Carbohydrate Polymers 90 (2012) 1362–1370

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

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

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ARTICLE INFO

Article history:
Received 7 May 2012
Received in revised form 19 June 2012
Accepted 2 July 2012
Available online 11 July 2012

Keywords:
Nonwoven
Wound healing
Silver
Gellan
Hyaff®

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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http://dx.doi.org/10.1016/j.carbpol.2012.07.005

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.

Table 1
Ag+ and PVA content in the hydroalcoholic solution (80/20) for the different samples prepared. Borax crosslinking, when carried out, is also indicated.

Sample name	Soaking bath composition			Borax crosslinking
	Ag^+ (mg/ml)	PVA_{47k} (%, v/w)	PVA_{145k} (%, v/w)	
[Ag_1-H_2O]	1	–	–	–
[Ag_{10}-H_2O]	10	–	–	–
[Ag_1-$PVA47_{0.2}$]	1	0.2	–	–
[Ag_1-$PVA47_{0.2}$-B]	1	0.2	–	Yes
[Ag_{10}-$PVA47_{0.2}$]	10	0.2	–	–
[Ag_{10}-$PVA47_{0.2}$-B]	10	0.2	–	Yes
[Ag_1-$PVA47_1$]	1	1.0	–	–
[Ag_1-$PVA47_1$-B]	1	1.0	–	Yes
[Ag_{10}-$PVA47_1$]	10	1.0	–	–
[Ag_{10}-$PVA47_1$-B]	10	1.0	–	Yes
[Ag_1-$PVA145_{0.2}$]	1	–	0.2	–
[Ag_1-$PVA145_{0.2}$-B]	1	–	0.2	Yes
[Ag_{10}-$PVA145_{0.2}$]	10	–	0.2	–
[Ag_{10}-$PVA145_{0.2}$-B]	10	–	0.2	Yes
[Ag_1-$PVA145_1$]	1	–	1.0	–
[Ag_1-$PVA145_1$-B]	1	–	1.0	Yes
[Ag_{10}-$PVA145_1$]	10	–	1.0	–
[Ag_{10}-$PVA145_1$-B]	10	–	1.0	Yes
[GeH]	–	–	–	–
[$PVA47_1$-B]	–	1.0	–	Yes
[$PVA145_1$-B]	–	–	1.0	Yes

2.9. Solvent uptake experiments

According to British Pharmacopoeia (British Pharmacopoeia, 1993) sample specimens (~2.5 cm × 2.5 cm) were accurately weighed (W1), and then placed in a Petri dish: 10 ml of a NaCl 142 mM and $CaCl_2$ 2.5 mM, at 37 °C, were added, and allowed to stand for 30 min at 37 °C. Samples were then removed, suspended by one corner with tweezers for 30 s, in order to remove freely draining fluid, and reweighed (W2). The weight of fluid absorbed and retained per gram was calculated by (W2 – W1)/W1. All tests were performed in triplicate and the standard deviations were reported.

2.10. Dehydration rate evaluation

Using the same procedure described for the solvent uptake experiment, the samples (~2.5 cm × 2.5 cm) were accurately weighed, swelled at 37 °C in NaCl 142 mM and $CaCl_2$ 2.5 mM for 30 min, suspended by a corner for 30 s to remove the freely draining liquid, and then reweighed. The hydrated samples were laid on dry Petri dishes without lids and placed in a ventilated oven at 37 °C. The weight loss of each sample was measured every hour and the rate of weight loss calculated. All tests were performed in triplicate.

2.11. Antimicrobial activity assay

Antimicrobial activity of the samples was determined on two reference strains: *Staphylococcus aureus* ATCC6538P and *Pseudomonas aeruginosa* ATCC27853. The strains were maintained at −80 °C in LB broth containing glycerol (20%, v/v). For assays strains were inoculated in LB broth from colonies isolated on LB agar plates and incubated 18 h at 37 °C with vigorous shacking (250 rpm). Broth cultures were refreshed 1/50 in pre-warmed LB broth and incubated at 37 °C with vigorous shacking until they reached an $OD_{600\,nm}$ of 0.7. Bacterial cells were then harvested by centrifugation (10,000 × *g* for 5 min) and suspended in the same pre-warmed medium at an $OD_{600\,nm}$ of 1.0. Aliquots of 1 ml of these cultures were seeded into 24 well plates containing 10 mm × 10 mm pieces of each sample and incubated 18 h at 37 °C. At 0, 1, 2, 3, 4, and 18 h of incubation 0.05 ml aliquots were sampled from each well and appropriately diluted in phosphate buffered saline (pH 7.4). Live bacterial cells were enumerated in dilutions of all samples by spot plating five 0.01 ml aliquots of three dilutions of each sample on LB agar plates. Growth of both strains in wells containing the non medicated patches was evaluated in parallel. Each experiment was performed twice in triplicate.

3. Results

3.1. Ag loading

In the Ag-slow-release nonwoven commercial products, silver is usually present as counter-ion of the polymer fibers, or dispersed as nanocrystals in the matrix. Preliminary tests carried out in order to exploit gellan fiber formation starting from Ag gellan hydrogels, did not fulfill the mechanical properties necessary to process a nonwoven tissue; the mechanical strength of Ag gellan fibers is lower than that of Na gellan fibers, probably due to the high steric hindrance of Ag ions that limit the interaction among the polymer chains, thus reducing their mechanical resistance to stresses.

For an homogeneous swelling of all the fibers, the GeH nonwoven patches were first imbibed with an hydroalcoholic solution, whose composition (H_2O/EtOH = 80/20) was chosen in order to balance the fibers shedding phenomenon, due to water uptake, and dehydration, due to alcohol, during the loading process. The wet nonwoven patches were then immersed into the loading solution. To modulate the silver content in the tissue, two different silver concentrations (1 or 10 mg/ml) were chosen.

In order to increase the Ag loading, to modulate the ion release and to increase the mechanical resistance of the medical device, PVA was added to the loading solution. PVA of two different M_w and at two different concentrations was used. During soaking, the patches were swelled by the hydroalcoholic solution containing Ag and PVA, and the following thermal treatment at 40 °C allowed the solvent evaporation while Ag and PVA remained on the patches.

The prepared nonwoven patches were also mechanically stressed in order to simulate a strong manipulation, as described in Section 2. The Ag content on the patches after the mechanical stress was determined and compared with that of the untreated ones.

In Fig. 1a and b the silver contents of the nonwoven patches treated with different loading solutions are reported.

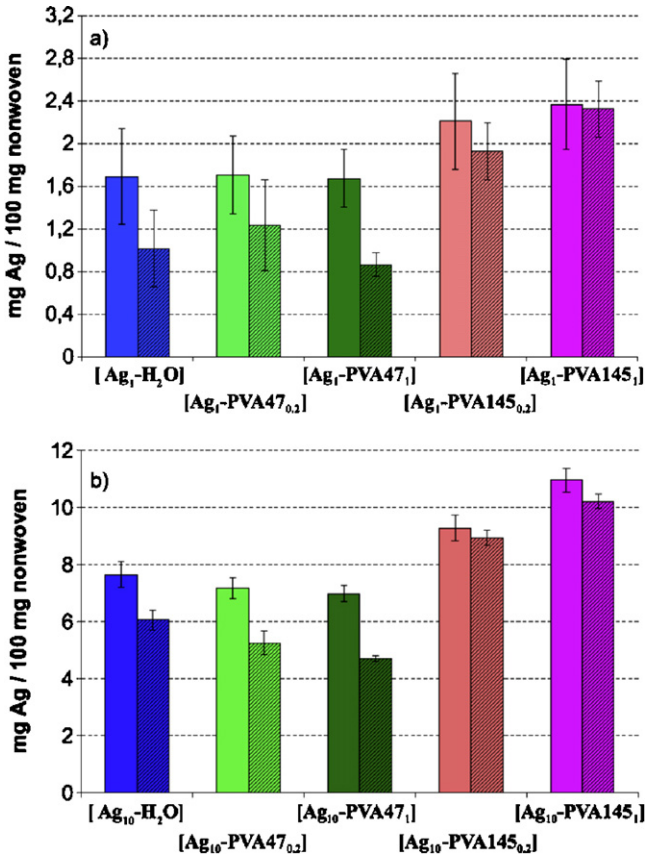

Fig. 1. Silver content of the loaded nonwovens treated with different loading solutions (silver concentrations: 1 mg/ml (a) and 10 mg/ml (b)). Left columns report the silver content of the unstressed nonwovens; right columns report the silver content of the stressed ones. Mean values ± SD of 3 different tests are reported.

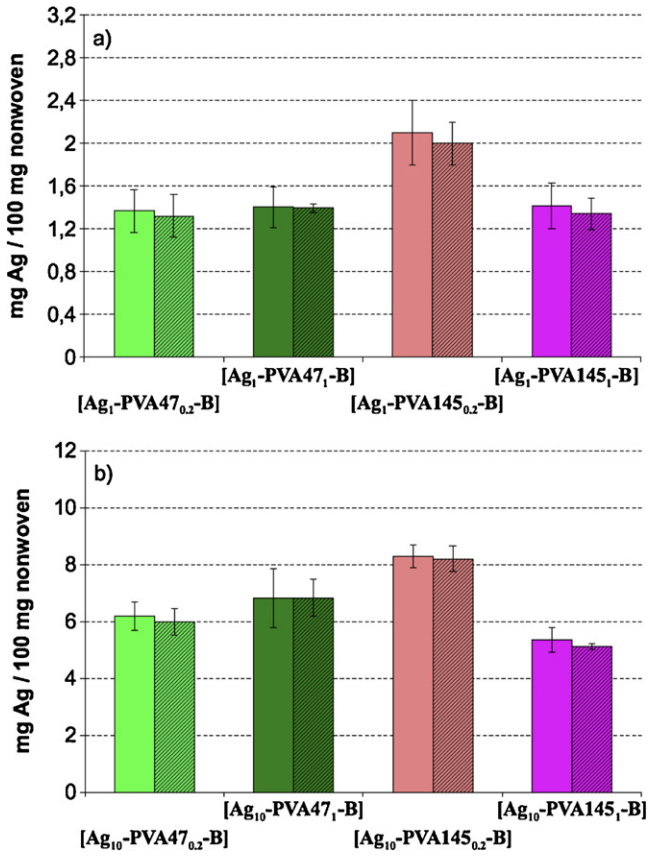

Fig. 2. Silver content of the loaded nonwovens treated with different loading solutions (silver concentrations: 1 mg/ml (a) and 10 mg/ml (b)) and then crosslinked with borax. Left columns report the silver content of the unstressed nonwovens; right columns report the silver content of the stressed ones. Mean values ± SD of 3 different tests are reported.

3.2. Borax crosslinking

The PVA containing patches were treated with borax to crosslink the system, in order to increase the mechanical resistance of the loaded patches as well as to modulate the Ag release from the patches.

In Fig. 2, Ag content for unstressed and stressed borax treated samples is reported.

3.3. Rheology of the loading solutions

Viscosity of the polymer solutions at $d\gamma/dt = 10\,s^{-1}$ obtained by rheological experiments, has been considered for the comparison among different samples. Because of the pseudoplastic behavior of the polymeric solutions, this shear rate value was chosen to mimic the loading conditions adopted in the present experimental setup during the PVA loading/rinsing process. The solutions containing PVA at low M_w (47,000) have viscosity values of 22 and 29 mPa s at concentrations 0.2 and 1% (w/v), respectively, while the solutions containing high M_w PVA (145,000) have viscosity values of 31 and 60 mPa s at concentrations 0.2 and 1% (w/v), respectively.

3.4. PVA content

The amount of loaded PVA seems to be dependent on the M_w and concentration of the polymer in the soaking solutions: for the samples [$PVA47_{0.2}$] and [$PVA47_1$], the polymer content is about 2.5 mg/100 mg GeH; the samples [$PVA145_{0.2}$] and [$PVA145_1$] show a higher amount of PVA, 4 and 8 mg/100 mg GeH, respectively. For all the crosslinked samples, the amount of loaded PVA was approximately halved, probably due to the soaking procedure of crosslinking.

3.5. Silver release

Silver release tests were performed in sink conditions (as described above) for the different loaded samples. In Fig. 3a and b the release profiles from nonwoven patches treated with different loading solutions are reported (silver concentration were 1 mg/ml and 10 mg/ml, respectively).

3.6. Solvent uptake and dehydration rate determination

The different loaded patches were immersed in NaCl 142 mM and $CaCl_2$ 2.5 mM, as described in the British Pharmacopeia for testing absorption capacity of the dressings. The solvent uptake (S) was then calculated as g_{H_2O}/g_{patch}. The obtained data are reported in Table 2. The wet samples were immediately put in a ventilated oven, and weighed every hour in order to evaluate their dehydration rate. In Fig. 4 the dehydration curves of the different samples are reported, and in Table 2 the dehydration rate (expressed as g_{H_2O}/h) and the time needed for a complete dehydration are reported.

3.7. Compliance matter

Finally, in the production of a silver loaded nonwoven tissues, it must also be taken into account how the medical device looks to the patients, in order to increase the patient compliance, as often

Table 2
Solvent uptake (S), dehydration rate and time of complete dehydration of the different patches and of the commercial materials. Mean values ± SD of 3 different tests are reported.

Sample name	S (g_{H_2O}/g_{patch})	Dehydration rate (g_{H_2O}/h)	Time of complete dehydration (h)
[GeH]	16.34 ± 1.63	7.83	2
[Ag_1-H_2O]	13.61 ± 0.22	6.32	2
[Ag_1-$PVA47_{0.2}$]	13.59 ± 0.62	3.86	3.5
[Ag_1-$PVA47_{0.2}$-B]	12.96 ± 0.14	3.21	5
[Ag_1-$PVA47_1$]	13.10 ± 0.17	3.68	3.5
[Ag_1-$PVA47_1$-B]	12.87 ± 0.24	3.42	5
[Ag_1-$PVA145_{0.2}$]	13.46 ± 0.26	3.81	3.5
[Ag_1-$PVA145_{0.2}$-B]	13.14 ± 0.38	3.74	4
[Ag_1-$PVA145_1$]	13.81 ± 0.35	3.92	3.5
[Ag_1-$PVA145_1$-B]	13.10 ± 0.08	3.72	4
Reference ***A***	6.01 ± 0.99	6.01	1
Reference ***B***	19.90 ± 1.28	6.63	3
Reference ***C***	17.35 ± 0.75	5.78	3

the consumer could not be prone to use unpleasant bad-looking products. Pictures of the Ag loaded patches are reported in Fig. 5.

3.8. Antimicrobial activity

In order to evaluate the antimicrobial activity of the samples, 10 mm × 10 mm of the different silver loaded patches (low Ag concentration soaking bath – c_{Ag} = 1 mg/ml) and of reference commercial materials were incubated in the presence of active cultures of two microorganisms that are common agents of skin and wound infections (*S. aureus* and *P. aeruginosa*). At time intervals ranging from 0 to 18 h, samples were taken from each culture and the number of colony forming units was evaluated by a simple cultural enumeration method. Untreated non-woven GeH was used as control. The results are reported in Fig. 6.

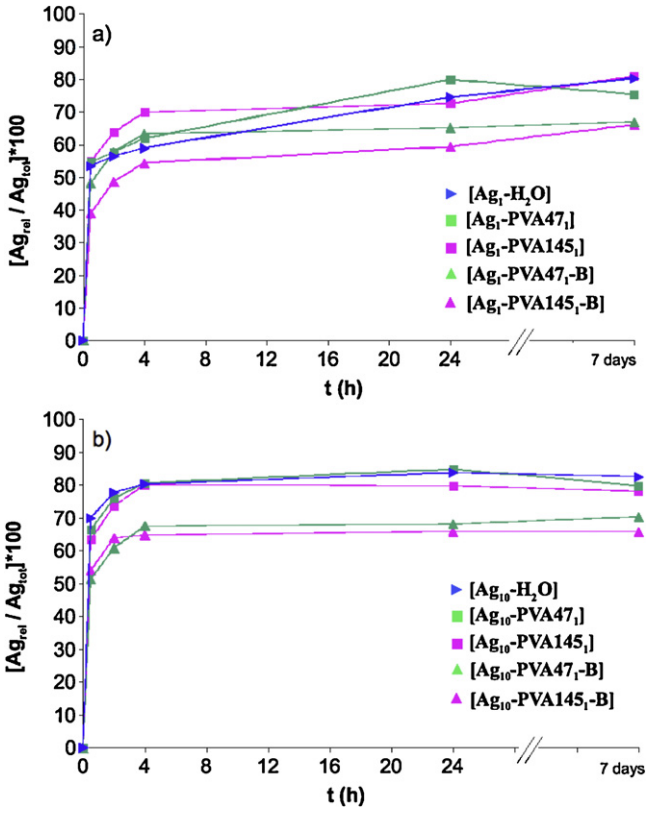

Fig. 3. Silver release profiles (37 °C, $NaNO_3$ 0.1 M) from loaded nonwoven with a silver concentration of 1 mg/ml (a) and 10 mg/ml (b). Ag-H_2O (▶), Ag-$PVA47_1$ (■), Ag-$PVA145_1$ (■), Ag-$PVA47_1$-B (▲) and Ag-$PVA145_1$-B (▲). All tests were performed in triplicate and the obtained values always lay within 10% of the mean.

4. Discussion

The polysaccharide support used in this work (GeH) was a nonwoven composed of Gellan and Hyaff® (98/2, w/w), an anionic polysaccharide and a benzyl derivative of hyaluronic acid respectively, see Fig. 7.

Gellan and Hyaff® fibers were obtained by extruding a paste of the mixed polymers in a NaCl hydroalcoholic bath; the fibers were then scattered on a conveyor where they rapidly set and, without any further intervention, formed a compact, three-dimensional matrix (Bellini, Terrassan, & Pavesio, 2007). Gellan gum possesses the well known ability to form a physical network, by means of structural rearrangement of its polymeric chains in a "double helix" conformation; these double helices can assemble together, leading to the formation of physical junction zones, whose strength and stability depends on the salt content, due to the physico-chemical interactions between junction zones and counter-ions (Crescenzi, Dentini, Coviello, & Rizzo, 1986; Grasdalen & Smidsroed, 1997; Miyoshi, Takaya, & Nishinari, 1996; Pollock, 2002). Sodium gellan fibers, and the corresponding nonwovens, are characterized by high mechanical strength, when they are in the dry form; while, in the presence of an aqueous medium, their resistance to traction and penetration decreases remarkably, due to their high swelling.

Hyaff®, an hydrophobic polymer, was added in order to decrease the swelling degree of the fibers, and the mechanical resistance of the wet nonwoven. Moreover, Hyaff®, as a derivative of the hyaluronic acid, is widely used in several biomedical devices, wound repair included (Chen & Abatangelo, 1999; Renier, Pavesio, & Callegaro, 1999).

The silver loading (Fig. 1a and b) is strongly dependent on the solution composition, as Ag_1-H_2O shows a loading of about 1.7 mg/100 mg whereas Ag_{10}-H_2O has a silver content of 7.8 mg/100 mg. A quite similar silver content is obtained using the loading solutions containing low M_w PVA. Also in this case, as expected, an increase of the Ag loading was observed when a soaking bath at high silver concentration was used. The nonwoven patches treated with the high M_w PVA loading solutions showed a higher silver content with respect to those loaded without PVA. This effect can be ascribed to the high viscosity of the polymer solution, which increases the solution imbibition, thus increasing the Ag loading of the tissues. This effect was observed for both the Ag loading bath (Ag_1 and Ag_{10}).

For a comparison, the silver content of the three different commercial wound dressings was evaluated: commercial ***A***, ***B*** and ***C***

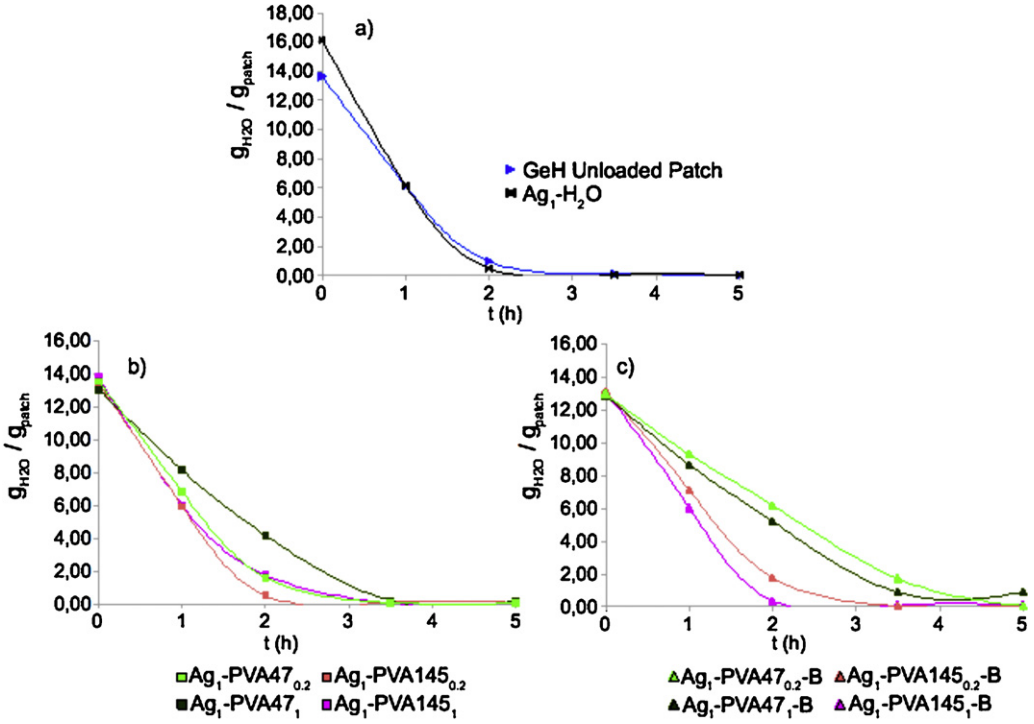

Fig. 4. Dehydration curves of the different patches: (a) GeH unloaded patch and Ag_1-H_2O sample; (b) patches treated with polymeric loading solutions; (c) borax crosslinked loaded patches. The tests were performed in triplicate, and standard deviations always laid within 10% of the mean.

have a silver content of 5.60 ± 0.23, 3.88 ± 0.28 and 0.23 ± 0.07 mg Ag/100 mg sample, respectively.

In Fig. 1, Ag content obtained for the nonwoven tissue after mechanical stress is also reported. In most cases the mechanical treatment reduces the Ag content not less of 30% in Ag_1-H_2O, Ag_1-$PVA47_{0.2}$ and Ag_1-$PVA47_1$ samples with respect to the unstressed tissue ones. The same trend is observed for the sample loaded with a higher silver concentration (Ag_{10}-H_2O, Ag_{10}-$PVA47_{0.2}$ and Ag_{10}-$PVA47_1$). Nevertheless, it must be pointed out that high M_w PVA in the loading bath reduces significantly the loss of Ag: actually for all these samples the silver reduction was never more than 10%. According to collected data, it can be suggested that for the nonwoven patches, immersed in a loading solution with higher viscosity, the payload is higher with respect to that obtained using the non polymeric loading solution. Moreover, when the loaded nonwoven tissue is dried, PVA forms a film, which can entrap more effectively the loaded Ag.

These data are confirmed by the results obtained for the PVA content evaluation and the rheological measurements of the different loading solutions: the viscosity of the polymer soaking baths increases with the M_w and the concentration of the PVA, leading to a higher polymer content in the treated nonwoven.

Borate ions have long been known for their ability to form complexes with diol groups (Grassi et al., 2009; Renn, 1999; Wang, Shyr,

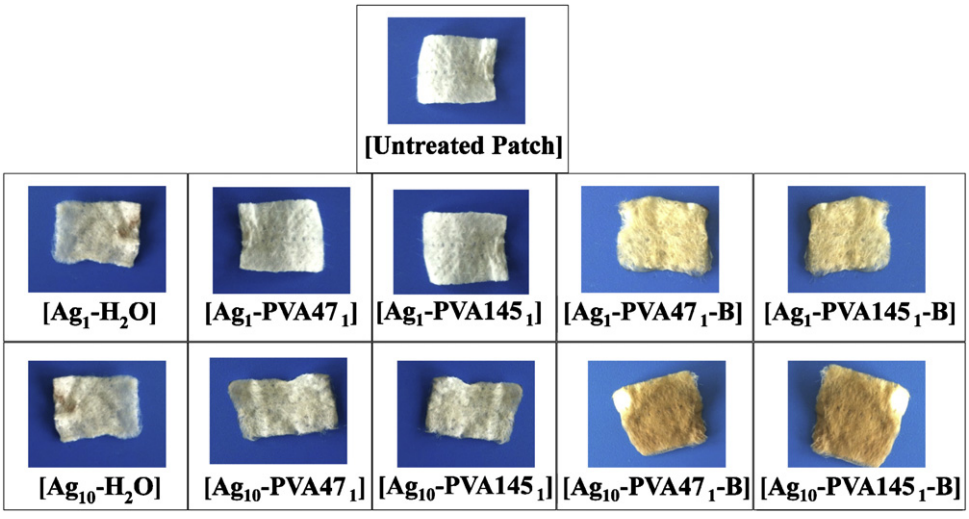

Fig. 5. Appearance of different Ag-loaded nonwovens, in comparison with the untreated samples.

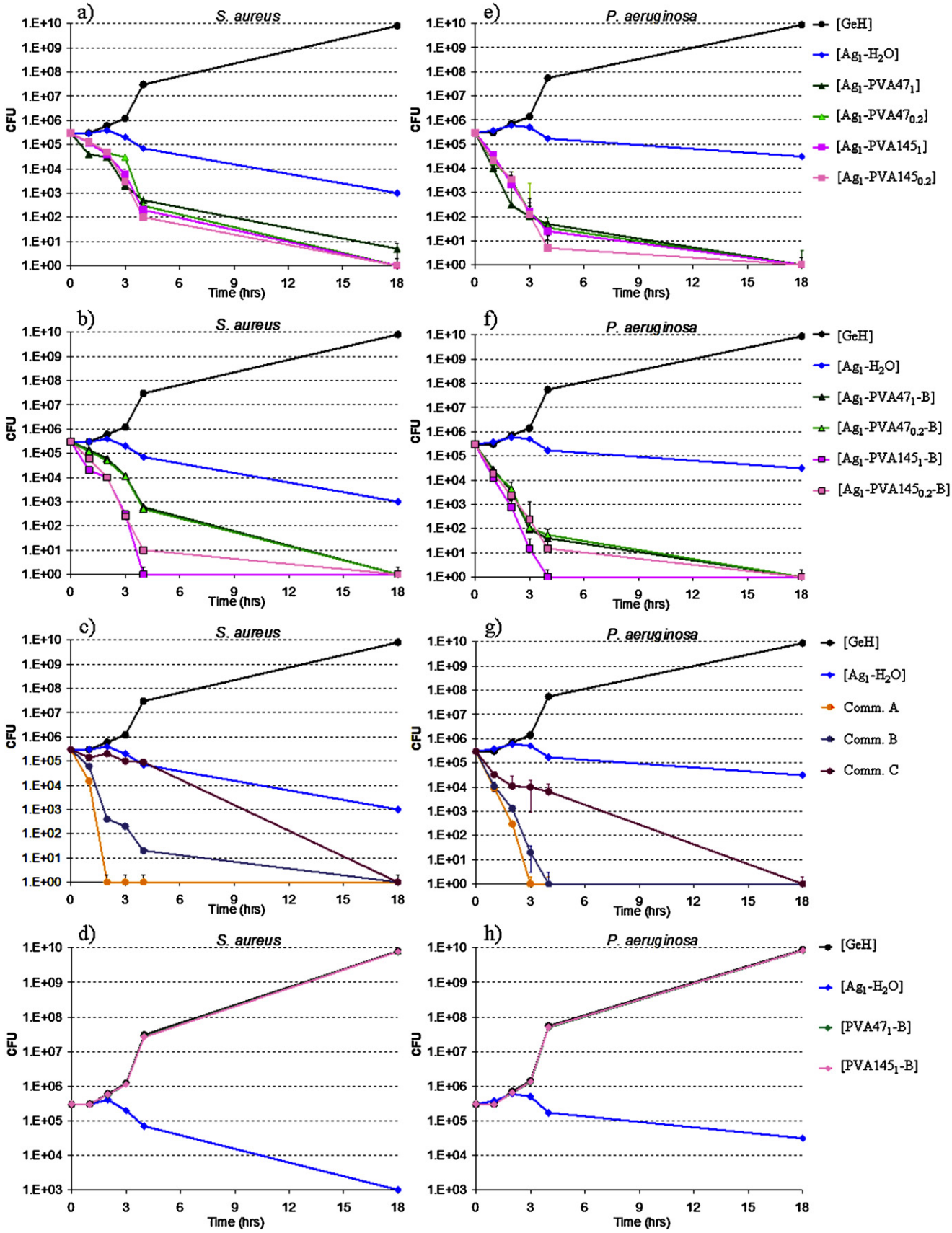

Fig. 6. Antibacterial efficacy of silver-containing dressings against *S. aureus* (a–d) and *P. aeruginosa* (e–h). Uncrosslinked samples Ag_1-PVA (a–e); borax crosslinked samples Ag_1-PVA-B (b–f); commercial samples (c–g); non medicated samples (d–h). Control (unloaded GeH) and Ag_1-H_2O samples were always reported for an appropriate comparison. Where not visible, standard deviation bars are contained within the symbols.

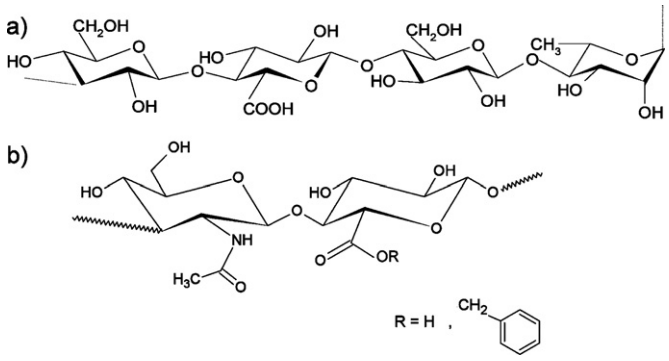

Fig. 7. Structure of repeating unit of (a) deacylated gellan gum (Ge); (b) Hyaff® (esterification degree = 75%).

& Hu, 1999) according to the scheme described below involving two successive reactions: monocomplexation (I) and dicomplexation (II) (Fig. 8a).

PVA possesses 1,3-diol groups which may form monodiol-borate or didiol-borate complexes; the formation of a interchain didiol-borate leads to the crosslinking of PVA, as depicted in Fig. 8b.

In the present case we are not able to discriminate the crosslinking chemistry of borax: actually, vicinal diols due to PVA as well as to polysaccharides are present. The borax concentration (10 mM in H_2O/EtOH = 80/20, corresponding to 0.2%, w/v) was chosen in order to guarantee an excess of crosslinker, in comparison with diols. According to the above described reactions, PVA crosslinking occurs leading to a polymeric film that can enhance Ag loading of the nonwoven patches and at the same time the mechanical resistance to stresses.

The borax treatment step led to a decrease of the silver content (Fig. 2) with respect of the not crosslinked patches. This effect can be ascribed to a dilution effect due to the immersion into the borax solution.

On the other hand, the borax treatment leads to a more stable Ag entrapment, especially for the nonwoven patches treated with the low M_w PVA loading solution. In this respect, it can be observed that for the samples Ag-PVA47-B, irrespective of the Ag and PVA concentration, the mechanical treatment reduces the Ag content only at a negligible extent.

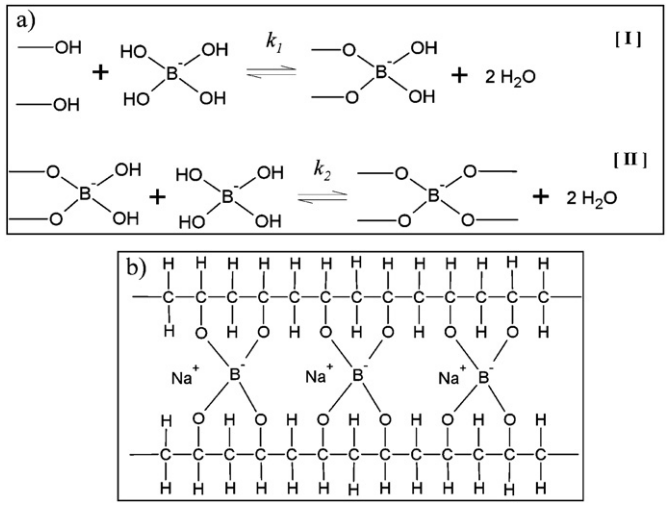

Fig. 8. (a) Scheme of reaction of mono- [I] and dicomplexation [II] of borate ions with diols groups; (b) structure of the didiols-borate complexes for PVA.

For both Ag concentrations, the release profiles of nonwoven treated with polymeric loading solutions, Ag-$PVA47_1$ and Ag-$PVA145_1$, are quite similar to the profile of the sample loaded in water, Ag-H_2O. While, the silver loaded samples treated with borax, Ag-$PVA47_1$-B and Ag-$PVA145_1$-B show lower release profiles, with a lower initial burst, most probably due to the higher stability of silver entrapment, in accordance with the stress experiment, as well as silver tetraborate insoluble salt formation. Moreover, the silver release seems to be independent on the PVA content: samples Ag-$PVA47_1$ and Ag-$PVA145_1$ show a very similar release profile, despite the quite different polymer content (2.5 and 8 mg/100 mg GeH, respectively).

The different samples show a solvent uptake lower than that of the untreated patch GeH, but quite similar to each other, irrespective of M_w and concentration of PVA. Finally, the borax crosslinked patches show an S value slightly lower than that of the corresponding not crosslinked samples.

The dehydration rate of the different samples is lower than that of the untreated nonwoven, probably due to the formation of a PVA film, which allows a longer water retention. This phenomenon is even more pronounced for the borax crosslinked samples, which, in two cases are completely dried after 5 h.

For a comparison, solvent uptake and dehydration rate were determined also for the three different commercial wound dressings. Also for these materials, results are reported in Table 2: commercial ***B*** and ***C*** have the higher solvent uptake values, due to their polysaccharide nature, but they reach the complete dehydration quite rapidly (3 h). Commercial ***A*** possesses a very low S value, and reaches complete dehydration after 1 h. These results show that our samples are characterized by a good solvent uptake, important for the absorption of the exudates, and by a slow dehydration rate, critical in order to avoid the painful adhesions on the wound site.

The appearance of samples soaked in low Ag concentration solutions (Ag_1-H_2O, Ag_1-$PVA47_1$ and Ag_1-$PVA145_1$) is quite the same of the untreated patch. Even the samples soaked in high Ag concentration solutions (Ag_{10}-H_2O, Ag_{10}-$PVA47_1$ and Ag_{10}-$PVA145_1$) have an appearance quite similar to that of the original nonwoven, even if a minimal darkening effect can be observed.

When the low silver concentration samples are treated with borax (Ag_1-$PVA47_1$-B and Ag_1-$PVA145_1$-B), a slight darkening of the matrix occurs, probably due to a partial precipitation of the loaded silver as silver tetraborate insoluble salt. The phenomenon is severe for the high silver content samples (Ag_{10}-$PVA47_1$-B and Ag_{10}-$PVA145_1$-B), where the matrices assume a quite unpleasant brown color.

The different samples are characterized by a high antimicrobial activity, which seems to be independent on the silver content: in fact, despite of the quite similar silver content in the different samples (1.6–2.4% for the uncrosslinked samples, 1.2–2.0% for the borax crosslinked samples), the Ag_1-H_2O patch shows a lower antimicrobial activity with respect to the PVA soaked samples (Fig. 6a and e). Moreover, the borax crosslinking, especially in the samples with high M_w PVA (Ag_1-$PVA145_{0.2}$-B and Ag_1-$PVA145_1$-B), seems to enhance the antimicrobial activity (Fig. 6b and f), that for these sample is comparable to that of the marketed dressings ***A***, ***B*** and ***C*** (Fig. 6c and g).

In order to understand this phenomenon, samples prepared with the same treatments described in Section 2, but without silver, were evaluated for their antimicrobial activity. These samples ($PVA47_1$-B and $PVA145_1$-B) did not show antimicrobial activity, and in Fig. 6d and h the results are superimposed with those of the control sample GeH: thus, the PVA and borax treatments are able to enhance the antimicrobial silver activity.

To summarize, we succeeded in the preparation of a novel dressing for wound healing. The silver content in the dressing

is tunable with the Ag concentration in the soaking bath. The hydrophilic behavior of the GeH nonwoven provides high water uptake capability, fundamental for the absorption of the wound exudates. The formation of a PVA film upon the nonwoven decreases the dehydration rate of the dressing: this fact could be exploited in reducing painful adhesions of dried dressings on the wound site. The borax loaded samples show a slower silver release, probably due to a more effective silver entrapment: this could be useful to reduce the frequency of patches re-applications, with a better patient compliance. The dressings exhibit a strong antimicrobial activity against both *S. aureus* (Gram-positive bacteria) and *P. aeruginosa* (Gram-negative bacteria), which are bacteria that can usually be found on contaminated wounds: the borax loaded samples show the strongest antimicrobial activity, with the PVA and borax that enhance the antimicrobial silver activity.

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