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Modification of cellulose non-woven substrates for preparation of modern wound dressings

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Abstract

Different ways are presented of modifying cellulosic non-woven substrates, which can serve as potential wound dressings with satisfactory antimicrobial and hydrophilic properties. For safe attachment of silver particles without a measurable release from the used materials, a sol–gel derived process was used. Alkaline and oxygen plasma treatments were used to improve the hydrophilicity of the materials. Their efficiency was determined by measuring contact angles and water retention values. Scanning electron microscopy (SEM) was used for determination of sample morphology prior to and after treatment. The efficiency of silver attachment and activity was evaluated by *in vitro* release studies and antimicrobial tests. Atomic force microscopy (AFM) and SEM, combined with dynamic light scattering, were used for determination of silver particle size. Additionally, we evaluated the influence of treatment on technological parameters, important for application performance, i.e. mechanical properties and air permeability.

Keywords

Viscose non-woven, silver nanoparticle attachment, alkali treatment, oxygen plasma, antimicrobial activity, hydrophilicity

Acute and chronic wounds that result from burns or pressure sores usually excrete high amounts of exudate. Although exudation is part of the body's natural defense, exudates often hinder wound healing and represent an efficient medium for bacterial growth.[1] Special treatment is needed for such wounds, since unsuitable treatment could lead to serious infections or even the patient's death.[2] The key for successful wound treatment is to ensure the surrounding area of the wound, as well as the healthy part of the skin, is protected from infection by opportunistic bacteria.[3,4] Antimicrobial creams, foams, hydrogels, hydrocolloid and polymer films, and textile medical dressings are currently used to prevent such infections.[5,6]

Even though silver has been used as an antimicrobial agent for more than 2000 years, the proof of its potential cytotoxicity is much more recent.[7–10] The latter has led to several studies in recent years, resulting in new technological solutions for achieving a safe silver attachment or alternative means of antimicrobial activity.[11–14]

One of the most important factors that influence the efficiency of wound healing is the presence of moisture in the wound during the healing process.[15] Functional materials that enable this are hydrophilic medical dressings which, due to their characteristics (increased absorption of the exudate's hydrophilic components), are capable of absorbing the exudate and some other impurities from the wound.[2,16] Cellulosic materials and

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their derivatives are common host materials in medical dressings for the treatment of pressure sores and burns. In order to improve the hydrophilicity of cellulosic materials, standard chemical procedures are used in the textile industry, i.e. mercerization. The literature reports on different other types of cellulose material modifications, e.g. for films, which result in a different hydrophilic/hydrophobic character afterwards. Among these are heat treatment, combination of cellulose materials with other moieties (for example silane derivatives), and regeneration of cellulose derivatives to pure cellulose.[17–19] An alkaline treatment that leads to a better and faster binding of reagents, as well as a softer feel, is one of the commonly used methods.[20,21] Alkaline treatment causes changes in the structure of the cellulose material, which lead to its increased hydrophilicity, but simultaneously affect the material's mechanical properties. The procedure demands the use of large amounts of chemicals (sodium hydroxide) and therefore presents an environmental burden.

Thermodynamic non-equilibrium physical processes, such as gas plasma, present more ecologically acceptable alternatives to chemical processes.[22–24] The plasma technique enables homogenous surface treatment; its main advantage is maintaining technological parameters (mechanical properties) while improving the hydrophobic and hydrophilic properties of the material,[25–28] depending on the input gases used.

The purpose of this study was to explore different methods for modifying the host cellulosic material in order to achieve satisfactory antimicrobial activity and suitable hydrophilicity.

A suitable and safe antimicrobial effect was achieved by using the sol-gel process, with which commercially available nanoparticles of silver chloride were bound to the host material. The choice of a suitable method for the attachment of silver chloride nanoparticles was based on one of our previous studies, where we compared the influence of differently chemically modified sol–gel systems on the adsorption of these nanoparticles and their release from viscose non-woven.[29] A commercial sol–gel system was used because the same formulation was already effectively attached onto a cellulose based fabric,[30,31] and due to the fact that such materials have a higher probability to be used in commercial wound dressings in the near future. The greatest attention was paid to finding the optimum modification processes of the cellulosic non-woven in order to enhance its hydrophilic properties. To verify our hypotheses, the following methods were used: scanning electron microscopy (SEM), *in vitro* release studies with Franz diffusion cell, and atomic absorption spectroscopy (AAS), antimicrobial activity testing, powder contact angle method, water retention value, atomic force microscopy (AFM), dynamic light scattering (DLS), and mechanical properties determination.

Experimental

Materials

Non-woven fabric, composed of regenerated cellulose fibers (i.e. viscose), was purchased from KEMEX, The Netherlands. The surface mass of the fabrics was 175 g/m^2 and the thickness was 1.7 mm.

Preparation procedures

Sol–gel silver binding. Silver nanoparticles in silver chloride form, iSys Ag (CHT, Germany) in combination with iSys LTX (CHT, Germany) as the organic–inorganic binders, were used to achieve an antimicrobial effect of the used materials. Kollasol CDO (CHT, Germany) was used as a wetting agent. Kollasol is a hydrophilic silicone surface-active substance mixed with higher alcohols.

Firstly, the aqueous solution of iSys LTX (5 g/L), iSys Ag (5 g/L) and Kollasol (0.7 g/L) was prepared. Viscose non-woven samples were impregnated in solution with a bath ratio of 1:30 at room temperature for 1 h (amount of dissolved silver was therefore 1.26 mg). After treatment, the viscose was wrung-out on a foulard (Werner Mathis AG, Switzerland) with a pressure of 4 bar between rollers and their speed of rotation at 0.5 m/min, oven dried in a stretched state at 80°C, and additionally condensed for 1 min at 150°C (Werner Mathis Ag, Switzerland).

Alkaline treatment

53 g/L of NaOH solution with pH 13.1 was prepared from analytical grade NaOH pellets (purchased from Merck). Viscose non-woven was impregnated in a NaOH solution with a bath ratio of 1:10 at room temperature and allowed to swell for 5 min. In order to completely remove the residual NaOH after the treatment, the viscose fibrous substrate was washed with deionized water until a constant conductivity was achieved. The as-prepared viscose non-woven was oven dried in a stretched state at 70°C (Werner Mathis AG, Switzerland).

Oxygen plasma treatment

Samples were air-conditioned at 20°C and 65% RH for 24 h, prior to plasma treatment. The samples were treated with oxygen plasma; a spherical cylinder with an inner diameter of 36 cm and the height of 30 cm. Plasma was created with an inductively coupled RF (radio

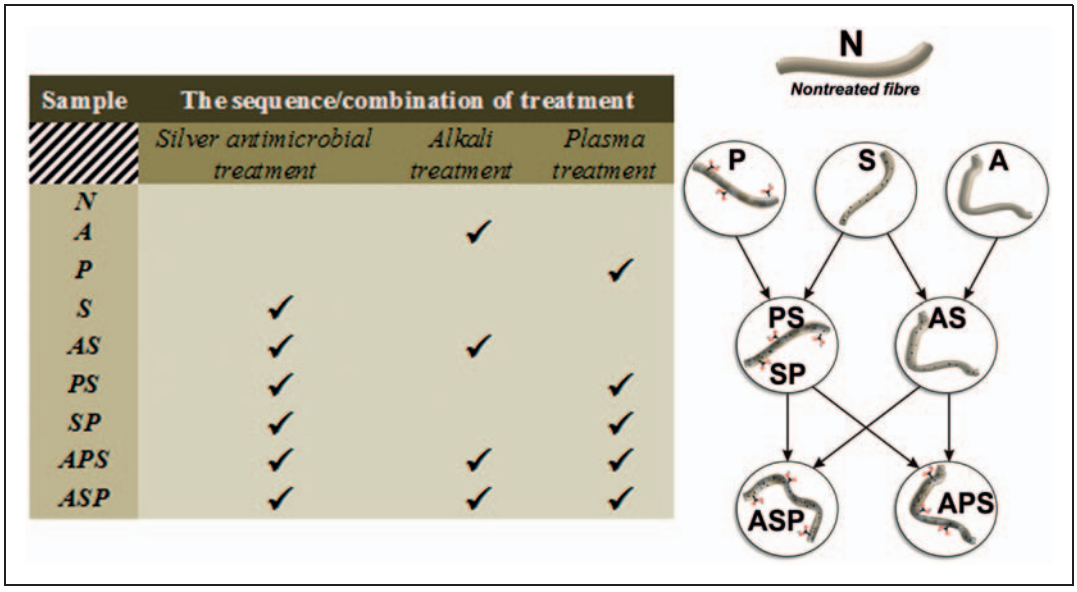

Figure 1. The sequence/combination of treatment procedures and corresponding sample notation. Sample notations are composed of the combined first letters of the used treatment procedures in the chronological order as performed (S – silver antimicrobial treatment, A – alkali treatment, P – plasma treatment and N – untreated sample).

frequency) generator, operating at a frequency of 27.12 MHz and output power of about 500 W. During the experiment, the pressure was fixed at 75 Pa. More detailed description of the treatment procedure can be found elsewhere.[32–34] The samples were exposed to oxygen gas for 10 min.

The sequence of treatment procedures and the sample notations are listed and schematically depicted in Figure 1.

Methods

Evaluation of surface topography and morphology

Scanning electron microscopy (SEM). In order to observe possible changes in material surface morphology after different treatment methods scanning electron microscopy was used. Prior to imaging, several single fibers were withdrawn from all samples and pressed on a double-sided adhesive carbon tape (SPI Supplies, USA). Micrographs were taken using a field emission scanning electron microscope (FE-SEM, Supra 35 VP, Carl Zeiss, Germany) operated at 1 keV. Although several magnifications were used to analyze the samples' morphology, only two are shown for each sample.

Additionally, SEM analysis was performed on as-purchased silver chloride particles (iSys Ag, CHT, Germany) for the means of their size determination. A droplet of the silver nanoparticle suspension was added directly onto the metal disks, used for SEM imaging. The metal disks were oven dried at 80°C for 1 h and imaged at the same conditions as written above.

During all preparation steps prior to imaging, extreme caution was put into the assurance of complete absence of light, which could alter the silver particle morphology and consequently also their size.

Determination of silver chloride concentration on fibrous non-wovens

For the determination of silver concentration on the viscose non-woven, the sample was weighted (approximately 0.5 g) prior to its digestion in 20 mL of HNO_3 by refluxing for 2 h. Solution was cooled and quantitatively transferred into 100 mL volumetric flask, and the volume was completed with distilled water. The digestions of each sample were performed in three parallels. Before the determination of the amount of silver on the atomic absorption spectrophotometer, the samples were filtered with Rotilabo®-syringe PTFE filters (Carl Roth, Germany) with pore size of 0.45 μm.

In vitro silver release studies

In vitro release studies were performed using static diffusion cells, according to Franz.[35] The receptor compartment was filled with milli-Q water (7.5 mL) and its temperature was fixed and maintained constant at 37°C during the experiment by stirring the thermostatic water around the Franz's diffusion cell. Due to a lack of standard method for evaluate the silver release from wound dressing, milli-Q water was chosen as the medium for testing; this allowed for a proper comparison of our silver release data with other published results.

Additionally, by using water as the medium, we avoided possible problems during silver detection with atomic absorption spectroscopy (AAS), which could arise due to the presence of sodium in all available simulated body fluids. The silver release from viscose fibrous samples with size 1 cm^2 was determined after 24 h as silver concentration in milli-Q solution using atomic absorption spectroscopy. All silver release studies were performed twice in parallel. In order to prepare the calibration line, the working standard solutions were prepared by dilution of a stock standard solution of silver (500 mg/L) in water with 1% (v/v) HNO_3. An atomic absorption spectrometer (Perkin–Elmer 1100B) equipped with an air-acetylene burner and silver hollow cathode lamp operating at 5 mA was used for determination of silver without background correction. The operating conditions were as follows: wavelength: 328.1 nm, band pass: 0.7 nm, flow rate of acetylene: 2.5 L/min, and the flow-rate of air: 8 L/min. All measurements on AAS were performed thrice in parallel.

Silver nanoparticle size determination

Atomic force microscopy (AFM). AFM was used for size determination and possible morphology evaluation of silver nanoparticles. A droplet of the silver nanoparticle suspension was added directly onto round shaped V-1 Muscovite mica (SPI Supplies, USA) of 1 cm in diameter and dried under high grade nitrogen (5.0). The as-prepared samples were attached onto round shaped metal discs and mounted on the micrometer positioning stage of the Multimode V AFM (Veeco Instruments/Bruker). Tapping Mode AFM mode was used to simultaneously acquire height, phase, and amplitude images. Silicon AFM tips doped with antimony (FESPA tapping mode tips, Bruker) with a nominal spring constant of $k = 2.8$ N/m were used for imaging purposes for all samples. Images of size $2.5 \times 2.5\,\mu m^2$ were recorded with a resolution of 2048×2048 pixels. All images were processed and the corresponding roughness was calculated using WSxM software.[36]

Dynamic light scattering (DLS). The size of silver nanoparticles was additionally measured by DLS (dynamic laser scattering) using a Malvern Instrument Zetasizer Nano ZS laser scattering system with a 632 nm laser source. The intensity of scattered light was detected at 173° to minimize the effect of multiple scattering. The investigated suspensions were put into 12 mm diameter polystyrene cuvettes, the minimum sample volume required for the experiment was 1 mL. The analysis was run at 25°C in three parallels of 10–100 repetitions (the number was defined according to the overall quality of the sample, which was automatically evaluated each time a fresh cuvette had been inserted into the system). All the results are reported as average values with standard errors bars along with the appropriate size distributions.

Determination of antimicrobial activity

The antimicrobial activity of untreated and modified viscose samples was evaluated using the standard method AATCC 100 – 1999.[37] The two gram-negative bacteria, i.e. *Escherichia coli* and *Pseudomonas aeruginosa*, and the two gram-positive bacteria, i.e. *Staphylococcus aureus* and *Enterococcus* faecalis, were used as test species. These challenging bacteria were chosen, since they are the most frequently isolated micro-organisms from skin wounds in humans.[38–40]

Circular pieces (4.8 cm in diameter) of viscose fibrous samples were put into a 70 mL container and inoculated with 1.0 mL of inoculums containing $1–2 \times 10^5$ colony forming units of bacteria. After incubation at 37°C for 24 h, the bacteria were eluted from the samples by shaking in 50 mL of sterilized water for 1 min. 0.1 mL of these suspensions (2×10^2 CFU) and diluted suspensions with physiological solution (2×10^1 CFU) were plated on trypticase soy agar (TSA) and incubated at 37°C for 24 h. The number of bacteria was counted and used to determine the reduction of bacteria, R, as:

$$R = \frac{B - A}{B} \times 100[\%] \quad (1)$$

where A is the number of bacteria recovered from the inoculated piece of the viscose sample in the plastic container incubated over the desired contact period (24 h) and B is the number of bacteria recovered from the inoculated piece of the viscose sample in the container immediately after inoculation (at "0" contact time).

Hydrophilic/hydrophobic properties determination

The powder contact angle method. The hydrophilic/hydrophobic character of samples, i.e. their wetting ability with water as a test liquid, was studied by contact angle measurements. The samples were cut into circular pieces, 2.5 cm in diameter, and suspended on a sample holder of a Krüss K12 processor Tensiometer. Prior to measurement, the container with the liquid (*n*-heptane; water) was raised until the sample edge touched the liquid surface.

The change in sample mass (m), as a function of time (t) during the water adsorption phase, was monitored. The initial slope of the function $m^2 = f(t)$ is known as the capillary velocity, from which the contact angle

between the solid (polymer sample) and the water was calculated using a modified Washburn equation:[41]

$$\cos\theta = \frac{m^2}{t} \cdot \frac{\eta}{\rho^2 \cdot \gamma \cdot c} \quad (2)$$

where θ is the contact angle between the solid and liquid phases, m^2/t is the capillary velocity, η is the liquid viscosity, ρ is the liquid density, γ is the surface tension of the liquid, and c is a material constant. For a more detailed description of this experimental method, see Peršin et al.,[42] and also Zemljič et al.[32,33]

Determination of water retention values. The water retention values of porous polymer material have been determined according to standard DIN 53 814. This method determines the quantity of water that the sample can absorb and retain under controlled conditions. This property is expressed as a ratio between the mass of a sample after soaking (2 hours in non-ionic wetting agent) and centrifuging (20 min; 8000–10 000 $m^2 s^{-1}$; 3000 rpm), and the mass of an absolute dry sample. The sample was dried in an oven (Kambič, Slovenia) at 105°C for 4 h and cooled in a desiccator before weighing. All measurements were performed in four parallels, accordingly all data is depicted as average values with standard errors.

Determination of mechanical properties

The determination of tensile properties. The tensile properties of non-woven fabrics were determined according to standard SIST ISO 13934-1. The maximum force and elongation at maximum force, i.e. at the moment of sample rupture, were determined using the strip method.

The measurements were performed on samples with a size of 50×350 mm; dynamometer Textechno Statigraph M was used. Clamped samples were stretched until breaking point, while the software controlled dynamometer automatically recorded the number of measurements, the minimum, mean, and maximum breaking force and elongation (N), the elongation at break (%), standard deviation, and coefficient of variation. Five measurements were performed for each sample under standard conditions (20 ± 2°C, 65 ± 2% RH). Following test parameters were used: gauge length 200 mm, clamping with pretension 200 cN, and rate of extension 100 mm/min.

Air permeability determination. Air permeability of non-woven fabrics was determined according to DIN 53 887 with the Karl Schroeder apparatus. Clamping surface of the used specimen was 20 cm^2. The three-stage air-flow meter was calibrated at 20°C and 1013 mbar, while the temperature in the room during the measurement was 21°C and the barometric pressure 1023 mb. 1 mbar negative pressure was created by air, trapped in the specimen and remained constant during the measurement. The reference air permeability was calculated using the following equation:

$$V_N = f \cdot V_G \cdot \sqrt{\frac{P_U \cdot T_N}{P_N \cdot T_U}} \quad (3)$$

where V_N, P_N, and T_N are the reference air permeability, pressure, and temperature, respectively, while V_G is the air permeability and P_U and T_U are room air pressure and temperature. f is a factor for calculation, corresponding to a defined surface area.

Results and discussion

Surface topography and morphology

In Figure 2, longitudinal views of single fibers are shown, as imaged by SEM using a low magnification. It is clear that no site-specific changes can be observed on any sample surface. All surface changes as a result of the used treatments seem to be more or less evenly distributed over the whole fiber surface. Several fibers from different parts of the treated non-woven samples were examined with SEM and mentionable discrepancies could not be found (additional images not shown).

Figure 3, on the other hand, shows samples imaged with a higher magnification, where the differences between the treatment procedures are more pronounced. The extent of change, resulting from different fiber treatment, varies from no morphological changes at all to highly altered surface features. The most observable changes appear for samples P, SP, and ASP, where plasma treatment was the final procedure (Figure 3). This is due to the fact that plasma treatment does not result only in surface functionalization, but it also affects the surface due to etching, which accompanies the plasma treatment procedure. Among these samples, sample P exhibits holes on the surface with a diameter of a couple hundred nm. The other two samples, where plasma treatment was the final preparation step (SP and ASP), experienced less rigorous changes.

Samples where the final procedure was the attachment of silver chloride nanoparticles via the sol–gel procedure (S, AS, PS, APS) exhibit a smoother surface compared to samples where the treatment was not applied. The same is also true for samples where plasma treatment was used prior to the attachment (samples PS and APS). In these samples, the plasma-induced changes are less pronounced when compared to the samples where the silver nanoparticle attachment was not applied. This can be observed especially on the

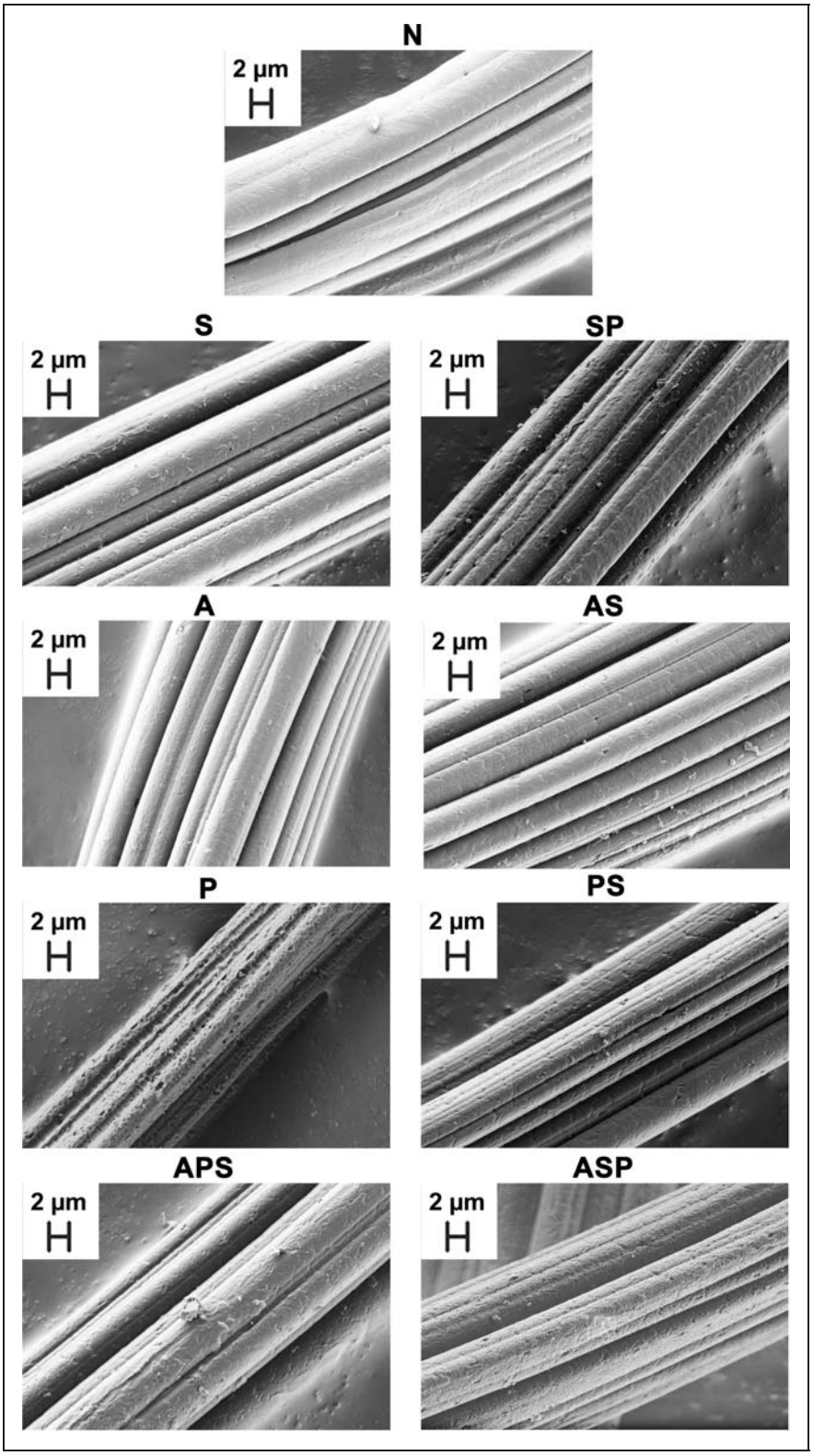

Figure 2. SEM micrographs of the untreated and differently treated viscose non-woven with low magnification.

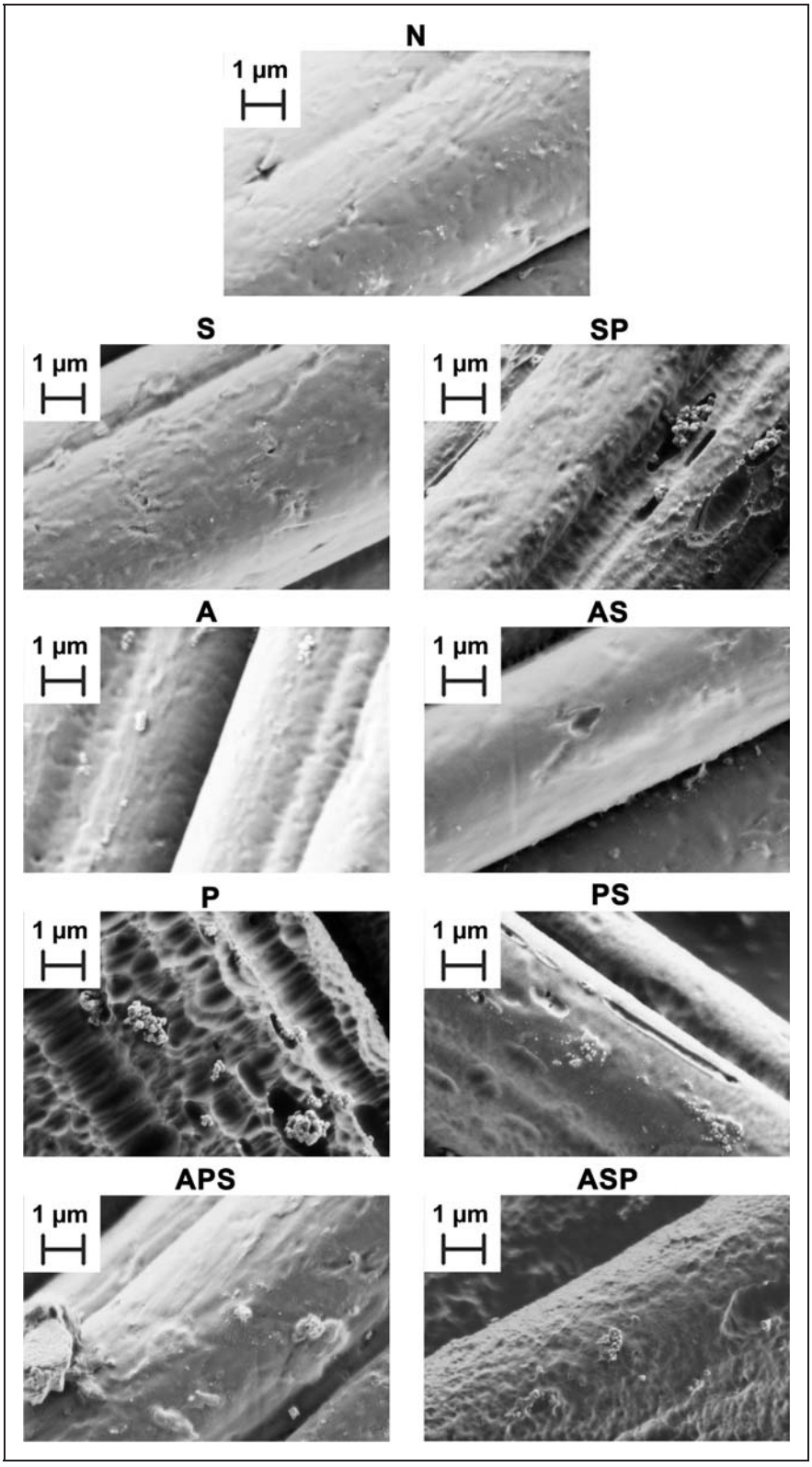

Figure 3. SEM micrographs of the untreated and differently treated viscose nonwoven with high magnification.

edges of holes and surface bumps, where these appear to be more rounded. Regions where different features (like holes, bumps and channels) could be observed prior to treatment are now for the most part covered with thin surface films. This effect results in an overall decreased surface roughness when compared to the untreated sample (in the case of S and AS) or the samples P and AP (in the case of samples PS and APS). The latter is a result of the used sol-gel procedure for the attachment of silver particles (the surface of fibers is first covered with a thin layer of the sol–gel solution, which is evenly distributed over the surface; this is then transformed to the gel state and the result is a smoother surface, as observed in Figures 2 and 3). The least pronounced effect on the surface is due to treatment with sodium hydroxide, which exhibits no observable effect on the surface of treated fibers. The treatment with sodium hydroxide results in a reaction with cellulose, forming Na-cellulose. Silver particles could not be observed on the surface of the fibers, probably due to their incorporation inside the surface sol-gel films. Additional EDS and FTIR analyses were performed to add to the overall interoperation of structural and morphological changes. Application of EDS was not successful due to the experimentally proven threshold for observation of silver on the surface, which was proven to be above 600ppm (results not shown); the incorporated amount in our samples is far less, while FTIR could not deliver explicit proof about any surface changes due to the complex nature of the cellulose-based samples and the absence of observable interactions between silver and cellulose.

Determination of surface silver concentration and in vitro silver release evaluation

Determination of the amount of the incorporated silver showed that all silver-treated samples (S, PS, SP, AS, ASP, and APS) exhibited a concentration above the threshold, necessary for the antimicrobial effect (0.060 mg/g).[43] The silver concentration for these samples was between 0.077 and 0.141 mg/g.

In vitro silver release studies

The cytotoxicity of silver is a relatively new finding that has recently boosted the search for alternative methods of achieving an antimicrobial effect, i.e. by amino acid-based antimicrobial agents,[44–48] or finding ways to safely attach silver in wound care products.[2,13,49] Regarding the latter, it is particularly important to ensure that after long-term use of the product, the silver is not released to an extent, which could lead to a cytotoxic effect. Burd et al. have explored the limit value of silver release from commercial medical dressings at which an antimicrobial effect was still observed; they showed that if less than 2 mg/L of silver is released within 24 h, no significant cytotoxic effect is noticed.[10] This value was taken into account during preparation of silver-coated fibrous substrates in such a way that its release would not exceed the limit value, and thereby a safe antimicrobial effect is achieved. In order to safely bind silver nanoparticles on cellulose fibrous material, sol–gel process was used. This proved to be extremely efficient since the silver release from treated samples (as determined with Franz diffusion cells) and the measurements of the silver concentration in the solution after the release showed that, regardless of the sequence/combination of the used sample treatment processes, the concentration of silver release was very low. All measurement data showed values of silver concentrations below the detection limit (LOD), which is less than 0.05 mg/L.

The same study also established that silver cytotoxicity is not only dependent on the concentration of the bound silver in the dressing, but on other parameters too, especially the method of silver binding in the dressing and its affinity to moisture.[10] Medical dressings with greater hydrophilicity thus release less silver into the wound and are also less toxic to human cells since the bacterial kill zone in such instances is transferred to the interior of the dressing. On this basis, it is possible to conclude that a lower silver release was achieved in samples that had been alkaline and plasma treated in order to increase hydrophilicity, as opposed to those without pretreatment prior to sol–gel coating. To be completely safe in regard to silver's potential cytotoxic effect, further steps were taken. Attention was also paid to the size of the attached silver nanoparticles, since it is known that the skin presents a rather big barrier for larger particles (the limit for transition through sebaceous glands is 120 nm). Used commercial nanoparticles offer themselves as a suitable choice since their size, ranging from 100 nm to 500 nm, had been reported in literature.[30] Several methods were used to confirm the size of silver nanoparticles in a suspension iSys Ag. Size values of silver particles were determined using SEM, AFM, and DLS. Images obtained with both mentioned microscopy techniques, together with the particle size distribution, are shown in Figure 4. The results obtained with different methods match and can also be confirmed with data from the published literature; therefore, even if silver chloride particles would be released from our materials, they could not penetrate into the body.

The silver release and silver measurement methods used can be confirmed as suitable for evaluating the safety of medical dressings with attached silver; however, it does not provide an answer to a more scientific question in present study; namely, what is the effect of treatments for enhancement of hydrophilicity on the

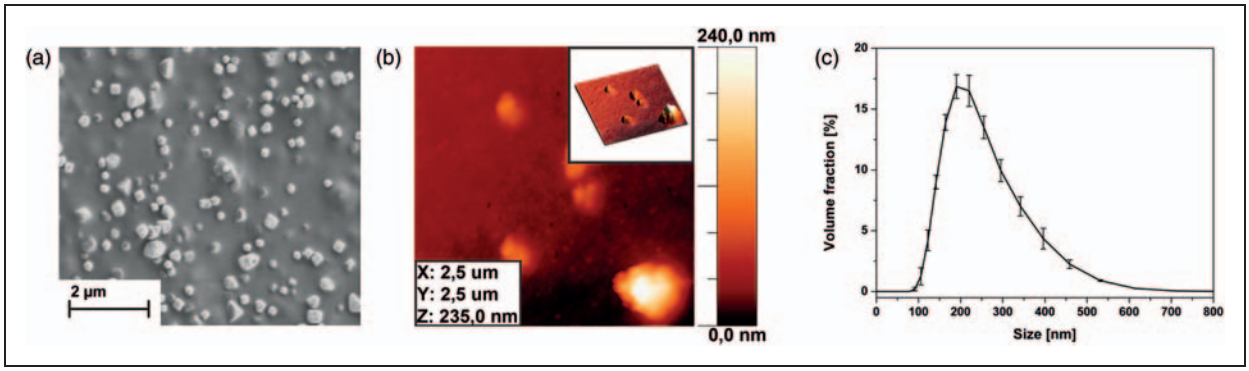

Figure 4. Silver particle size determination with: (a) SEM, (b) AFM and (c) DLS.

Table 1. Reduction R (%) of the bacteria, present the most in the infected wound, for untreated and differently treated viscose samples

	Reduction, *R* (%)			
Sample	*Staphylococcus aureus* (G+)	*Escherichia coli* (G−)	*Enterococcus faecalis* (G+)	*Pseudomonas aeruginosa (G−)*
N	No reduction	No reduction	77	No reduction
A	99	No reduction	No reduction	No reduction
P	>99	No reduction	>99	No reduction
S	>99	>99	>99	95
AS	98	>99	>99	>99
PS	90	>99	>99	>99
SP	>99	>99	>99	98
APS	>99	>99	98	>99
ASP	>99	99	>99	74

release of silver from fibrous viscose non-woven. This aspect is further elaborated in the following sections of the article.

Reduction of bacteria

The results of antimicrobial activity for the non-treated and treated viscose fibrous samples are presented in Table 1.

As expected, no reduction in the bacteria *S. aureus*, *E. coli*, and *P. aeruginosa* was found for the untreated viscose sample, while the reduction of *E. faecalis* was 77%. The alkaline-treated sample of viscose non-woven also does not show antimicrobial activity against *E. coli, E. faecalis*, and *P. aeruginosa,* whereas the reduction of *S. aureus* was 99%. Plasma treatment successfully reduced the number of *S. aureus* and *E. faecalis* bacteria, while an increased antimicrobial effect on *E. coli* and *P. aeruginosa* was not observed.

Since no antimicrobial effect is to be expected after the oxygen plasma treatment and after the alkaline treatment where the alkali was rinsed from the material, the obtained results could be seen as an error in the chosen antimicrobial testing method. According to the standard, after the bacterial suspension has been incubated with the sample, bacteria are excluded from the material by adding 100 ml of distilled water and shaking for 1 min. Thereby discrepancies in the number of bacteria released into the water from more or less hydrophilic samples could occur. Despite the hydrophobic properties of the bacterial cell membrane,[50] the result obtained with our antimicrobial test indicates the adsorption of bacteria to the viscose non-woven, since the plasma-treated sample that is the most hydrophilic, showed a reduction in two out of four bacterial strains. In the case of the alkaline-treated and the reference samples, the reduction was shown in only one bacterial strain.

There is certainly no doubt about the antimicrobial effect of all the silver-treated samples. Silver, which is known to effectively destroy numerous microorganisms (broad-spectrum effect), also destroyed the *S. aureus* bacteria in our study. These bacteria can cause several human diseases, from previously mentioned skin infections (impetigo or scabbing, postural rash, boils, carbuncles, skin abscesses etc.) to life-threatening diseases

such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia and septicemia. *S. aureus* is one of the five most common pathogens in hospital infections and often causes postoperative wound infections.[51] All the silver-treated dressings also inhibited the *E. coli* bacteria, which is best known as intestinal bacteria, but can cause infection in almost any organ or anywhere in its host due to its opportunistic character; namely, it takes advantage of other bacterial infections in the body for its own growth. All infections can lead to bacteremia and later to sepsis and septic shock.[52] All samples of viscose non-woven, except the alkaline-treated sample A, successfully reduced *E. faecalis* bacteria, which are connected to endocarditis and bacteremia, urinary tract, blood circulation and postoperative wound infections.[53,54] In addition, they are also resistant to many types of antibiotics.[55] Another good characteristic of silver-treated medical dressings is that they destroy *P. aeruginosa* bacteria, which play a key role in the infection of burns and open wounds.[56] The sol–gel system without silver may also contribute to a lesser extent to the antimicrobial activity, as some studies represent the silanols as a novel class of antimicrobial agents.[57–59]

With regard to the results of our and other studies,[2,6,11,13,29,40,49] and because of silver's great efficiency, we believe that finding better methods for binding of silver, while simultaneously ensuring suitable hydrophilicity, is a more favorable approach than completely denouncing silver due to its possible cytotoxicity. This is possible by using superhydrophilic materials in combination with silver binding techniques using the sol-gel process.

Water contact angles and water retention values

Hydrophilicity is an important characteristic of medical dressings, since it enables absorption of exudates, bacteria and other impurities from a wound. The hydrophilic properties of the untreated and differently treated samples are presented as water contact angles and water retention values (shown in Figure 5). At least ten measurements were performed for each sample in order to obtain appropriate statistical significance. The water retention experiments were carried out in quadruplicate, accordingly the results are presented as averages with standard errors.

It is clear from Figure 5 that all the treatment combinations improved the hydrophilic properties of viscose non-woven; however, each treatment contributed differently to the reduction in the contact angle between the material and water (samples A, P and S). The combination and sequence of treatments also had different effects on the contact angle; contribution of an individual treatment in combination with others is not the same as the result of an individual treatment.

Only the alkaline-treated sample A showed a 35% reduction in the contact angle compared to the reference sample. The biggest reduction in the contact angle is noticeable in the plasma treatment (sample P) and amounts to 67%. Hydrophilic properties were the least influenced by the silver antimicrobial treatment (sample S) where silver was incorporated to viscose non-woven

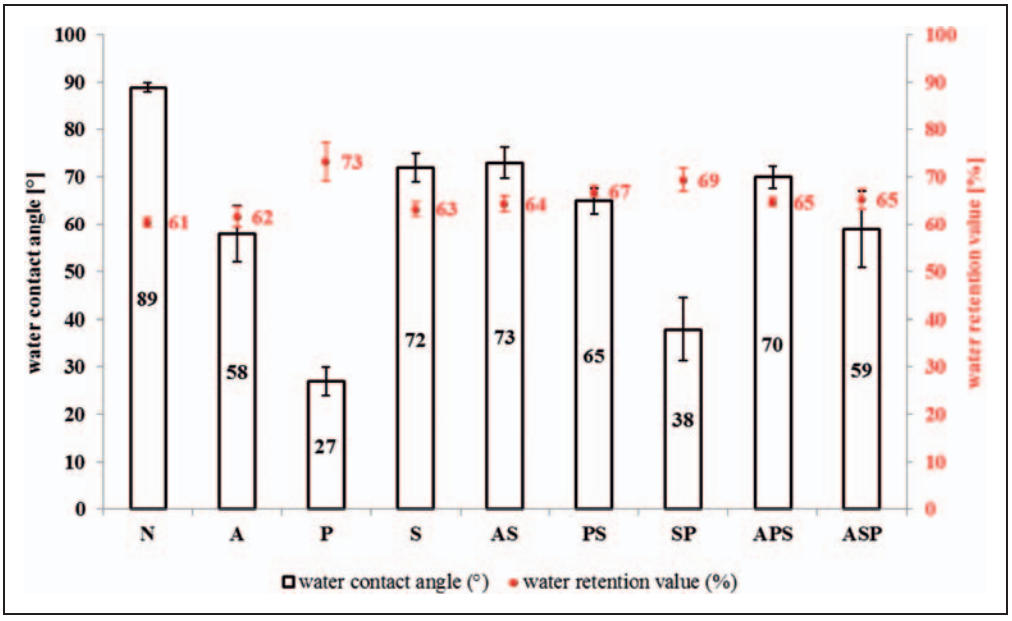

Figure 5. The hydrophilic properties for the untreated and differently treated samples of cellulose non-woven.

using the sol-gel process; the contact angle was reduced by 19%.

To ensure wound healing a medical dressing has to have an excellent hydrophilic as well as antimicrobial activity; this is why our research also focused on finding the right combination of the mentioned treatments.

As shown in Figure 5, the sample AS, which first underwent alkaline and then antimicrobial treatment, does not exhibit such good hydrophilic properties as it did after the alkaline treatment alone (sample A). Compared to the reference sample the contact angle was reduced by 18%, which is practically the same as in the sample S. It can therefore be concluded that the sol–gel process undid the effect of the alkaline treatment, which expanded the structure of cellulose fibers and thereby the access to the amorphous regions of the fibres interior.[60] A probable cause of this is the overlaying of viscose fibers with the sol-gel coating, which prevented water penetrating through the surface of the fibers and into their interior. Similar results can be seen when comparing the samples P and PS, which were plasma treated in order to achieve hydrophilicity. Applying oxygen plasma with a high density of neutral oxygen atoms that react with the surface of the viscose material, results in two different effects. Namely, in formation of new oxygen-rich functional groups (among other -COOH groups), and in etching, which enlarges the openings on the fiber surface.[22,23] This can be observed in Figures 2 and 3 (especially for sample P). Combined effect of the etched surface and the presence of hydrophilic functional groups on the surface is an excellent hydrophilicity of the modified material. A subsequent treatment of such material with the sol-gel system would most likely, similar to the sample AS, render the fiber surface more waterproof. Also, the combination of both techniques to increase the material's hydrophilicity and subsequent treatment using silver (sample APS) did not significantly reduce the contact angle. Different results were obtained for the sample SP that was first treated with the sol-gel system and subsequently plasma-treated. The results of contact angle measurements and the SEM micrograph analysis showed that the oxygen plasma treatment lead to changes of the previously sol-gel coated fibers. Compared to the previously untreated material, sample P, the changes were less significant. The reason for this is most likely in the chemical composition of the iSys LTX binder, whose components are of organic–inorganic origin.

Since it was established that a satisfactory reduction in the contact angle (57%) can be achieved with a combination of treatments, i.e. first a sol–gel coating, followed by a plasma treatment (sample SP), the same or improved results were expected in the case of the sample ASP. However, the alkaline pretreatment of the sample did not improve the hydrophilic properties, since the contact angle of the finally treated sample was reduced by only 34%. The cause of the increased contact angle – as compared to the sample SP – could lie in the greater concentration of the sol–gel system that was adsorbed to the previously alkaline-treated material. Preliminary studies have shown an up to threefold increase in the concentration of the adsorbed sol–gel reaction mixture; however, additional studies are needed to determine a statistically significant correlation between the treatment effect and the mentioned sol–gel adsorption. The alkaline treatment, which is known to effect the intermolecular hydrogen bonds between cellulose chains, is expected to loosen the fibers' structure and open its pores.[60,61] The latter could have had an effect on the greater sol-gel precursor absorption that plasma could not modify as successfully as the untreated viscose surface. A similar effect was already noticed by comparing the samples P and SP.

The comparison of the water retention results, shown in Figure 5, mostly confirms the effect of various treatments on the contact angles, as described above. The water retention value for the sample A stands out, since it could be expected to be slightly by higher, given the results of the contact angle measurements. The highest percentage of retained water was obtained with sample P. The difference in the degree of retained water and contact angle values, resulting from alkaline and plasma treatments, obviously stems from the fact that the latter has a more pronounced effect and creates hydrophilic groups not only on the material's surface but also in its interior. In order to achieve greater hydrophilicity with alkaline treatment, a higher alkali concentration should be used. The high drying temperature that followed the alkaline treatment (70°C) could have had an effect on the closing of the pores of the cellulosic fibers that were initially enlarged with alkaline swelling. The influence of high temperature on cellulose was also reported by Mohan et al.,[17] who found that heat treatment increases surface hydrophobicity and reduces swelling capacity of cellulose model thin films.

No significant improvement in water retention was observed in any of the samples with applied antimicrobial coatings since the sol–gel system covered the fiber surface and blocked its pores; only plasma slightly modified their hydrophilicity (sample SP).

Mechanical properties

The tensile properties of differently treated viscose samples were determined as breaking force and elongation. These parameters are particularly important since this study aims to result in development of medical dressings that must not tear during placement or removal

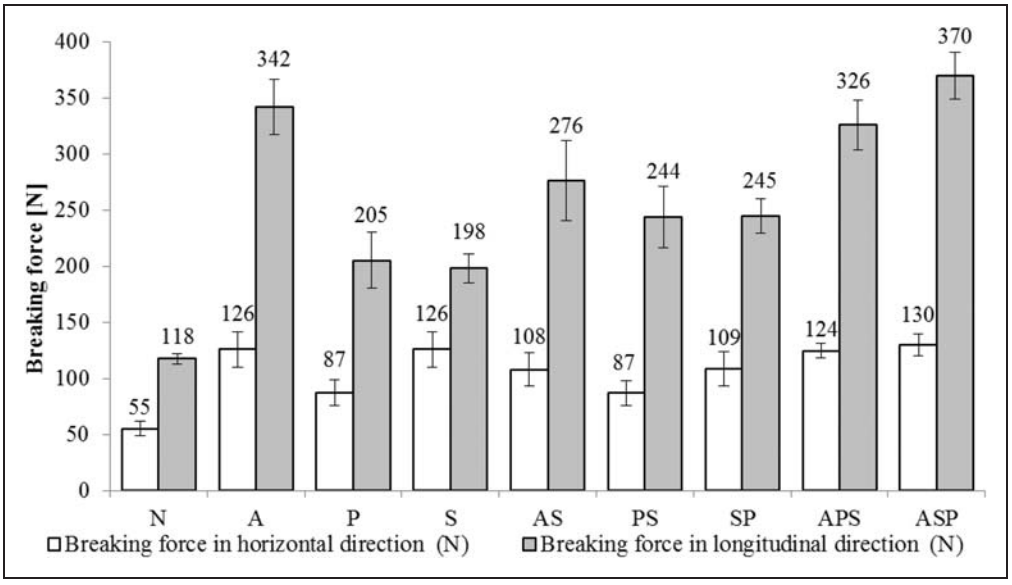

Figure 6. The breaking force in horizontal and longitudinal direction of untreated and treated viscose samples.

from the wound; not only does this cause additional pain for the patient, but also further damages the already sensitive wound and surrounding tissue. The breaking force for differently treated viscose samples, measured in horizontal and longitudinal directions, is presented in Figure 6.

In Figure 7, the elongation in longitudinal and horizontal directions is presented for all samples, depending on the treatment used.

Comparison of the untreated sample with the modified ones shows that all treatment procedures increased the breaking force of the material. Among them the sample ASP, which underwent first the alkaline, then the antimicrobial and in the end the plasma treatment, showed the highest breaking force in the horizontal and the longitudinal direction. The breaking force increased to the same extent for samples A and APS, followed by the sample AS. All these samples underwent alkaline treatment which most likely tangled together the viscose fibers in the non-woven fabric during the intensive rinsing of the alkali solution, rather than damaged the smooth surface of the fibers. This is why during breaking force testing increased friction between the rough surfaces of individual fibers appears, and it becomes more difficult to separate the tangled fibers, which consequently leads to increased breaking force. Subsequent application of the sol–gel system (sample AS, Figures 2 and 3) most likely fills in the rough surface of the fibers and softens the fibers due to the addition of a wetting agent. This also improves the smoothness of the fibers.

Based on the results, we can conclude that etching of the fiber surface by plasma treatment (sample P) increased the friction between the fibers during the breaking force testing, even though this effect is not as significant as in the case of the alkaline treated fibres. The plasma treatment that follows the alkaline treatment additionally etches the surface, which fails to become smooth even after the sol–gel treatment (sample APS, Figures 2 and 3), unlike in the case of the sample AS. If the plasma treatment is used after the application of sol–gel coating, it increases the roughness of the sample surface and thereby increases friction between fibers. It can also be concluded that the sol–gel coated fibers are not completely smooth since the breaking force value of the sample S has increased almost twofold in comparison to the untreated sample.

As was expected due to the breaking force measurement results all the treatment methods reduced the elongation at break of the viscose non-woven (Figure 7); however, the differences are not as pronounced. The plasma treatment had the biggest effect on the reduction of the elongation at break of the viscose non-woven, as well as the greatest morphological effect on the surface of the fibers (Figures 2 and 3). The antimicrobial treatment affected the elongation at break the least, among all treatments.

The viscose non-woven that was chosen as the input material has very good tensile properties on its own since it is used in the production of medical dressings. The most important finding from the performed set of tensile testing is that none of the treatments worsened these properties. Overall, all the treatment combinations improved the breaking force. The alkaline treatment had the greatest effect in this regard, therefore, the samples AS, APS and ASP are most suitable for a potential medical dressing that includes antimicrobial

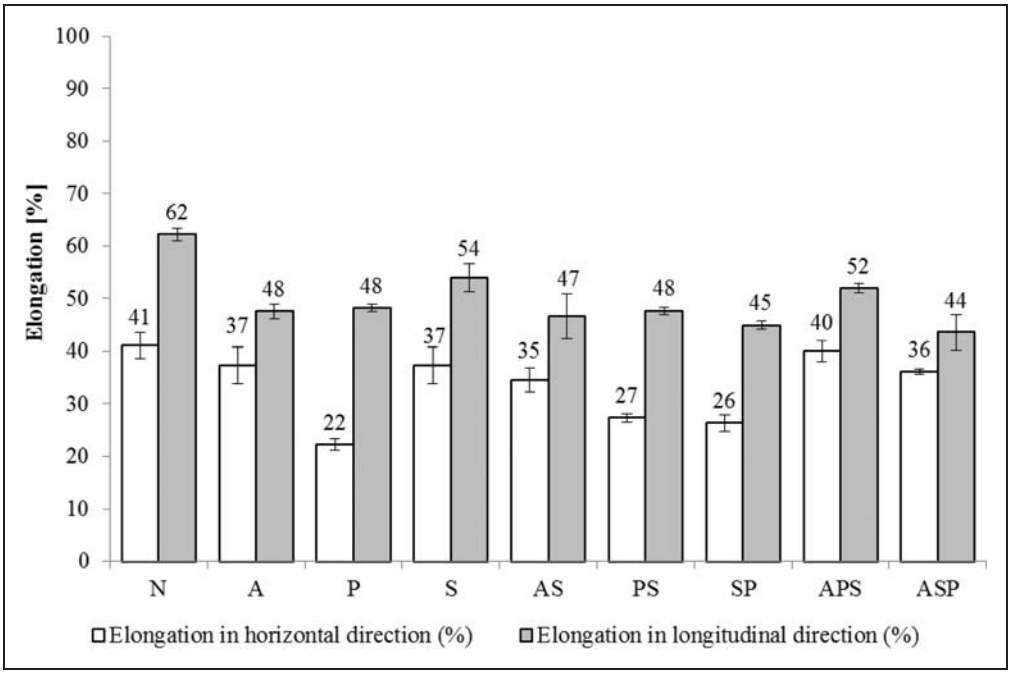

Figure 7. The elongation at break in horizontal and longitudinal direction of untreated and treated viscose samples.

treatment. Among these samples, the most pronounced reduction in the elongation at break was observed with the sample APS; from the viewpoint of tensile properties, the sequence of treatment used with this sample seems to be the most promising.

Air permeability

Keeping the wound's surface moist is the fundamental principle of open wound treatment.[1] Besides hydrophilicity, air permeability also affects the moisture of the wound, since it significantly contributes to an optimum 'breathing' of the wound and the evaporation of fluids from the wound. A medical dressing with excessive air permeability could dry out the wound, leading to cells dying, in growing of the dressing into the wound and generally have an unfavorable effect on the wound's healing rate.[62] Figure 8 shows the air permeability values of untreated and differently treated samples of viscose non-woven.

Sample A permeated the least air in comparison to the reference sample; air permeability was reduced by 62%. The reason for this lies most likely not only in the effect of the alkaline treatment on the cellulosic material, which caused fiber swelling, but in the entire alkaline treatment process. Fibers in the non-woven fabric got tangled during intensive rinsing of alkali swelling solution and thereby reduced the permeability of air due to the closing of fiber-to-fiber interstices. This assumption is confirmed by comparing all the samples, whereby it is clear that all the samples that included alkaline treatment have lower air permeability than the reference sample.

The air permeability of the material was increased the most by the plasma treatment (sample P). This phenomenon is most likely due to in the etching of the fiber surface. The plasma may have even burned away thinner fibers and thereby decreased the density of a fiber web. Comparison of samples S, AS and PS also indicates that plasma has a permanent effect on air permeability since it was maintained even after the sol–gel treatment. Comparison of samples APS and ASP with the sample A revealed that the plasma treatment did not significantly increase the air permeability of the previously alkaline-treated samples, where rinsing caused a permanent entanglement of the fibers.

Treating material with the sol–gel process had the smallest effect on air permeability. The air permeability was only minimally reduced, which is speculated to result from the addition of Kollasol wetting agent and a slight thickening of the fibers due to a layer of a binder and nanoparticles. But nonetheless, applied sol–gel coatings preferably embed individual fibers only with little or no surplus silane coatings filling out the inter-fiber voids in the fibrous network.

Since hydrophilicity and consequently their ability to absorb exudates, bacteria, and other impurities from a wound, while also keeping a wound's surface moist,[2] are important characteristics of medical dressings, we believe that the reduction in air permeability is an advantageous aspect that contributes to a faster wound healing; in this regard, the samples AS, APS

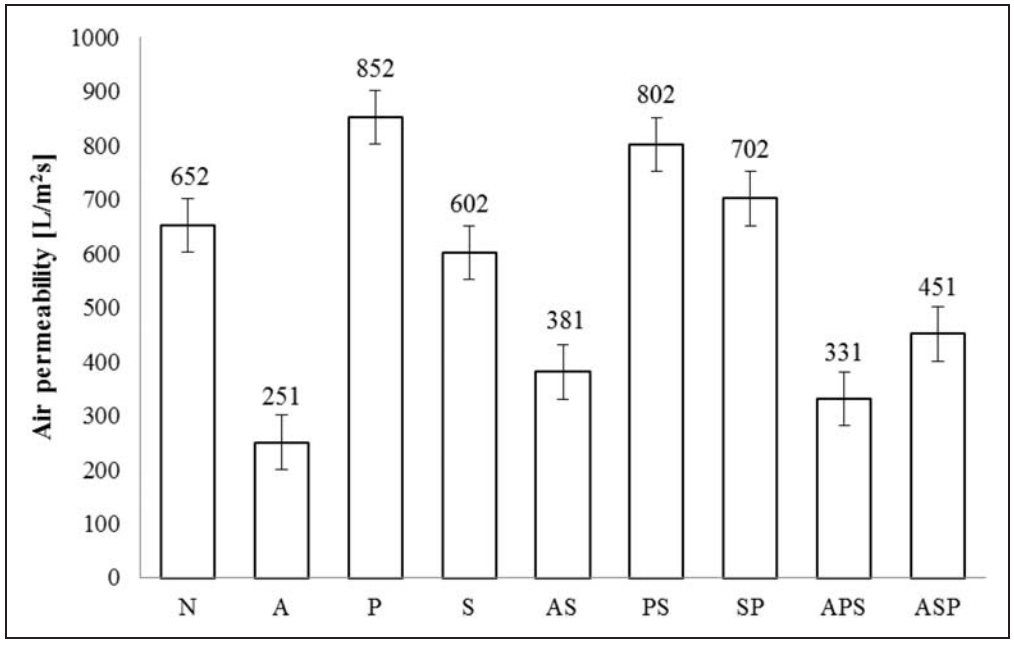

Figure 8. Air permeability for untreated and differently treated viscose samples.

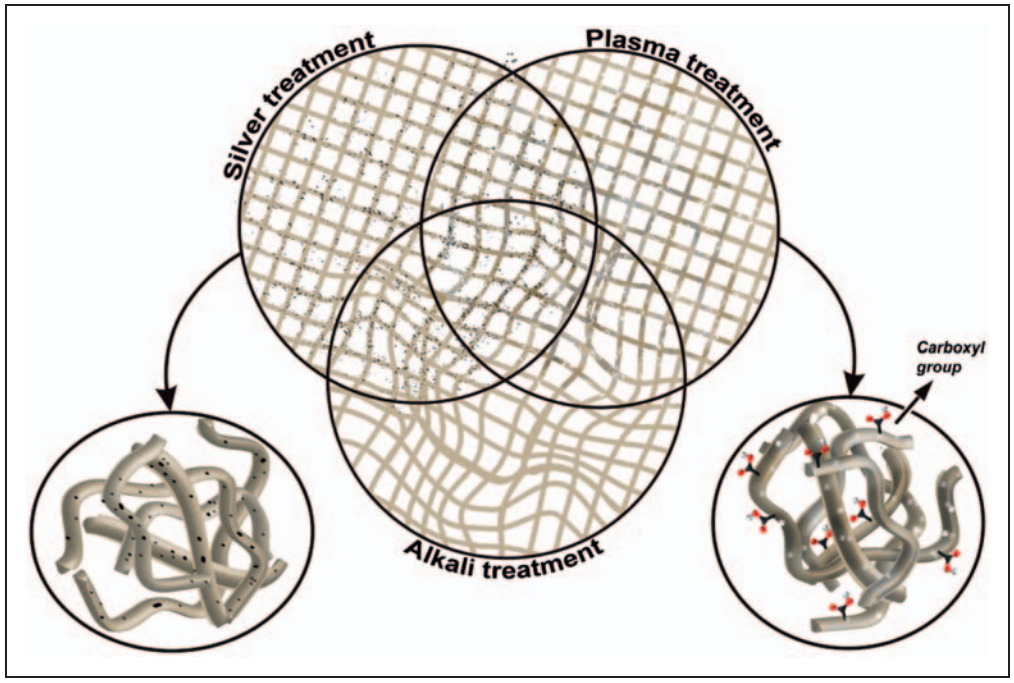

Figure 9. Scheme of the prepared samples by using different treatments and their combinations.

and ASP present themselves as best candidates for potential medical dressings.

Conclusion

In the present study, effect of different modifications, performed on the host cellulosic material to achieve satisfactory antimicrobial and absorbency properties on the basis of increased hydrophilicity, was explored (Figure 9).

The surface topography and morphology studies (SEM) showed that plasma treatment has the greatest effect on the morphological changes of the fibers. XPS studies of polymer surfaces, treated by oxygen plasma

can be found elsewhere.[63] *In vitro* silver release studies and antimicrobial tests showed, that a safe and suitable antimicrobial effect of a potential medical dressing can be achieved by binding silver nanoparticles with the sol-gel process, even when the material is modified in order to increase hydrophilicity. Independent of the initial concentration of attached silver chloride particles, all silver-containing samples showed no measurable silver release, providing a safe antimicrobial activity. Investigation of contact angle and water retention ability showed that the plasma treatment has the greatest effect in increasing sorption properties of the previously untreated (67%) or antimicrobial-treated viscose non-woven (57%). Measurements of the mechanical properties showed that the alkaline treatment has the most pronounced effect on increasing the breaking force and air permeability of the material, but this could be a consequence of the alkali rinsing. The plasma treatment is the most effective in reducing the elongation at break and air permeability of the viscose non-woven fabrics.

After an extensive study of carefully selected methods for the modification of the cellulosic fibrous material, it can be concluded that the combination of all three methods imparts all the characteristics a medical dressing should have. By varying the order of methods, dressings with different hydrophilicity and air permeability properties can be prepared, which enables treatment of a wide range of wounds. Thus, a combination of an antimicrobial coating, followed by a plasma treatment, is an excellent solution for wounds with excessive exudates, whereas it would be best to include an alkaline treatment in the preparation of a dressing meant for the final healing stages. Finally, binding silver with the sol-gel system enables its safe usage, and this is the most important achievement in the development of modern medical dressings.

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