Mechanical properties and in vitro characterization of polyvinyl alcohol-nano-silver hydrogel wound dressings


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Polyvinyl alcohol (PVA) hydrogels are materials for potential use in burn healing. Silver nanoparticles can be synthesized within PVA hydrogels giving antimicrobial hydrogels. Hydrogels have to be swollen prior to their application, and the common medium available for that in hospitals is saline solution, but the hydrogel could also take up some of the wound’s fluid. This work developed gamma-irradiated PVA/nano-Ag hydrogels for potential use in burn dressing applications. Silver nitrate (AgNO3) was used as nano-Ag precursor agent. Saline solution, phosphate-buffered solution (PBS) pH 7.4 and solution pH 4.0 were used as swelling media. Microstructural evaluation revealed an effect of the nanoparticles on PVA crystallization. The swelling of the PVA-Ag samples in solution pH 4.0 was low, as was their silver delivery, compared with the equivalent samples swollen in the other media. The highest swelling and silver delivery were related to samples prepared with 0.50% AgNO3, and they also presented lower strength in PBS pH 7.4 and solution pH 4.0. Both PVA-Ag samples were also non-toxic and presented antimicrobial activity, confirming that 0.25% AgNO3 concentration is sufficient to establish an antimicrobial effect. Both PVA-Ag samples presented suitable mechanical and swelling properties in all media, representative of potential burn site conditions.

1. Introduction

Developments in burn healing, improvements of skin grafting materials and the development of skin replacement surgeries have clarified some aspects of skin healing [1]. The concept of maintaining a moisturized environment during healing was introduced around the 1960s and it has been shown to diminish the incidence of scars [2,3]. Hydrogels are suitable for wound dressings, because they keep a moist environment, they provide a cool sensation diminishing pain, and their high water uptake allows them to be applied in the swollen state and still absorb some fluid from the wound site, presenting some characteristics of an ideal dressing [4]. This swelling also allows some debris and bacteria entrapment, and these gels usually act as barriers to bacteria penetration [5].

Poly(vinyl alcohol) (PVA) hydrogels have been used as dressings for at least three decades [6]. They are suitable due to their transparency, mechanical resistance, biocompatibility and biodegradability, they keep a moisturized environment and, when cross-linked, they can remain on site for 4 days [7,8]. PVA hydrogels’ characteristics allow the monitoring of healing with fewer dressing changes, as they keep their structural integrity when hydrated.

Cross-linking is necessary in order to maintain gel integrity and to ensure a reasonable strength. PVA can be cross-linked via chemical or physical routes. Chemical routes can be divided into two main types: (i) irradiation and (ii) processes that use cross-linking agents [9]. The second type refers to
the addition of chemicals, e.g. glutaraldehyde and formaldehyde, which react with PVA chains connecting them via covalent bonds [10]. However, residual chemicals remaining within the matrix can be delivered to the body and they are potentially hazardous [7]. Physical cross-linking does not require any chemical addition and its cross-linking method differs from the chemical routes. When the PVA solution is freeze–thawed, it allows crystallite formation which anchors the amorphous chains [11]. Radiation techniques are clean and effective, they cross-link the PVA chains through covalent bonds that are formed between the groups originally in the polymer chains and they also sterilize the gels [12].

Among the developments in hydrogels for wound healing in the past decade have been polymeric hydrogels loaded with antibiotics, which prevent wound infection. Several commercial dressings for different applications are available, e.g. Acticoat, dressing with nanocrystalline silver used for burn healing, and Tegaderm CHG, a polymeric gel dressing loaded with chlorhexidine gluconate to cover and protect catheter sites. Systems based on PVA combined with silver have been studied to be used as biosensors, as burn dressings, etc. PVA-Ag systems can present antimicrobial characteristics [13,14] and non-toxicity [15].

The effectiveness of silver as an antibiotic for burns was recorded by Moyer in the 1960s [16]. The bactericidal effect of 0.50% silver nitrate (AgNO₃) aqueous solution on the bacteria most commonly present in burns, e.g. *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, with no detrimental effect on the tissue re-epithelization, was shown [16]. Numerous micro-organisms can be found on burn sites: Gram-positive bacteria, such as *S. aureus* and *Enterococcus faecalis*, Gram-negative bacteria, such as *E. coli*, *Pseudomonas* spp. and *Klebsiella* spp., and some fungi, such as *Candida albicans* [17,18].

Silver nanoparticles are a more effective antimicrobial than ionic silver owing to their better permeation and retention effects. In addition, silver nanoparticle-loaded PVA gels have been extensively studied, because PVA is a remarkable capping agent for silver nanoparticle synthesis [19]. PVA is used in silver nanoparticle synthesis to control the particle size and to avoid agglomeration. Silver nanoparticles can be synthesized via chemical route, but this method can result in toxicity, or via irradiation (gamma radiation, ultraviolet radiation, etc.), a clean and effective method [9]. When a silver salt is mixed with PVA, the ionic silver is attracted by the chains’ hydroxyl groups. When gamma-irradiated, silver is reduced, and nanoparticles are formed close to the OH⁻ groups, remaining entrapped in the PVA network [7,20]. In addition, through the hydroxyl groups, the PVA chains can be cross-linked when irradiated [11,21]. The concentration of silver loaded in PVA gels should be controlled, because silver can inhibit bacteria by interacting with their DNA, and it could do the same to eukaryotic cells [9,22]. The main difference between bacteria and eukaryotic cells, related to the silver action, would be that the bacteria are much smaller and less complex organisms than eukaryotic cells. As a consequence, a therapeutic window is established, where the silver is incorporated within a range that inhibits bacteria without reaching levels toxic to human cells [23]. Studies on the use of gamma radiation in PVA-Ag systems with the final goal of obtaining nanoparticles [24,25], and studies on PVA blends that were gamma-irradiated and loaded with silver to be used in wound healing have been developed recently [9,26]. PVA hydrogels loaded with a controlled concentration of silver could combine the hydrogel property of keeping a moisturized environment, that stimulates healing, with the silver (antibiotic) effect of inhibiting or killing the bacteria on site and, consequently, avoiding infection.

The specific goal of this work is to develop PVA hydrogels loaded with low amounts of silver, to confirm their antimicrobial activity and non-cytotoxicity, and to analyse the effects of three relevant fluid environments on their swelling, mechanical and silver release behaviour. These gels were produced via gamma-irradiation, because this process would cross-link the polymer, synthesize silver nanoparticles and sterilize the gels. The bacterial tests analysed the gel barrier properties, and the antibacterial effect of these materials on different organisms, on *E. coli*, Gram-negative bacteria, on *S. aureus*, Gram-positive bacteria, and on *C. albicans*, fungi. Cell *in vitro* tests analysed the cytotoxicity of these materials to epithelial cells. The hydrogel dressings would usually be hydrated when placed on a burn site. The most common medium available in hospitals to hydrate these gels is sterile saline solution. In order to mimic these materials properties when in place, the silver delivery, the mechanical and the swelling properties of these materials were studied in saline solution and in phosphate-buffered solution (PBS) pH 7.4, which mimics the inorganic part of the human plasma. PBS pH 7.4 was used with its original neutral pH and also with low pH, solution pH 4.0, which represents the inflammatory reaction.

### 2. Material and methods

#### 2.1. Materials

Hydrogels were manufactured using PVA, M₆₈ 85 000–124 000 and degree of hydrolysis 99%, and AgNO₃, both purchased from Sigma-Aldrich, analytical grade, used without further purification.

#### 2.2. Preparation of the samples

PVA aqueous solutions were obtained by dissolving 10 g of PVA in 100 ml of distilled and deionized water at 90°C for 2 h, under mechanical stirring. For PVA-Ag samples, 10 g of PVA was dissolved in 70 ml of distilled and deionized water at 90°C for 2 h, under mechanical stirring, and the AgNO₃ solutions were obtained by dissolving the correct amount of AgNO₃ in 30 ml of distilled and deionized water at room temperature in the dark, under magnetic stirring for 2 h. The AgNO₃ solutions were mixed with the PVA solutions under mechanical stirring, in the dark, at room temperature for 30 min, resulting in solutions containing 10 g of PVA and 0.00%, 0.25% or 0.50% AgNO₃ (relative to the weight of the polymer), in 100 ml H₂O. The resulting solutions were exposed to ultrasonic waves (Ultrasonic Clear, USC 750, Unique) in the dark for 30 min to remove the remaining air from the solution. Then, 20 ml of the final solutions were poured into Petri dishes (diameter = 150 mm) and dried in the dark, at room temperature, under constant air flow for 48 h. After drying, the samples were irradiated under ambient conditions with a Co-60 γ-source at a dose rate of 1.5 kGy h⁻¹ for 10 h. The samples without silver were named ‘PVA’; the samples with 0.25% AgNO₃ were named ‘0.25’; and the samples with 0.50% AgNO₃ were named ‘0.50’.

#### 2.3. Microstructural characterization

Microstructural characterization of dry samples was performed using X-ray diffraction (XRD) analysis, with the diffraction angle range set between 5° and 60° (XRD 6000 Shimadzu)...
differential with CuKa at 30 kV and 30 mA, step length of 0.02°, step time of 1 s) and Fourier-transform infrared spectroscopy (FT-IR, Nicolet 6700 spectrometer, 32 scans per sample, wavenumber range of 550–4000 cm⁻¹).

2.4. Atomic force microscopy
The morphology of the PVA and of PVA-Ag hydrogels swollen in PBS pH 7.4 against E. coli (ATTC 25922), S. aureus (ATTC 6538) and C. albicans (ATTC 10231) were evaluated using the disc diffusion method [9]. Overnight grown cultures of E. coli, S aureus and C. albicans were diluted and plated on Mueller Hinton agar inoculated with approximately 10⁹ colony forming units per ml. The hydrogel samples consisting of different compositions were cut (circular, diameter = 1.80 cm), kept in 5 ml of PBS pH 7.4 overnight and then placed on the plates. The plates were incubated at 37°C for 18 h, and the zones of inhibition were measured. For the antimicrobial penetration test, autoclaved test tubes containing 10 ml of nutrient broth were covered with the hydrogels, a sealed tube was used as the negative control, and an open tube was used as the positive control. The turbidity of the media was observed for up to one month.

2.5. Antimicrobial activity assay
Antimicrobial activities of PVA and of PVA-Ag hydrogels swollen in PBS pH 7.4 against E. coli (ATTC 25922), S. aureus (ATTC 6538) and C. albicans (ATTC 10231) were evaluated using the disc diffusion method [9]. Overnight grown cultures of E. coli, S aureus and C. albicans were diluted and plated on Mueller Hinton agar inoculated with approximately 10⁹ colony forming units per ml. The hydrogel samples consisting of different compositions were cut (circular, diameter = 1.80 cm), kept in 5 ml of PBS pH 7.4 overnight and then placed on the plates. The plates were incubated at 37°C for 18 h, and the zones of inhibition were measured. For the antimicrobial penetration test, autoclaved test tubes containing 10 ml of nutrient broth were covered with the hydrogels, a sealed tube was used as the negative control, and an open tube was used as the positive control. The turbidity of the media was observed for up to one month.

2.6. Cytotoxicity evaluation
The cytotoxicity test was performed according to the standard ISO 10993-5:2009, using a multi-parametric method to evaluate three parameters of cell viability, cell metabolic activity (mitochondrial activity (XTT) and cell density (CVDE)) and neutral red membrane integrity assay (NR). Mouse fibroblast cells, 3T3 line, obtained from the cell bank of the Clinical Research Unit of the Fluminense Federal University, Brazil, were cultured in a humidified 5% CO2 atmosphere with a strain rate of 10 mm min⁻¹ and resonance frequencies of 160 kHz range. Scanning was carried out at the free cantilever oscillation frequency and different amplitudes, depending on the stability and contrast obtained. The amplitude was set higher than 80 nm, and the set point was fixed at 10–30% of the free oscillation amplitude to guarantee that the microscope was operating in intermittent contact mode. Samples were fixed on double-sided adhesive tapes, and the AFM images of the external surface were obtained in air. ImageJ software was used to process images [27].

2.7. Swelling tests and drug delivery analysis
Swelling tests and degradation tests were performed gravimetrically for each sample composition (n = 5). The degradation tests followed the procedure described in the standard ISO 10993-9:1994. The samples were immersed in three different media, sterile saline solution (Sigma-Aldrich, 0.9% NaCl), PBS pH 7.4 (Sigma-Aldrich, 0.01 M phosphate buffer, 0.0027 M KCl, 0.137 M NaCl), and aqueous solution pH 4.0, to simulate the local inflammatory environment. To produce this solution, the pH of PBS pH 7.4 was lowered using lactic acid (Sigma-Aldrich). Samples of approximately 5 cm², weight normalized, were placed in 5 ml of each media at 37°C. The samples remained immersed for 4 days, being weighed at regular time intervals (30 min, 1 h, 2 h, 3 h, 4 h, 1 day, 2 days, 3 days and 4 days) after removing the excess of water on the surface with filter paper. After 4 days, the samples were dried and weighed. The swelling degree (SD) of the samples was calculated according to equation (2.1), where W₀ is the swollen weight (weight of the sample at each time interval) and W₀ is the dry weight before swelling [9,28]:

\[ SD = \frac{W_s - W_D}{W_D}. \]  

(2.1)

To calculate the weight loss (Wₚ) equation (2.2) was used, where W₀ is the weight of the dried samples after swelling tests:

\[ W_p = 100 \times \frac{(W_D - W_{DS})}{W_D}. \]  

(2.2)

To analyse the silver delivery from the hydrogels, triplicates (n = 3) of each sample composition (approx. 5 cm², weight normalized) were immersed in 5 ml of saline, of PBS pH 7.4 and of solution pH 4.0 at 37°C. The medium was removed after 4 days and analysed via a UV–visible spectrometer (Perkin-Elmer, lambda 25), from 400 to 500 nm, using polystyrene cuvettes. The qualitative analysis was based on the characteristic band of the silver nanoparticles, around 420 nm.

2.8. Mechanical characterization
Tensile tests were performed after 24 h of swelling in the three different media (saline solution, PBS pH 7.4 and solution pH 4.0), and the samples were cut into a dog bone shape. The samples were attached to the grips of a Zwick 2005 tensile test machine with the help of sand paper, using a 500 N load cell. The initial length of the samples was approximately 30 mm. Each sample's thickness was measured using a digital calliper, and the samples were measured in three different points. The samples' thickness was (0.16 ± 0.07) mm and their width was (4.52 ± 0.31) mm. The tests were conducted at room temperature with strain rate of 10 mm min⁻¹ until failure. For each test, at least 10 samples of each composition (n = 10) were used, in accordance with ASTM D882-00.

2.9. Statistical analysis
The statistical analysis of all results obtained was performed using the program Origin Pro 8 with a two-way ANOVA analysis, significance level of 95%, to evaluate the significance of the two factors (type of medium and concentration of AgNO₃) and three levels of each factor. The levels of the factor 'type of medium' were (saline solution, PBS pH 7.4 and solution pH 4.0). The levels of the factor 'concentration of AgNO₃' were 0%, 0.25% and 0.50% AgNO₃. When the population means results were significant to the evaluated parameters, a Tukey honestly significant difference test was conducted. It reveals if there was a significant difference in the analysed property values between each pair of levels compared, p < 0.05, sig. = 1. A one-way ANOVA statistical analysis, significance level of 95%, was used to evaluate the antimicrobial activity results and the cytotoxicity results, because in these tests the concentration of AgNO₃ was the only parameter evaluated. A Tukey honestly significant difference test, significance level of 95%, was used to determine whether the difference between the levels studied was significant.

3. Results and discussion

3.1. Microstructural characterization
The PVA’s diffraction characteristic peak at 2θ = 19.6°, related to the (110) reflection, was observed in all diffraction patterns,
3.2. Atomic force microscopy

The macroscopic aspect of the samples suggested the formation of silver nanoparticles after irradiation. It was observed that the colour change of PVA samples varied from pale yellow for pure PVA to yellowish brown for the sample produced with higher concentration of AgNO₃ (0.50). This difference in colour was in agreement with the observation of other authors [36].

In order to confirm the distribution of silver nanoparticles on the gel surface, the samples were analysed by AFM (figure 2). Comparing the cross-section plot of PVA and of 0.25 obtained from topography images, figure 2a,b, higher and wider peaks were present in the 0.25 plot that can be related to the silver nanoparticles. Based on this image, the nanoparticles are of approximately 90 nm in diameter. AFM phase contrast images are produced by changes in phase angle of the cantilever probe and have been shown to be sensitive to stiffness, viscoelasticity and chemical composition of material surfaces [31]. The PVA contrast phase image shows a uniform material, figure 2c, believed to be a polymeric matrix. A second phase, different from the polymeric matrix, is observed in the image of the 0.25 sample (figure 2d). The dark regions in the phase image of the 0.25 sample are thought to be related to silver nanoparticles surrounded by PVA matrix, clearer regions.

3.3. Antimicrobial activity assay

The antimicrobial tests (figure 3) revealed that the samples containing silver presented significant \( p < 0.05 \) inhibition against all micro-organisms studied. The response of *S. aureus* and of *C. albicans* to the two concentrations of silver tested was statistically equivalent; however, for *E. coli*, high inhibition was observed for the samples produced with high concentration of silver. In summary, the samples containing silver presented antimicrobial activity against both Gram-positive and Gram-negative bacteria and against fungi. The antimicrobial properties of silver have long been recognized. Silver nanoparticles are thought to disinfect via a number of mechanisms, including causing damage to the cell membrane and the generation of reactive oxygen species [32].

The antimicrobial penetration test could determine whether the samples are able to protect a wound from a secondary bacterial infection [33]. The test revealed that all samples were barriers to microbial penetration. After one month of test tube coverage, the solution in the tubes covered by all the samples and the solution of the negative control presented no turbidity, whereas, in the positive control, high turbidity was observed (images not shown). No difference was encountered between samples with and without silver. The microbial penetration prevention is probably related to the polymer network that blocks/entrap the microbials [5].

3.4. Cytotoxicity evaluation

Mouse fibroblasts were exposed to the samples’ extracts for 24 h, and cell response results are compiled in figure 4. The cell density of the samples after contact with the samples’ extracts remained in the same range as those of the negative control. The cells’ density of the positive control value was considerably lower than the others. Besides cell density, the membrane integrity of the cells remained high even when in
contact with the samples’ extracts. In addition, the cells’ mitochondrial activity was not affected by the presence of the extracts. All samples are non-toxic, because the studied parameters of the cells after contact with the samples’ extracts were close to those of the negative control.

The concentration of AgNO₃ used in this work allowed the production of non-cytotoxic PVA/silver nanoparticle samples with antimicrobial activity [34]. This could suggest that the amount of silver present in samples is located inside the therapeutic window level for silver.

3.5. Swelling tests and drug delivery analysis

Figure 5 shows that all the samples immersed in all media swelled at least 300%. A peak of media uptake can be observed at the beginning of the immersion in all curves.
This might be related to the media entrance, which stretches the chains until they reach a relaxed state. The equilibrium swelling degree (ESD) occurs when the hydration forces (the network stretching by the water uptake in the first time) and the elastic force of the cross-linking reach the equilibrium [35]. The ESD was observed after 1 day of immersion and these values are displayed in figure 6. In all media, the SD of 0.50 samples was higher than that of 0.25 samples in all media. Comparing the samples with the same composition, the swelling was lower when the samples were immersed in the lower pH solution (solution pH 4.0).

The ANOVA analysis revealed that all factors (type of media and concentration of AgNO₃) and their interaction were significant to the swelling, \( p < 0.05 \). Based on the Tukey test, there is a relevant difference regarding the concentration of AgNO₃. The difference occurs between the samples containing 0.50 and the others. In all media, the highest swelling was observed for the samples containing the highest amount of silver. When the samples were irradiated, the radiation cross-linked the PVA and also reduced the silver [24]. Because the cross-link occurs at the same site where the Ag⁺ attaches to PVA, silver nanoparticle formation could compete with cross-link formation. High silver concentration represents more nanoparticles synthesized and fewer hydroxyl groups available to cross-link, potentially resulting in a low degree of cross-linking. This network would be able to expand more when in aqueous media, increasing the gel swelling capacity [11, 21, 35].

There is also a difference (\( p < 0.05 \)) between the samples’ swelling behaviour according to the media used. The SD was lower for solution pH 4.0, the inflammatory reaction pH. It is worth saying that no difference was observed between the swelling in saline solution and in PBS pH 7.4. Saline solution would be the medium used by hospitals to swell dry wound care gels before applying them [37], and PBS pH 7.4 has similar characteristics to human plasma. In addition, it is anticipated that the gels could be used for 4 days maximum and, during this period, not only could the polymer degrade, but also some silver delivery could take place. The weight loss of the dried samples after 4 days of immersion in all media was calculated (figure 7).

The ANOVA analysis revealed that at the 0.05 level, both factors (type of medium and concentration of AgNO₃) and their interaction are significant. The post hoc Tukey test for type of medium revealed that the weight loss in solution pH 4.0 was lower than in the other media used. The Tukey test results for the concentration of AgNO₃ showed that the weight loss of the 0.50 samples was significantly higher than that of the 0.25 samples when in saline and in PBS pH.

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**Figure 5.** Swelling degree of the samples in (a) saline solution, (b) PBS pH 7.4 and (c) solution pH 4.0.

**Figure 6.** ESD of (a) the different samples immersed in each medium (saline solution, PBS pH 7.4 and solution pH 4.0) and of (b) sample composition (PVA, 0.25 and 0.50) in the different media. *\( p < 0.05 \), sig. = 1. There is a significant difference between the levels indicated by arrows.
7.4, although the weight loss of the 0.50 samples in solution pH 4.0 was similar to weight loss of the 0.25 samples.

The gels seem to have low affinity for the acidic pH, presenting low SD which could affect the silver delivery and also the PVA chains’ movement, diminishing the polymer degradation. The higher weight loss of the samples containing the highest amount of silver in saline and in PBS pH 7.4 could be related to the PVA degradation and also to some contribution of the silver delivery. The network of the 0.50 samples could be more open, and the high water uptake could facilitate the silver leaching to the media.

The antimicrobial activity of the gels could be related to the silver delivery from the hydrogels [9]. Silver nanoparticles present a characteristic band in the UV spectra around 420 nm, related to the spontaneous formation of silver nanoparticles due to the direct redox between PVA and Ag⁺ when there is no other reducing agent in the system [24, p. 342] which is responsible for the yellow/brownish colour of the samples containing silver. The drug delivery profiles of the swelling media, between 400 and 500 nm (figure 8) after 4 days, were qualitatively analysed and a band around 450 nm was found in all PVA-Ag profiles.

Silver nanoparticles of 20 nm present a characteristic UV–visible band at 420 nm; when this band is shifted to longer wavelengths, it indicates that the nanoparticles are bigger than 20 nm [9]. There was a difference in the absorbance values of the 450 nm band according to the composition and according to the media used (figure 9). The silver in the PVA-Ag hydrogels would be in nanoparticle form, although they might be bigger than 20 nm. This is in agreement with AFM results (figure 2). The statistical analysis on these relative results, two-way ANOVA, revealed that, at the \( p < 0.05 \) level, both parameters, ‘type of medium’ and ‘concentration of AgNO₃’, have a significant effect on silver delivery, although their interaction is not significant. The Tukey test for type of medium revealed that there is a difference between the silver delivery in solution pH 4.0 and the other media. The lowest absorbance occurred for solution pH 4.0, meaning low delivery of nanoparticles in acid pH. The Tukey test for the concentration of AgNO₃ showed a difference between the silver delivered by the 0.50 samples and the 0.25 samples. There was a high absorbance for the 0.50 sample media, indicating high silver nanoparticle delivery by these samples independently of the media used.

It can be noted that 0.50 samples delivered more silver, which is consistent with the possibility that this network...
Silver nanoparticle delivery (approx. 450 nm): as the EDS, in figure 10, average tensile curves are displayed in figure 10. Mechanical properties could be also related to the silver retention by the samples. In pH 4.0 medium, where the low degradation in pH 4.0 assumption is in agreement with the sample weight loss behaviour that could explain the limited leaching of the silver by the medium. Nonetheless, the PVA-Ag hydrogels of this work presented high mechanical strength and high elongation, suitable properties for application in wound coverage [36]. The PVA-Ag samples' strength, their swelling capacity and Ag delivery, their antimicrobial activity and barrier properties as well as their non-toxicity behaviour in all media make them suitable for application as burn dressings.

4. Conclusion
PVA samples and PVA-Ag hydrogel samples were studied in this work to assess their potential in wound dressing applications. The presence of silver in PVA gels causes slight microstructural differences, such as lower crystallinity. In addition, all samples were effective barriers to microbial penetration and the samples containing silver presented antimicrobial activity against *E. coli*, *S. aureus* and *C. albicans*. No sample was toxic to mouse fibroblasts.

Hydrogel dressings are usually applied hydrated. The samples' swelling capacity in saline solution and in PBS pH 7.4 is similar, independent of their composition. However, all samples presented low swelling in a solution with inflammatory pH levels. The low affinity of the samples to an acidic solution (pH = 4.0) led not only to low swelling capacity but also to low weight loss and low silver delivery. The samples in the pH 4.0 solution presented low levels of medium uptake which could explain the limited leaching of the silver by the medium.

The swelling capacity of the 0.50 samples was higher than that of 0.25 in all media. This fact, coupled with their degradation in the various media being similar or higher, their silver delivery after 4 days immersed in the various media being similar or higher, their samples' swelling capacity and Ag delivery, their non-toxicity behaviour in all media make them suitable for application as burn dressings.

Figure 9. Silver nanoparticle delivery (approx. 450 nm): (a) various sample compositions in the same media and (b) the same sample composition in the different media. *p < 0.05, sig. = 1. There is a significant difference between the pairs of levels indicated by arrows.

3.6. Mechanical properties
Silver in PVA hydrogels could have diminished the PVA crystallinity and also the samples' cross-linking degree. If so, it could alter the mechanical strength of these gels. The gels average tensile curves are displayed in figure 10r–c, as well as the EDS, in figure 10d. It can be noted that the 0.50 sample in PBS pH 7.4 seems to present a lower modulus and to fail under low stress compared with the other samples. The gel mechanical properties are shown in figure 11, where the secant modulus was calculated at a strain of 50%.

The failure strength values were above 1 MPa, and the secant modulus values were above 80 kPa for all tests performed. The failure strain was above 300% for all tests. The statistical analysis on the failure strength, table 1, included the two factors, concentration of AgNO₃ (%AgNO₃) and type of medium and their respective levels. The ANOVA analysis revealed that each factor (type of medium and concentration of silver nitrate) and their interaction are significant to the failure strength, p < 0.05. The Tukey test revealed that there is a difference between the mechanical strength of the samples swelled in pH 4.0 medium versus the other media for each composition. The strength at failure is higher in solution pH 4.0 for PVA and 0.25 samples, although for 0.50 samples the highest strength at failure was related to the saline solution. The Tukey test revealed that, regarding the amount of silver, there is a difference between the mechanical strength of the 0.50 samples and the others, figure 9, where the 0.50 samples are less mechanically resistant than the 0.25 samples. Assuming that the sites in PVA chains used for the formation of nanoparticles are the same that allow cross-link formation, more silver could mean a lower cross-linking degree. In addition, the nanoparticles affect the PVA crystallization and, consequently, the 0.50 samples would be expected to present a lower strength [38].

4. Conclusion

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Hydrogel dressings are usually applied hydrated. The samples' swelling capacity in saline solution and in PBS pH 7.4 is similar, independent of their composition. However, all samples presented low swelling in a solution with inflammatory pH levels. The low affinity of the samples to an acidic solution (pH = 4.0) led not only to low swelling capacity but also to low weight loss and low silver delivery. The samples in the pH 4.0 solution presented low levels of medium uptake which could explain the limited leaching of the silver by the medium.

The swelling capacity of the 0.50 samples was higher than that of 0.25 in all media. This fact, coupled with their degradation in the various media being similar or higher, their silver delivery after 4 days immersed in the various media being higher and their mechanical strength being lower or similar is consistent with the possibility that synthesis of silver nanoparticles has interfered with the formation of cross-links. A lower degree of cross-linking, and also the observed lower degree of crystallinity, could be responsible for the more elastic mechanical behaviour of the 0.50 samples.

The 0.25 and 0.50 samples presented suitable mechanical and swelling properties in all media, representative of potential conditions for a dressing applied to a burn site. Both samples could expand more in aqueous solution and more silver could be leached out to the media. In addition, the high weight loss after 4 days of immersion in saline and in PBS pH 7.4 could be related to the polymer degradation and to a minor effect of silver delivery. The low silver delivery to the acid pH medium could be related to the low swelling of the samples in pH 4.0, which could affect the silver delivery by the samples. This assumption is in agreement with the sample weight loss behaviour in pH 4.0 medium, where the low degradation in pH 4.0 could be also related to the silver retention by the samples.
Figure 10. Average tensile curves for the various sample compositions in (a) saline, (b) PBS pH 7.4 and (c) solution pH 4.0 after 1 day of immersion (n = 10) and (d) gels’ equilibrium swelling degree (ESD) values.

Figure 11. Mechanical properties of the gels: (a) secant modulus $E$ at 50% strain, (b) strain at failure $e$, (c) strength at failure $s_f$ for each sample composition in different media and (d) strength at failure in each medium for the various sample compositions.
were also non-toxic and presented antimicrobial activity, confirming that 0.25 concentration is sufficient to establish an antimicrobial effect. PVA hydrogels with silver nanoparticles at these concentrations can therefore be considered potential platforms for further development towards antibacterial dressings for wound healing.

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