibit wound healing [82]. Another study measuring the inhibitory effect of the nanocrystalline silver on keratinocyte growth concluded that Acticoat is cytotoxic to cultured keratinocytes and should not be applied as a topical dressing on cultured skin grafts [20]. Acticoat also appeared to specifically retard re-epithelialization [14]. Silver keratinocyte cytotoxicity was recognized in Moyer's original report [83]. Epithelial regeneration appears to be inhibited when the concentration of $AgNO_3$ exceeds 1% which is why it was recommended to continually wet silver nitrate dressings with 0.5% $AgNO_3$ at 2-h intervals to prevent increase in concentration of the $AgNO_3$ to caustic concentrations (more than 2%) through drying [11].

Experimental study on wound healing efficacy as evaluated in a partial thickness burn mouse model covered by keratinocyte cultures suggests also that epidermal growth factor (EGF) is a useful agent in the retardation of wound healing caused by silver sulfadiazine (Ag-SD) [17]. Yet in another study, various silver dressings were applied to the centre of culture plates that were then seeded with keratinocytes at an estimated 25% confluence. Effects of Silvazine (Sigma Pharmaceuticals, Melbourne, Australia) 1% silver sulphadiazine (Flamazine (Smith & Nephew Healthcare, Hull, UK)) and a silver-based dressing (Acticoat (Smith & Nephew Healthcare, Hull, UK)) were compared. In this in-vitro study Silvazine was found to be the most cytotoxic agent. Seventy-two hour exposure to Silvazine in that study resulted in almost no keratinocyte survival at all and a highly statistically significant reduction in cell survival relative to control, Acticoat and Flamazine ($P < 0.001$, $P < 0.01$, $P < 0.01$, respectively). Flamazine is associated with a statistically significant reduction in cell numbers relative to control ($P < 0.05$), but is much less cytotoxic than Silvazine ($P < 0.005$) [2]. Effects of incorporating antimicrobial silver-zeolite on the in vitro cytotoxicity of five tissue conditioners against the living dermal model, which consisted of normal human dermal fibroblasts in a collagen lattice, were also evaluated. The results suggest that the highest cell viability is observed with the smallest silver-zeolite concentration [22], proving once again that irrespective of the form of silver delivery, its cytotoxicity is directly proportional to its concentration.

Absorption of silver from wound care products and dressings by cells of the wound margin is not documented in most clinical studies [45]. In the wound bed, silver ion is biologically active and avidly combines with proteins, cell surface receptors (and sulphydryl groups) and wound debris [45]. We do know also through experimental and clinical work, that silver permeating into the wound bed is taken up by epidermal cells at the wound margin and is accumulated in the wound debris and passes into the peripheral circulation to be deposited in the liver and kidney, with some voided in the urine [45]. Studies concerned with the absorption of silver from partial- and full-thickness burn wounds (5% body surface area) showed that most of the silver is associated with the superficial eschar and very little is absorbed into deeper layers [84,85]. In contrast to these findings, Wang et al. [86], Boosalis et al. [87], and Sano et al. [88] demonstrated significant absorption of silver from large burn wounds (40% body surface area) treated topically with silver sulfadiazine, so there is the possibility of silver toxicity occurring [84].

There are very few reports in the literature of silver toxicity despite large exposures to silver in the treatment of burn wounds [34]. Argyria, a permanent disorder caused by silver deposition in the skin's micro vessels in patients who are exposed to chronic silver toxicity [89,90], is only seen following large oral or inhaled intakes of silver dust or colloidal silver over an extended period of time and has never been reported as a result of topical application [34]. Transient skin discoloration related to the acutely elevated blood silver levels has recently been described following the use of nano crystalline silver for local treatment of 30% TBSA burn [25]. Liver function abnormalities have also been observed following acute silver toxicity due to nano crystalline silver [25]. Silver toxicity in the brain producing refractory myoclonic status epilepticus has also been reported following ingestion of colloidal silver [7].

No evidence has been provided so far to show that silver influences the immuno-suppressed state commonly seen in burns [45]. However, allergic responses to silver have sometimes been noted [34] affecting a small proportion of patients treated with topical silver nitrate. Although not specifically identified so far, the possibility of allergic reactions arising through the use of newer silver wound treatments should be considered, and may prove a contra-indication for their use in some patients. Other complications including leucopenia, bone marrow toxicity and renal or hepatic damage through silver deposition, as seen rarely with silver nitrate of silver sulphadiazine, are likely to be of marginal significance [45].

6. Conclusion

The only constant with wounds is that they are constantly changing. Practitioners should stay vigilant about the adverse effects of bacteria in wounds and keep in mind the role of infection in producing wound healing failure [27]. However, due to substantial experiences with adverse silver sulfadiazine reactions and side effects, it is appropriate to keep the possibility of a toxic silver effect in burn patients treated with slow sustained release silver-coated newly developed wound dressings in mind [25]. Silver levels in plasma and/or urine should be monitored [25]. The dilemma remains however, in product development to produce an agent and system of delivery which maximizes the lethal effect for bacteria and minimizes the damage to human cells. Ultimately, no matter how sophisticated the delivery system the agent, silver, cannot be expected to make a selective kill [23] even though it has been reported that its toxicity towards bacteria was quite a bit greater than that towards the human cells [91]. It is clear also that the effects of the various silver products available on wound infection and wound healing are variable. Understanding the characteristics of these products and dressings may enable them to be targeted more appropriately according to the specific requirements [92]. A word of caution, however, about extrapolating too directly from laboratory studies to clinical application, nevertheless, based on available evidence it is recommend at present that silver-based products should be avoided if possible as a topical antimicrobial strategy where rapidly proliferating keratinocytes are exposed as in donor sites, superficial partial thickness wounds and undifferentiated cultured keratinocyte applications [23]. The ultimate goal remains the choice of a product with a superior profile of antimicrobial activity over cellular toxicity [75].

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