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Preparation and characterization of antimicrobial wound dressings based on silver, gellan, PVA and borax

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ABSTRACT

Silver-loaded dressings are designed to provide the same antimicrobial activity of topical silver, with the advantages of a sustained silver release and a reduced number of dressing changes. Moreover, such type of dressing must provide a moist environment, avoiding fiber shedding, dehydration and adherence to the wound site. Here we describe the preparation of a novel silver-loaded dressing based on a Gellan/Hyaff® (Ge-H) non woven, treated with a polyvinyl alcohol (PVA)/borax system capable to enhance the entrapment of silver in the dressing and to modulate its release. The new hydrophilic non woven dressings show enhanced water uptake capability and slow dehydration rates. A sustained silver release is also achieved. The antibacterial activity was confirmed on *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

1. Introduction

Absorptive fibrous dressings are among the most widely used products for wound treatments. The first existing materials, such as gauze, suffered from severe limitations, such as fiber shedding and easy dehydration, with subsequent painful adherences to the wound site.

The ideal wound dressing should provide a moist but not saturated environment to enhance healing process and patient compliance. At the same time it should be pointed out that the moist, warm, and nutritious environment of wounds is an ideal medium for microbial growth.

In case of superficial wound bioburden, in order to prevent an infection of the deeper tissue, topical antimicrobial agents are the usual initial treatment. Furthermore, with the step-up of bacteria resistant to multiple antibiotics, topically delivered antiseptic agents are being increasingly used (Jones, Bowler, Walker, & Parsons, 2004).

Silver products have been used for years as antimicrobials on wounds from burns, traumas, and diabetic ulcers. Unlike antibiotics, silver is toxic to multiple components of bacterial cell metabolism. These include damage to the bacterial cell wall, and membrane permeability leads to gross cellular structural changes, blockage of transport and enzyme systems such as the respiratory cytochromes, alteration of proteins and binding of microbial deoxyribonucleic acid and ribonucleic acid that prevent transcription and division (Leaper, 2006).

Topical creams or solutions containing silver (e.g., silver sulfadiazine) have long been used as a mainstay of wound management in patients with serious burns, who are especially susceptible to infection. However, disadvantages to their use include skin-staining and toxicity. In addition, the need for frequent removal and re-application of silver sulfadiazine, due to the development of pseudoeschar, is both time consuming for professionals and painful for patients (Innes, Umraw, Fish, Gomez, & Cartotto, 2001; Parsons, Bowler, Myles, & Jones, 2005; Silver, Phung, & Silver, 2006).

Dressings that can sustain silver release do not need to be changed so often, thereby representing a nursing management time benefit. Furthermore, a reduced number of dressing changes could affect positively a patient's quality of life, particularly in burn treatments.

In recent years, several slow-Ag-release wound dressings have been marketed, including Acticoat (a multilayer of polyesters and antiadherent polyethylene covered with silver nanocrystals), Aquacel Ag (a nonwoven of Na-CMC with ionic silver), Actisorb Silver (a patch of activated carbon and metallic silver), Silverlon (a silver coated fabric), and others. This new class of dressings is designed to provide the antimicrobial activity of topical silver in a more convenient application (Parsons et al., 2005). In these products the silver can be present as polymer charges co-ions or is simply impregnated

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in the non-woven. Moreover, many wound dressings containing silver nanoparticles are also described (Abdel-Mohsen et al., 2012; Juby et al., 2012; Tijing et al., 2012).

In this work, we prepared a novel silver-impregnated Gellan/Hyaff® (Ge-H) non woven, treated with a polyvinyl alcohol (PVA)/borax system, capable to enhance silver entrapment in the dressing, and to modulate its release. The rationale of this choice is the enhancement of the dressing antibacterial activity, because of borax, that, at the same time, acts as a cross-linker between the polysaccharide chains and PVA, thus imparting enhanced mechanical properties. PVA and borax, because of their film forming properties, are able to improve the silver release profile from the new dressings.

2. Materials and methods

2.1. Materials

Polyvinyl alcohol $M_w \sim 145{,}000$ (PVA_{145k}) and $M_w \sim 47{,}000$ (PVA_{47k}) were provided by Sigma–Aldrich. Silver nitrate, sodium nitrate, nitric acid and borax were provided by Carlo Erba Reagents. Reference marketed dressings were: ***A*** from Smith & Nephew (a multilayer composed of absorbent material and polyethylene covered with nanocrystal silver); ***B*** from Systagenix (a nonwoven composed of alginate, carboxymethylcellulose and silver-coated nylon fibers); ***C*** from Convatec (a nonwoven of carboxymethylcellulose with ionic silver). Nonwoven Gellan-Hyaff® (GeH) patches were kindly provided by Anika Therapeutics. More information on the commercial samples is available from the authors.

All other chemicals were of analytical grade and used without further purification.

2.2. Preparation of the Ag-loaded nonwoven Ge-H patches

The nonwoven patches (≈2.5 cm × 2.5 cm, ≈35 mg) were immersed for 5 s in 10 ml of a hydroalcoholic solution (H_2O/EtOH = 80/20), at room temperature, and then in a hydroalcoholic solution containing $AgNO_3$ at two different concentrations (i.e., 1.57 and 15.7 mg/ml, corresponding to ion concentration of 1 and 10 mg/ml respectively) for 5 s.

In order to prepare samples loaded with Ag and PVA, hydroalcoholic solutions containing $AgNO_3$ at two different concentrations (i.e., 1.57 and 15.7 mg/ml) and PVA of two different M_w (47,000 and 145,000) and at two different concentrations (c_P = 0.2 and 1%) were used. The nonwoven patches were then washed for 5 s in 10 ml of hydroalcoholic solution (H_2O/EtOH = 80/20), for 5 s in 10 ml of acetone, and finally dried at 40 °C overnight. An overview of the prepared samples is reported in Table 1.

2.3. Preparation of the crosslinked Ag-loaded nonwoven gellan patches

To crosslink PVA after its loading on the nonwoven patches, an intermediate step during the sample preparation was carried out. After the immersion in the hydroalcoholic PVA and $AgNO_3$ solutions as above described, the nonwoven patches were immersed for 5 s in 10 ml of a borax hydroalcoholic solution (borax 10 mM/EtOH = 80/20). The patches were then washed in a hydroalcoholic solution and in acetone, and finally dried at 40 °C, according to the procedure described above. The prepared samples are reported in Table 1.

2.4. Rheology of loading solutions

Rheological tests were performed under shear conditions to determine steady shear viscosity values of all the hydroalcoholic polymeric solutions used and prepared as previously described. Flow curves of the solutions were performed using a cone-plate geometry (Haake CP60Ti: diameter = 60 mm; cone = 1°; gap = 0.053 mm) in the range 0.001–1000 s^{-1}. A stepwise increase of the stress was applied, with an equilibration time of 30 s. All measurements were performed at 25 °C. For an appropriate comparison among all the tested solutions, shear viscosity data at $d\gamma/dt = 10\ s^{-1}$ were collected.

2.5. PVA content evaluation

In order to evaluate the amount of loaded PVA, the GeH patches were soaked using the same procedures above described, using PVA hydroalcoholic solutions without silver. The GeH patches were accurately weighted before (W1) and after (W2) the loading treatment. The weight of PVA loaded in the nonwoven was calculated by (W2 − W1)/W1. All the tests were performed in quadruplicate; standard deviations always lay within 10% of the mean.

2.6. Silver content evaluation

The silver content of the samples was evaluated by means of Direct Potentiometry with a Ion-Meter Crison GLP 22+, provided with a silver-selective electrode (Crison, 9654) and a reference electrode (Crison, 5044), using $NaNO_3$ 0.1 M as Ionic Strength Adjustment Buffer (ISAB), in order to buffer at the same extent the ionic strength in the calibrating standard solutions and in the samples.

Calibration curves were obtained preparing standard solutions of $AgNO_3$ (Ag^+ in the range 10^{-3} to 10 mg/ml) in $NaNO_3$ 0.1 M. To 1 ml of each standard solution, 0.5 ml of HNO_3 were added, in order to provide all the silver in the Ag^+ form; these solutions were then diluted to 50 ml with $NaNO_3$ 0.1 M, and the Ag^+ content was potentiometrically evaluated.

The silver-loaded non woven patches were digested in 2.5 ml of HNO_3 to dissolve the dressing matrix and to release and oxidize all the silver present to Ag^+. These acid solutions were then diluted 250 times with $NaNO_3$ 0.1 M, and the silver content evaluated by potentiometry. The silver content (as mean of three different measurements, ±SD) is reported as mg Ag/100 mg of nonwoven patch.

2.7. Silver release

The silver loaded patches were immersed in 20 ml $NaNO_3$ 0.1 M at 37 °C. At different times (30 min, 1 h, 2 h, 4 h and 7 days) aliquots of the dissolution medium (1 ml) were withdrawn and replaced by an equal volume of fresh solution. 0.5 ml of HNO_3 was then added in order to oxidize all the silver to Ag^+; these acid solutions were diluted to 50 ml with $NaNO_3$ 0.1 M, and the silver content was evaluated by means of a potentiometer. After 7 days, the silver, still present in the nonwoven patches, was evaluated as above described. The silver release is reported as a mean of three measurements as the percentage of the total amount present in each patch.

2.8. Mechanically stressed nonwoven patches

In order to evaluate the effectiveness of silver entrapment, the loaded nonwoven patches were mechanically stressed. The different samples were beaten with a pestle for about 1 min, and then shacked off, in order to remove the dust containing silver.

The silver content of these stressed samples was determined and compared with the content of the unstressed ones, following the same procedures described above.