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Development and application of methods for the determination of silver in polymeric dressings used for the care of burns

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ABSTRACT

Open vessel and microwave digestion methods have been developed for the determination of total silver in six commercial dressing used for the treatment of skin burns. An extraction method using TMAH has also been developed to determine the amount of silver present in the exudates found on the surface after dressing removal so an estimation of the patient dose can be made. All microwave methods had a quantitative recovery, whereas the open vessel had recoveries that ranged from 80 to 100%. The silver concentrations were determined by inductively coupled plasma mass spectrometry using an external calibration. In the absence of suitable reference materials, isotope dilution analysis was applied to validate the accuracy of results obtained by external calibration. All the products had a total Ag content that agreed with the values declared by the producer, which ranged from 10 to 0.2% Ag by weight. One of the methods was applied to the indirect determination of Ag released *in vivo* by Acticoat™ Flex 3, a dressing composed of silver nanoparticles on a polymer net. Silver levels were determined in used dressings after application to patients with partial thickness skin burns. A maximum of 62% of the silver was found to have been released onto the patient where hemopurulent exudate occured, indicating that the dressing was virtually exhausted after 3 days of use. We conclude that the Ag released into the patient's tissues is closely correlated with the local severity of the wound.

1. Introduction

The use of silver (Ag) as an antimicrobial and medicinal agent has a long history [1,2]. Silver is a broad spectrum antibacterial agent characterized by multiple toxic effects on many components of the bacterial cellular wall. Unlike antibiotics, this reduces the development of bacterial resistance [3,4]. For centuries Ag has been used to control infection and avoid septicaemia in the care of burns and chronic wounds. Due to the local interruption of blood flow in burns, systemic infection prophylaxes have little effect, so topical antimicrobial treatments become decisive. In the 17th century, silver nitrate became the first Ag salt to be systematically applied in the treatment of chronic wounds and ulcers. After the publication by Moyer et al. [5] in 1965, the uses of Ag in the treatment of burns and other cutaneous wounds diversified into a wide variety of chemical forms, including colloidal Ag, chloride and sulphate solutions, and the complex with the antibiotic sulfadiazine [6]. In recent years a new class of products consisting of dressings either coated or impregnated with Ag have entered the market [7]. These products are mainly differentiated by the composition of the base polymeric material used as a scaffold and by the chemical form of Ag, which can be present either as a salt or metallic nanoparticles (NPs).

As commercial Ag-dressings are classified as medical devices, their regulation follows a distinct pathway compared to drugs, where no tight preclinical tests and clinical trials are required for approval. So, despite a wide range of studies that have demonstrated their antimicrobial efficacy *in vitro* and their contribution to wound healing *in vivo* [3,8–11], the metabolic routes of Ag and the biochemical mechanisms in which it is involved are still unknown. The toxic forms of silver are believed to be the Ag^+ ion or Ag^0 nanoparticles, while all other species are considered as carriers of the element. They are characterized by different distributions within dressings and tissues, and varying release kinetics for the ion Ag^+ [7,12].

The multiple toxic effects of silver on bacteria are known to consist of various actions involving the blockage of respiratory pathways along with alteration of DNA and the cell wall structure [13]. The potential side effects of Ag for patients are a more controversial issue. Topical application of massive levels of Ag salts can cause Argyria, a blue–grey discolouration due by deposition of the metal in the skin

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[14,15], but the consequences for patient seem to be mainly esthetic. Although systemic toxicity for humans is relatively low, recent studies have shown that Ag can have some toxic effects *in vitro* on keratinocytes or fibroblasts [10,11,16], *ex vivo* on reconstituted human epithelium [17] and *in vivo* on skin [16]. Originally it was believed that Ag was not absorbed across the skin, but this has changed since a study by Coombs et al. [18] that showed a significant level of Ag in serum in patients with burns being treated with the Ag–sulfadiazine complex. Silver preparations generally have minor toxic effects for patients relative to their potential benefits [19] and allergies to the metal are very rare [20]. However, considering the enormous use of Ag-containing dressings and the potential impacts of these unknowns on the care of burns and chronic wounds, more thorough biological/chemical studies could lead significant improvements in product design or application protocols, that can be optimized to the specific characteristics of each patient.

The few studies on these dressings available until now have been limited to low sample size case-control designs or clinical approaches [21]. Quantitative approaches to silver release have not been implemented and analytical chemistry methods for Ag determination in clinical and biological matrices are a virtually ignored topic. Although there is still interest in the analysis of seawater [22–24] trace levels and metallurgic materials (bullion, ores and jewellery) at high purities [25–27] there is little published on the analysis of materials with silver levels below 10% w/w. The use of Ag and Ag-NPs is spreading nowadays in a wide variety of consumer products including textiles, cosmetics, food containers, household items and many others [28,29], so that new methods for the determination of total Ag and its species in different matrices can find broad application.

With the aim of investigating the systemic absorption of Ag from dressings, this paper presents the development of a set of methods for the determination of the Ag concentrations in six different commercial products. Several strategies were explored and compared for sample preparation, including: dry ashing, solvent dissolution, open-system and microwave mineralizations. Instrumental parameters have been optimized and quantification by both isotope dilution analysis and external calibration was carried out to robustly validate the methods in a field where CRMs and standard methods are unavailable. In the final part of this paper the application of one of the methods is presented by a pilot study for the indirect estimation of the quantities of Ag released into the wounds of two patients who have undergone therapy for partial thickness skin burns. The work provides an analytical basis for the study of Ag release kinetics is solution [30], and complements bio-chemical studies carried out on skin biopsies [31] which has already been published by our group, as well as further *in vitro* and *in vivo* studies currently in progress.

2. Materials and methods

2.1. Products and patients information

Sample dressings were collected at the University Hospital of Padova (Italy). For method development 6 products were chosen among those most used at the Burns Center, namely: Acticoat™ Flex 3 (Smith & Nephew, Milan, Italy), Actisorb® Silver 220 (Johnson & Johnson, Rome, Italy), Aquacel® Ag (ConvaTec, Rome, Italy), Urgosorb® Ag (Fidia, Abano Terme, Italy), Mepilex® Ag (Mölnlycke Health Care, Gallarate, Italy), Cellosorb® Ag (Fidia, Abano Terme, Italy). The composition of the scaffold and the expected form of Ag are summarized in Table 1.

For the applicative study, samples of Acticoat™ Flex 3 were collected after application on two patients who had undergone hospitalization and treatment for partial thickness burns. The samples were collected after the third day of application (substitution of the dressing). The therapy did not include the use of silver sulphadiazine cream or the application of physiological solution to maintain the wound humidity. Informed consent was obtained and the study was performed in conformance with the Declaration of Helsinki ethical guidelines.

2.2. Reagents

High purity de-ionized water (18 MΩ cm^{-1} resistivity) was produced using a Purelab Ultra unit, (Elga, High Wycombe, UK). All reagents used were of analytical grade. The nitric acid (Sigma-Aldrich Milan, Italy) was doubly distilled using a two-step sub-boiling distillation unit (Milestone, Sorisole, Italy), H_2O_2 30% w/w solution, NH_4OH 28% w/w solution, NaOH, tetramethylammonium hydroxide (TMAH) dichloromethane, toluene, tetrahydrofuran (THF), dimethyl sulfoxide (DMSO), acetone, chloroform and ether were purchased from Sigma-Aldrich (Milan, Italy). A silver ICP-MS grade 1000 ng g^{-1} standard solution was obtained from Ultra Scientific (Bologna, Italy). Metallic isotopic ^{109}Ag was obtained from Spectra 2000S.r.l. (Rome, Italy), and was dissolved in purified HNO_3 and diluted to 100 μg g^{-1} in 2% v/v HNO_3. All subsequent dilutions of the isotopic spike were prepared in NH_4OH 28 or 2.8% w/w. We underline the importance of working with reagents with low Cl levels to avoid the formation of insoluble AgCl precipitates, where possible.

2.3. Instrumentation

Preliminary morphological characterization of the dressings was carried out by scanning electron microscopy (SEM) using a Philips XL-40 instrument equipped with an energy dispersive X-ray microanalytical system (EDAX-EDS).

For acid mineralization of the dressings, two instrumental set-ups were tested. The first was the use of ceramic crucibles on a hot plate (Velp Scientific, Usmate, Italy) and the second was an Ethos*1* microwave oven from Milestone (Sorisole, Italy) with high pressure vessels. A muffle furnace, LB series (Focus, Vicenza, Italy) was used for dry ashing.

Total Ag determinations were carried out by inductively coupled plasma-quadrupole mass spectrometry (ICP-QMS). An instrument without a collision cell was used when quantifying by external calibration (model 7500 is), while an instrument equipped with collision-reaction cell (CRC, model 7500cx) was

Table 1
Main characteristics of the dressings: composition and structure of the scaffold, and expected form of Ag.

Dressing	Scaffold	Ag form
Acticoat™ Flex 3	Polyester	NPs
Actisorb® Silver 220	Viscose–rayon based activated carbon, nylon	metallic
Aquacel® Ag	Na-carboxymethylcellulose	ionic
Urgosorb® Ag	Ca-alginate and Na-carboxymethylcellulose	Ag^+ Na^+ $Zr_2(PO_4)_3^-$ salt
Mepilex® Ag	Polyurethane foam with a silicone layer on the contact surface	Salts
Cellosorb® Ag	Polyester mesh impregnated with hydrocolloids and vaselline, polyurethane foam pad and polyurethane backing	Ag_2SO_4