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Safety evaluation of silver-ion dressings in a porcine model of deep dermal wounds: A GLP study

David J. Barillo[a,*], Claire R. Croutch[b], Anthony R. Barillo[a], Frances Reid[b], Al Singer[c]

[a] Disaster Response/Critical Care Consultants LLC, Mt Pleasant, SC, 29465, United States
[b] MRIGlobal Medical Countermeasures Division, Kansas City, MO, 64110-2299, United States
[c] Singer & Associates Toxpath Consulting Solutions, LLC, Westerville, OH, 43082, United States

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ABSTRACT

Introduction: Silver ion has strong antimicrobial properties and is used in a number of wound dressings. In burn models, silver-nylon dressings produce elevated silver levels in the wound along with minimal systemic effect. We evaluated systemic toxicity in a non-burn wound model to see if a similar pattern of silver ion distribution would occur.

Methods: Eight deep partial-thickness wounds each were created on the dorsum of 40 Gottingen minipigs using a Er-YAG Laser. Half were treated with a 21-day course of silver-nylon dressings (Silverlon®) and half were treated with moist gauze dressings. Wound, blood, liver and kidney silver levels, along with blood chemistry and hematology data were obtained at appropriate intervals.

Results: All wounds healed well with healing enhanced by silver-nylon dressings. Silver ion was demonstrable in all wounds treated with silver-nylon at day 21 and after 14 days of no further treatment. Silver ion was not detected in blood, liver or kidney of any animal treated with silver-nylon or control dressings. Liver and kidney function remained normal in all animals.

Conclusion: A 21-day application of silver-nylon dressings to a non-burn dermal wound produces no systemic or local toxicity in Gottingen minipigs.

1. Introduction

In developed countries, chronic wounds are found in 1–2 % of the population (Gottrup, 2004). The treatment of chronic wounds is estimated to cost in excess of 25 billion dollars a year (Sen et al., 2009). While the pathogenesis of chronic wounds is multifactorial, three elements that are commonly seen include prolonged microbial colonization, biofilm formation, and poor vascular perfusion, which limits delivery of systemic antibiotics to the wound site. When a bacterial species resistant to most or all common antibiotics appears on a hospital antibiogram, it is almost axiomatic that the source patient came from a nursing home, had chronic open wounds, and had been treated for months or years with empiric topical and systemic antibiotics, without regard to stewardship. For these and other chronic wounds, an ideal topical antibiotic would have high and sustained antimicrobial activity at the wound site, along with minimal or no systemic absorption or effect.

A number of metals, including mercury, zinc, gold, cadmium, magnesium, copper, lead and palladium have antimicrobial properties, often at extremely low metal-ion concentrations (Barillo et al., 2017; Goetz et al., 1940). In the 1890s, the Swiss botanist Carl von Nageli described the antimicrobial properties of copper ion in water at mass concentrations of 10^{-8} or less as the *oligodynamic effect* (Barillo et al., 2017; Goetz et al., 1940). Silver ion is also oligodynamic and has microbicidal effects at levels of one part per million or less (Barillo et al., 2017; Marx and Barillo, 2014; Hermans, 2006; Lansdown, 2006).

While the earliest medical applications of silver can be traced back two millennia (Barillo and Marx, 2014) the modern age of silver antimicrobial therapy began in the 1960s with the introduction of 0.5 % aqueous silver nitrate and 1 % silver sulfadiazine for the management of burn wounds (Fox, 1968; Moyer et al., 1965; Barillo, 2008). In the last 25 years, a number of new wound dressings based on silver ion technology have been developed.

The antimicrobial properties of silver-nylon fabric were established by MacKeen, Deitch and others in the 1980s (MacKeen et al., 1987; Deitch et al., 1983). 1n the 1980s and 1990s the US Army Institute of Surgical Research / US Army Burn Center extensively investigated the utility of silver-nylon dressings both with and without applied direct

* Corresponding author at: Disaster Response/Critical Care Consultants, LLC, PO Box 683, Mt Pleasant, SC, 29464, United States.
E-mail address: dbarillo@gmail.com (D.J. Barillo).

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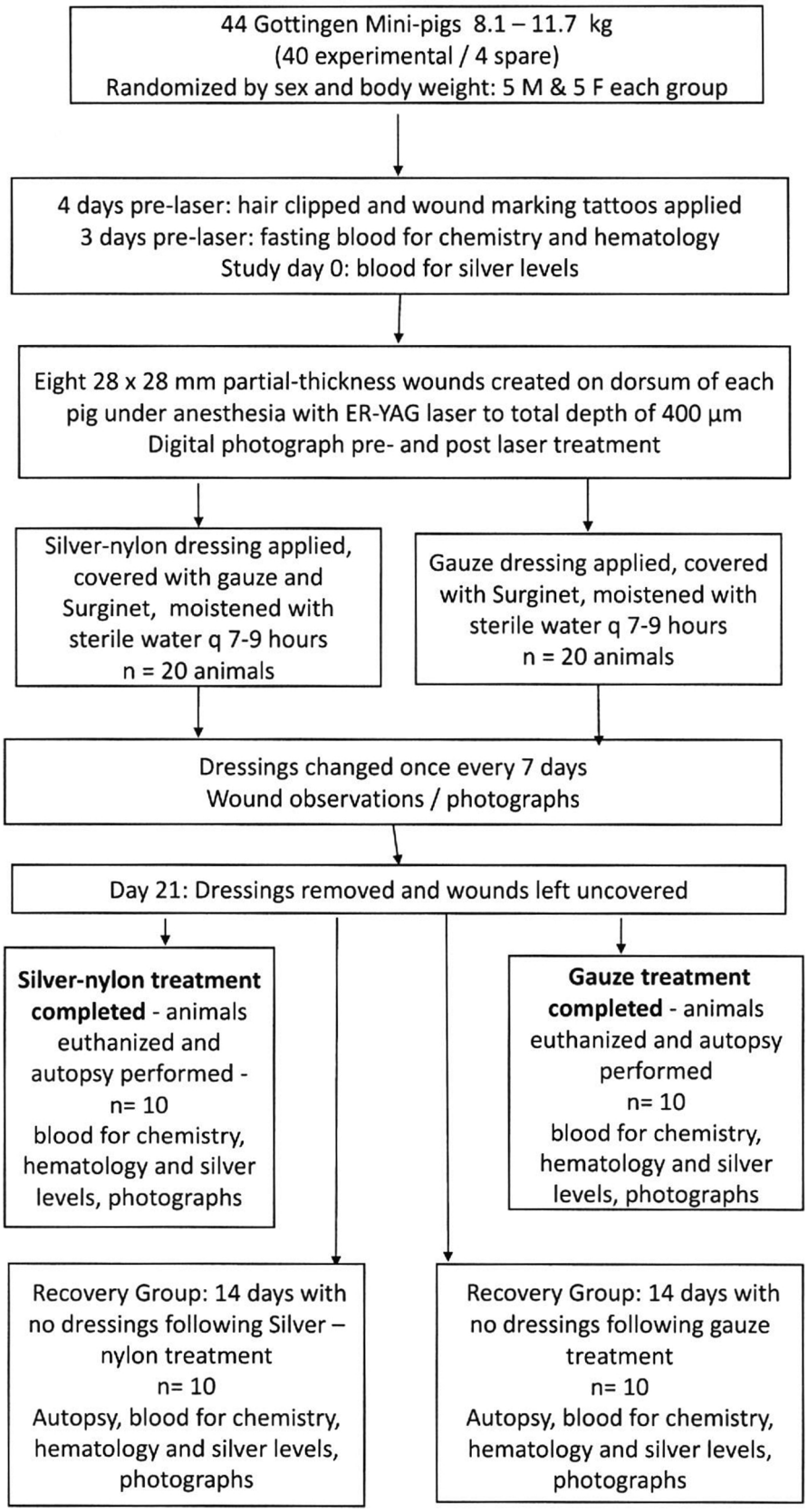

Fig. 1. Experimental Scheme.

Re-epithelialization:
0 – epithelium does not completely cover wound (wound is still open)
1 – wound is completely closed with monolayer of epithelial cells
2 – wound is completely covered by at least 2 layers of epithelial cells
3 – wound is completely covered by at least 2 layers of epithelial cells and includes at least the presence of some stratum corneum
4 – wound is completely covered by greater than 2 layers of epithelial cells and includes a normal stratum corneum

Abnormal epidermal cells: (dyskeratosis, apoptosis, pyknosis, karyorrhexis)
0 – present (over and above control background levels)
1 – absent (as compared to control background incidence)

Basement membrane:
0 – basement membrane is not completely intact
1 – basement membrane is intact with abnormal architecture
2 – basement membrane is intact with normal architecture

Hair follicles:
0 – absent/abnormal as compared to control background
1 – present/normal as compared to control background

Dermal inflammation:
3 – no increase in leukocytes over that seen in normal untreated skin
2 – increased leukocytes noted in papillary dermis
1 – increased leukocytes in papillary dermis and upper half of reticular dermis
0 – diffuse increased leukocytes extending to all layers of the dermis

Rete ridges:
0 – absent
1 – present

Vascular proliferation:
1 – absent
0 – present

Hemorrhage:
1 – absent
0 – present

Fig. 2. Histopathology Scoring System.

current in a number of wound models including skin flaps, partial and full-thickness burns, donor sites, infected burn wounds, and composite skin grafts (Chu et al., 2002; Shirani et al., 1993, Barillo1995; Chu et al., 1988, 1991; Chu et al., 1996, 1999; Matylevich et al., 1996; Chu et al., 1997, 1990; Chu et al., 1995, 2000; Chu et al., 2005). Silver-nylon dressings became commercially available in 2003 (Silverlon®, Argentum Medical, Geneva, IL), and were rapidly introduced into combat practice in both Afghanistan and Iraq (Brandt et al., 2004; Cancio et al., 2005; Barillo et al., 2014). Presently, with 16 years of use in combat settings, Silverlon® dressings have become a military standard of care for burns, traumatic wounds, open amputations, and in-flight wound care (Barillo et al., 2014).

We have previously investigated the distribution and concentration of silver ion following application of silver-nylon dressings to burn wounds in a number of animal species including rat, hairless guinea pig, and Gottingen minipig models of partial thickness, full thickness, thermal and chemical burns (Barillo et al., 2017, 2013; Barillo et al., 2016). A consistent finding is the presence of high levels of silver ion in the wound along with negligible or non-detectable levels in blood, liver or spleen. While we have investigated only burn injury, there is evidence that the rate of silver absorption may vary depending on the type of wound (Elliot, 2010; White and Cooper, 2005). Since silver-nylon dressings are employed in all types of acute and chronic wounds other than burns we performed the following study to establish a safety profile in wounds other than burn injury.

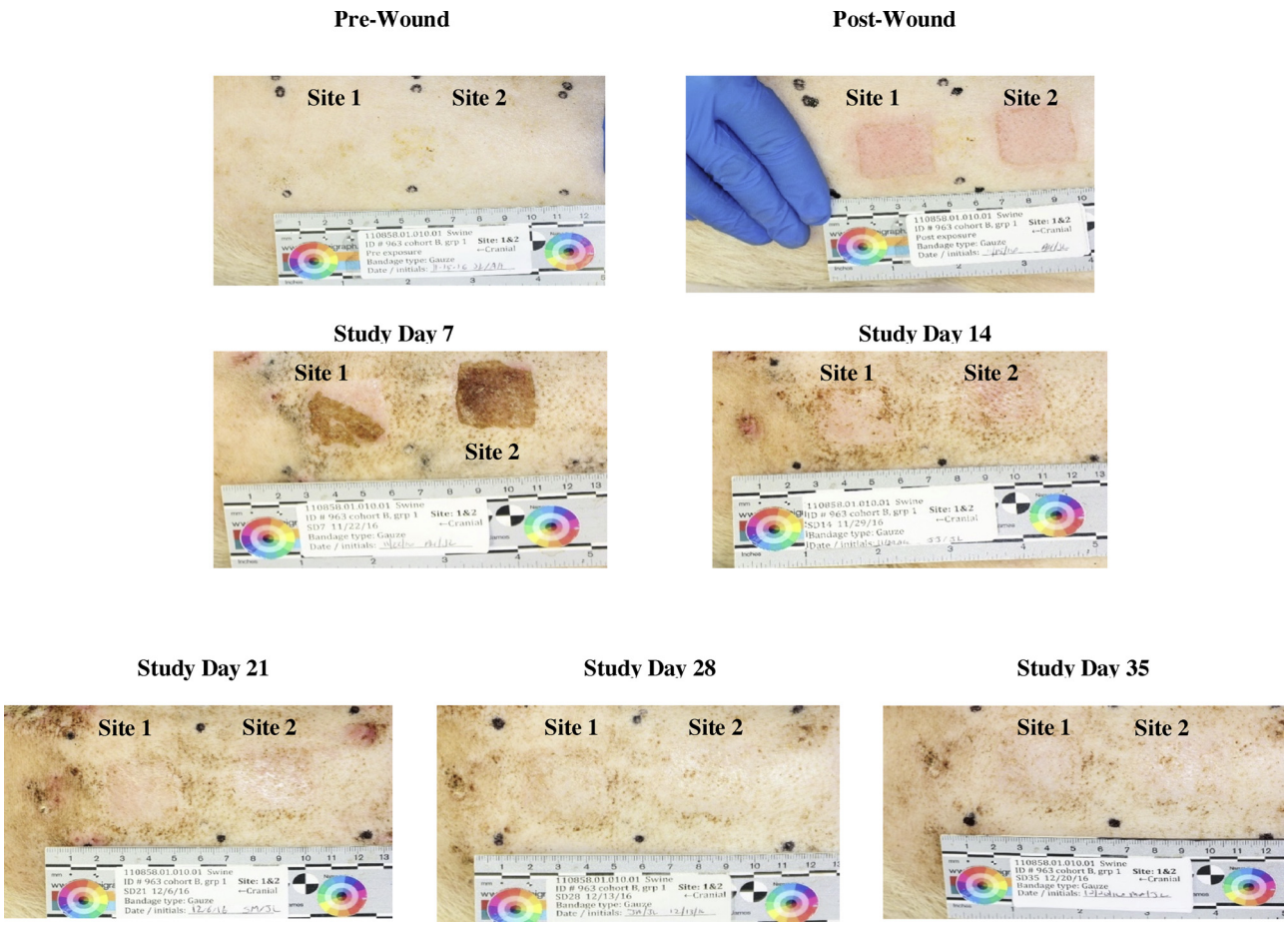

Fig. 3. Typical Wound Appearance for Gauze Dressings.

2. Methods

This study was conducted in compliance with the Food and Drug Administration Good Laboratory Practice Regulations (FDA GLP), 21 CFR Part 58; Final Rule effective September 4, 1987 and as updated where specified. All studies were carried out in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) approved facility and were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC).

Forty-four Gottingen minipigs (22 male, 22 female) at 5–6 months of age were procured from Marshall Farms (North Rose, NY), and were quarantined and acclimated for a minimum of seven days, examined by the Attending Veterinarian and released to the study. The minipigs were randomized by sex and body weight into two cohorts (silver-nylon dressings and gauze dressings). There were 10 males and 10 females in each group (Fig. 1). Pre-injury bloodwork was drawn and the outline of the planned wounds was marked by tattoo. Under anesthesia, a total of 8 deep partial thickness wounds per animal were created on the right and left dorsal flank approximately 40 mm from the spinal column by using a Sciton 2940 nm Er: YAG Contour TRL Resurfacing Laser set to the maximum ablation depth of 200 µm. Two passes were made on each wound to produce a total depth of 400 µm. Each wound measured 28 mm x 28 mm, with a minimum separation of 2 cm between wounds. At the age of this model, approximate normal skin thickness would be 63 µm for the epidermis and 2.28 mm for the dermis (Harvey and Danks, 2010).

Upon completion of laser treatment, wounds were gently cleaned of debris using saline-soaked gauze and covered with either a gauze bandage (control article) or a silver-nylon dressing (Silverlon® BCD Bandage, Argentum Medical Geneva, IL). Both control and silver-nylon sites were then covered with additional gauze, followed by a stretch mesh dressing (Surginet®). All sites were then moistened with sterile water (total of 80 – 120 ml per 8 sites) every 7–9 hours. Dressings were changed once every 7 days.

On Study Day 21, bandages were removed from all animals. Half of the animals in each group (5 male and 5 female each) were then euthanized, had blood drawn for chemistry, hematology and silver levels, and necropsy was performed. The wound sites were excised and submitted for histopathologic analysis by a Board-Certified veterinary pathologist who was blinded to the treatment group.

The remaining twenty animals (10 silver-nylon and 10 gauze dressing) were followed for an additional 14 days with no dressings applied (Fig. 1). The animals were euthanized, underwent necropsy and had blood, wound and organ harvest performed identical to the first (21 day) cohort. As all wounds were completely healed at this point, histopath scoring was not performed.

A 15-point scale was used by the pathologist to objectively describe the pathophysiology (Fig. 2). On this scale, a higher score indicates better healing with a score of 15 indicating normal skin architecture. The primary endpoint of the study was mean total histopathology score. Secondary endpoints were the individual components of the histopathology score. Statistical method was Analysis of Variance (ANOVA)

Body burden of silver ion was assessed by blood assay at up to six time points (Pre-injury and post-injury Days 1, 20, or 21, 34 or 35). At autopsy, samples of the wounds, liver, kidney and spleen were taken, flash- frozen in liquid nitrogen, stored at -80 °C, and then shipped overnight on dry ice to a reference laboratory. (Chemical Solutions, LTD, Harrisburg, PA). Silver ion analysis was performed using inductively coupled plasma-mass spectrography. The Lower Limit of Detection (LLD) was determined experimentally as 0.03 µg/gram for

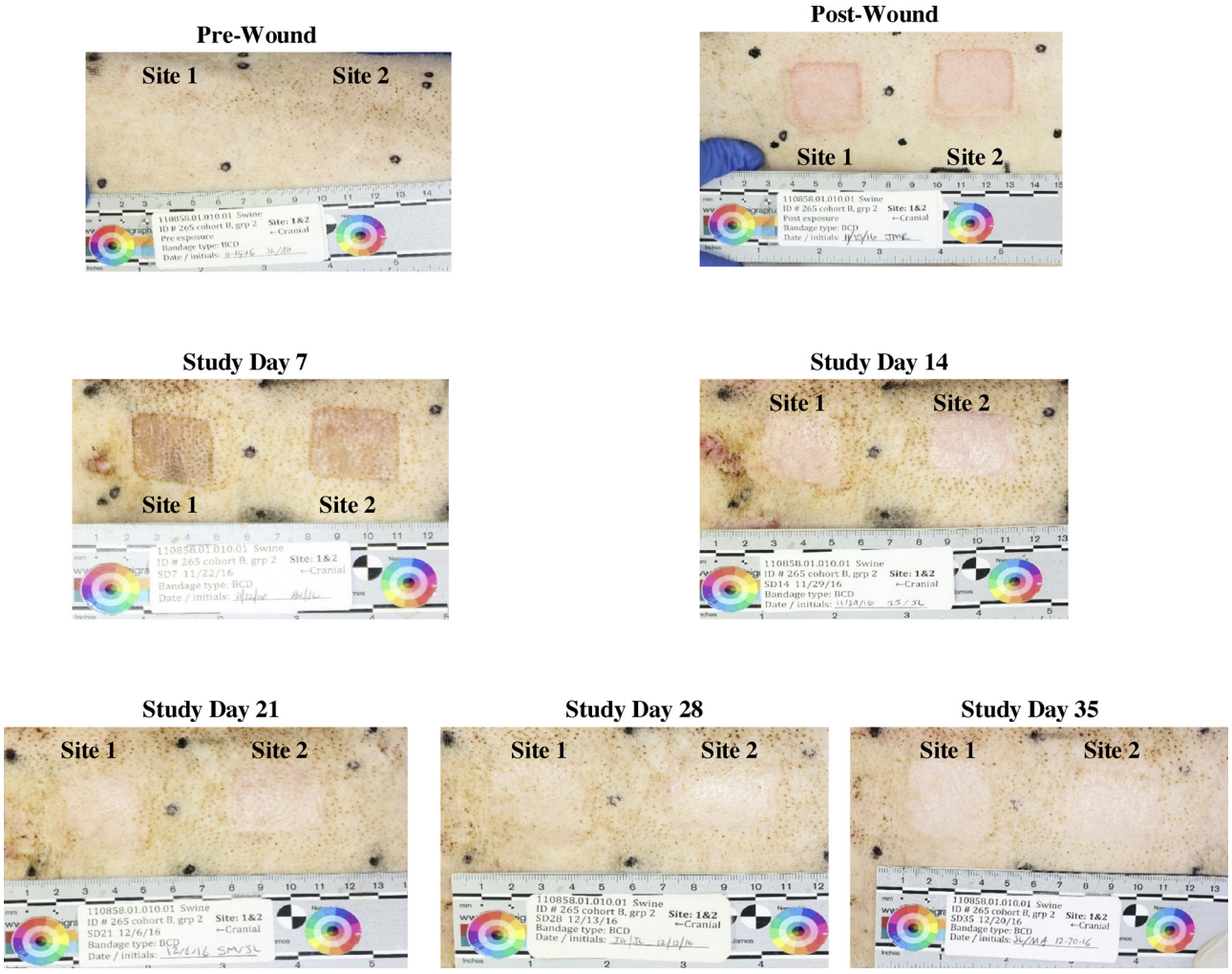

Fig. 4. Typical Wound Appearance for Silver-Nylon Dressings.

blood and internal organs and at 0.3 μg/gram for skin biopsy specimens.

While samples were collected from all animals at the indicated time points, only a representative subset of the collected samples were sent for silver ion analysis. This included 145 wound samples, 106 blood samples, 19 liver, 18 kidney and 18 spleen samples.

Blood for chemistry and hematology assay was obtained pre-study, on day 21 and on day 35. Chemistry assay included serum sodium, potassium, chloride, carbon dioxide, blood urea nitrogen, creatinine, total bilirubin, alkaline phosphatase, lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyl transferase. Hematology indices included hemoglobin, hematocrit, white blood cell count, leukocytes, lymphocytes, monocytes, basophils, neutrophils and eosinophils.

3. Results

There was no morbidity or mortality in any animal during the study. Regardless of dressing type, all animals on study exhibited normal porcine behavior, normal weight gain, and normal appetite.

All wounds healed well regardless of type of dressing, physical location on the back of the pig, sex, or group assignment. There were no signs of wound infection in any group. There was no skin discoloration observed in the silver-dressing wounds. Typical wound appearance at various stages of the study are seen in Figs. 3 and 4.

On histopathologic evaluation using our standard scale, wound healing was enhanced by application of silver-nylon dressings. At the time of study completion, mean histopathology scores for the silver-nylon group were 14.50 (standard deviation 0.42) compared to 13.85 (standard deviation 0.45) for the control group ($p = 0.0005$ by Analysis of variance (ANOVA)). Statistical analysis of secondary outcomes (individual components of the histopathology scoring system) showed significantly higher scores in silver-nylon-treated animals for the parameters of basement membrane appearance ($p = 0.0173$ by ANOVA) and Dermal Inflammation ($p = 0.0007$). No significant difference in scores was observed between groups for Re-epithelialization, Abnormal Epithelial Cells, Hemorrhage, or Rete Ridge formation (Fig. 5).

There was no silver ion detected in any blood, liver, kidney, or spleen sample analyzed from any treatment group, including the silver-nylon cohort in this study. There was no silver ion detected in any wound in the control (gauze) treatment group. Conversely, silver ion was detectable in all wound specimens where silver-nylon dressings were employed (Table 1). Interestingly, in the silver-nylon treated group, silver ion was also detectable in all wound samples at day 35, which is two weeks after all silver-containing dressings had been removed.

Alterations in liver function have been reported following the topical use of nanocrystalline dressings (Acticoat ®, Smith & Nephew) (Trop et al., 2006). In the present study, we obtained serum markers of liver and renal function to see if silver-nylon dressings produced a similar effect. Biomarkers assessed were, for the most part, within the normal reference ranges for Gottingen minipigs with all observed group differences being attributed to normal variation and incidental

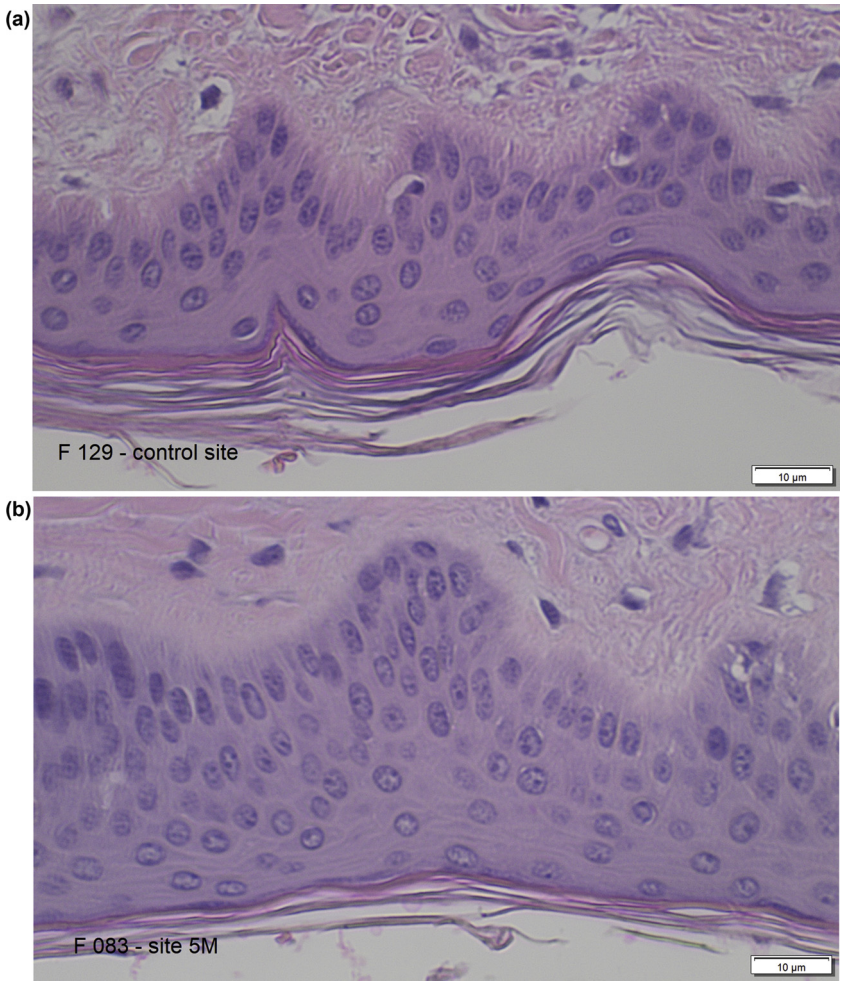

Fig. 5. Histopathology.
5A Wound treated with control dressings.
5B Wound treated with silver-nylon.

Table 1
Silver Levels in Wounds.

Group	n =	Mean	Minimum	Maximum	SD	# not detected
Silver-nylon day 21	49	6.79 µg/g	0.2 µg/g	30.0 µg/g	7.45	None (0)
Silver-nylon day 35	49	1.82 µg/g	0.32 µg/g	8.9 µg/g	1.82	None (0)
Gauze (control) day 21	42	None detected	None detected	None detected	–	All (n = 42)
Gauze (control) day 35	37	None detected	None detected	None detected	–	All (n = 37)

Notes: 1) SD = Standard Deviation.
2) Lower limit of detection for silver ion in wounds is 0.3 µg per gram.

spontaneous type I statistical error. Differences were characterized by most or all of the following: small magnitude, no relationship to treatment, inconsistency over time, and absence of correlative findings. In all remaining clinical pathology endpoints, there were no statistically significant differences when each dose group was compared both within treatment or time.

For hematology indices, leukocytes (LUC), white blood cells (WBCs), lymphocytes (LYM) monocytes (MON), basophils (BAS), neutrophils (NEU), and eosinophils (EOS) remained within the respective reference ranges throughout the duration of the study. In addition, there was no change in hematocrit (HCT) or hemoglobin (HGB) for any animal on study throughout the entire in-life.

4. Discussion

In this safety study, the use of silver-nylon dressings over partial thickness open wounds produced no adverse effects. There was no morbidity or mortality in any animal during the study. Regardless of dressing type, all animals on study exhibited normal porcine behavior, normal weight gain, and normal appetite. All wounds healed well regardless of type of dressing, physical location on the back of the pig, sex, or group assignment. There were no signs of wound infection in any group. There was no skin discoloration observed in the silver-dressing wounds. There were no significant alterations in liver or kidney function or in hematology indices. For wounds treated with silver-nylon, healing, as demonstrated by histopathology, was enhanced.

When silver-nylon dressings were placed, silver ion was

demonstrated in all wounds and was still present after two weeks of dressings being removed. There was no systemic absorption of silver ion into blood or deep organs (liver, kidney, or spleen). The pattern of high silver levels in the wound coupled with lack of systemic absorption is identical to results of our previous studies, and is consistent across several species (rat, Hairless Guinea Pig, Gottingen minipig); across two mechanisms of injury (thermal burn, chemical burn); and across three different depths of injury (full thickness, superficial partial-thickness and moderate partial thickness wounds). (Barillo et al., 2017, 2013; Barillo et al., 2016) Interestingly, this pattern is different from other silver-containing topicals such as silver sulfadiazine or nanocrystalline silver.

Silver ion (Ag^{+1}) is bacteriocidal against a broad spectrum of Gram-positive and Gram-negative bacteria, yeasts and true fungi, and certain viruses. Fifty years ago, the introduction of 0.5 % silver nitrate solution and 1 % silver sulfadiazine cream revolutionized burn care. At the same time, concerns were raised about the effects of silver on the human body. In humans, silver is not a required trace element or nutrient and appears to have no physiological role (Marx and Barillo, 2014; Lansdown, 2006). Nevertheless, silver can be normally found in the body secondary to natural inhalation and ingestion in the absence of medicinal or occupational exposure. Blood silver levels of less than 2.3 ug/L and urine excretion of up to 1–2 u g/day are considered normal (Marx and Barillo, 2014; Lansdown, 2006; White and Cooper, 2005; Boosalis et al., 1987; Melaiye and Youngs, 2005; Wan et al., 1991). Normal silver levels in the kidney and liver are 0.05 µg/g wet tissue (White and Cooper, 2005; Wan et al., 1991).

The application of certain silver compounds to open wounds or to burn injury is associated with elevated blood, urine or organ levels of silver. In animal models, Ag^{111}-tagged silver nitrate solution applied to full thickness (nonburn) wounds concentrates in the wound, liver, spleen and kidney (Constable et al., 1967). Following cessation of therapy, clearance is rapid with < 25 % of the isotope detected in the liver at 2 weeks (Constable et al., 1967). Wan et al. note that in burn patients treated with silver sulfadiazine, plasma silver levels may reach 50 µg/L within 6 h of treatment and can reach a maximum of 310 µg/L (Wan et al., 1991). Silver in urine is detectable after 1 day of treatment with silver sulfadiazine and reaches levels of 400 µg/day (Wan et al., 1991). In one burn patient who expired after 8 days of treatment, liver silver levels were 14 µg /g wet tissue and kidney levels were 0.2 µg /g (Wan et al., 1991).

Boosalis et al. Boosalis et al. (1987) evaluated 23 patients with second and third degree thermal burns treated with silver sulfadiazine and found that urine excretion of silver was markedly elevated. In patients with burns of > 60 % total body surface area (TBSA), mean urine silver excretion was 1100 µg/24 h (r34). Coombs et al. prospectively studied silver levels in 22 burn patients treated with silver sulfadiazine (Coombs et al., 1992). They found that silver was rapidly absorbed thru wounds and that elevated blood silver levels were detected in 20 of 22 patients or in all patients with burn size > 5 % TBSA. All patients with burns > 20 % TBSA had blood silver levels > 200 µg/L. The one patient in the study that expired (and thus was available for autopsy) had silver found deposited 'biochemically and electronmicrographically' in the kidney and liver (Coombs et al., 1992).

How do we explain why silver-nylon treatment results in elevated silver levels in the wound along with absence of systemic effect, while other silver dressings produce elevated silver ion levels in blood, urine and deep organs? At this point, there are theories rather than hard answers. Silver ion binds avidly to chloride and to proteins, both of which are readily available in open wounds, which would explain the elevated and persistent wound levels of silver ion seen with silver-nylon and other silver compounds. Systemic absorption of nanocrystalline silver may relate to the physical properties of nanotechnology, but data on this subject is lacking. When silver nitrate is placed on wounds, silver chloride and silver albuminate are formed. Silver albuminate is soluble in serum and is distributed to the tissues via the bloodstream (Wang et al., 1988).

Silver sulfadiazine has been extensively studied. There is no question that silver sulfadiazine is absorbed thru debrided partial thickness (second-degree) burns both as the intact compound and as the components (silver ion, sulfadiazine and vehicle (propylene glycol) (Lansdown, 2006; Coombs et al., 1992; Lazare et al., 1974, Kulick1985; Sano et al., 1981). Silver sulfadiazine that is absorbed intact then dissociates in the bloodstream into two portions (Sano et al., 1981), with the sulfadiazine component quickly excreted thru the urine and the silver portion remaining 'at a certain level in the body for a long time' (Sano et al., 1981). Thus, the reason that elevated blood and urine levels are seen following treatment with silver sulfadiazine may relate to both direct silver absorption and to carriage into the systemic circulation by the intact compound.

In conclusion, this study failed to demonstrate any evidence of toxicity, local or systemic, in any pig with the application of silver-nylon dressings (Silverlon®) to deep partial-thickness dermal wounds for 21 days. The pattern of elevated silver ion concentration at the wound site, coupled with negligible systemic absorption of silver ion that we have previously demonstrated in multiple burn models also applies to non-burn types of wounds or injuries. Based on these data, we conclude that a 21-day application of the silver-nylon dressings to a dermal wound produces no systemic or local toxicity in Gottingen minipigs.

Disclosures

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Declaration of Competing Interest

The authors report no declarations of interest.

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