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BINDING SILVER NANO-PARTICLES ONTO VISCOSE NON-WOVEN USING DIFFERENT COMMERCIAL SOL-GEL PROCEDURES

VEZAVA SREBROVIH NANO-DELCEV NA VISKOZNO KOPRENO Z RAZLIČNIMI KOMERCIALNIMI SOL-GELI

Tanja Pivec[1,2], Zdenka Peršin[2,3], Silvo Hribernik[3], Tina Maver[2,3], Mitja Kolar[2,4], Karin Stana-Kleinschek[2,3]

[1]Predilnica Litija d.o.o., Kidričeva 1, SI-1270 Litija, Slovenia
[2]Center of Excellent PoliMat, Tehnološki park 24, SI-1000 Ljubljana, Slovenia
[3]Faculty of Mechanical Engineering, University of Maribor, Smetanova 17, SI-2000 Maribor, Slovenia
[4]Faculty of Chemistry and Chemical Engineering, University of Maribor, Smetanova 17, SI-2000 Maribor, Slovenia
karin.stana@uni-mb.si

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The paper presents possible solution of Ag binding using commercial sol-gel systems which enable its low release into a wound, providing a good antimicrobial effect on those bacterial cultures that are most likely present in the wound. The influence of different sol-gel systems on the hydrophilic properties of carrier materials and the level of released silver has been studied. The results showed that sol-gel as binding-systems could provide proper hydrophilic properties of material, whilst binding silver strongly enough providing at the same time excellent antimicrobial activity of the treated viscose meterials.

Keywords: silver nano-particles, sol-gel, viscose non-woven, silver release, hydrophilicity, antimicrobial properties

V prispevku je predstavljana ena izmed možnih rešitev za uporabo srebra z majhnim sproščanjem v rano, pri čemer se je dosegel dober protimikrobni učinek na bakterijske kulture, ki se v rani najpogosteje pojavljajo. Uporabili smo komercialne sol-gel sisteme s katerimi smo inkorporirali srebrove nano-delce na viskozo. Spremljali smo vpliv različnih sol-gelov na hidrofilne lastnosti nosilnega materiala in raven sproščenega srebra. Rezultati so pokazali, da lahko uporaba sol-gel sistema zagotovi dobre hidrofilne lastnosti in obenem dovolj močno veže srebro, ki daje odlične protimikrobne lastnosti obdelanemu materialu.

Ključne besede: srebrovi nanodelci, sol-gel, viskozna koprena, sproščanje srebra, hidrofilnost, protimikrobne lastnosti

1 INTRODUCTION

The skin is an extremely effective biological barrier but if breached by trauma, the body becomes highly susceptible to microbial attack. Certainly, wound infection presents one of the major problems and can evolve into different serious medical complications. Treatment with silver-content wound dressings is becoming an increasingly popular strategy for eliminating the growth of opportunistic wound pathogens during the healing process. Silver has been known as a very effective natural antibiotic and for centuries has been in use for the treatment of burns and serious wounds. The antimicrobial property of silver is related to the amount of silver and the rate of silver released. Silver in its metallic state is inert but it reacts with the moisture in the skin and the fluid of the wound and becomes ionized. The ionized silver is highly reactive, as it binds to tissue proteins and causes structural changes in the bacterial cell wall and nuclear membrane (nucleolemma orkaryotheca), leading to cell distortion and death. Silver also binds to bacterial DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) by denaturin, and inhibits bacterial replication. Recently, due to the emergence of antibiotic-resistant bacteria and limitations on the use of antibiotics, clinicians have started to implement silver into wound dressings containing varying levels of silver[1–4]. Ag NPs (silver nano-particles) have emerged as an important class of nano-materials over a wide range of industrial and medical applications, but nevertheless they present a potential risk to human health. In vitro studies have reported that Ag NPs produce toxicity targeted at a variety of organs including the lungs, liver, brain, vascular system and reproductive organs. Ag NPs have induced the expressive level of those genes involved in cell cycle progression and apoptosis. The possible mechanisms of Ag NPs toxicity include the induction of ROS (reactive oxygen species), oxidative stress, DNA damage and apoptosis[5–8]. Therefore, silver release is very important for antimicrobial activity, but only in a controlled manner, due to toxicity.

The sol-gel process is one of the possible methods for incorporating Ag NPs onto cellulose matrices as viscose, and for the attainment of controlled Ag release. The sol-gel technique is a suitable method for incorporating homogeneously bioactive compounds, biomolecules and even whole living cells, into metal oxide matrices[9–13]. In comparison with organic matrices, inorganic sol-gel matrices provide higher mechanical, thermal, chemical and photochemical stability and are toxicologically and biologically inert, i.e., they are not a food source for microorganisms[13–15].

In presented study insignificant Ag release from treated samples in a controlled manner was attained, using commercial sol-gel systems for the incorporation of Ag NPs on viscose non-woven, which is sufficient for providing good antimicrobial activity, as well as a proper hydrophilicity of viscose non-woven.

2 EXPERIMENTAL

2.1 Preparation

2.1.1 Materials

A 100 % viscose non-woven (Kemex, The Netherlands) with a mass of 175 g/m^2 was used during the experiments. Ag NPs were incorporated onto viscose using different sol-gel procedures. Silver nano-particles in AgCl form, iSys Ag (CHT, Germany) were used as an antimicrobial agent in combination with iSys MTX or iSys LTX (CHT, Germany) being organic-inorganic binders. Kollasol CDO (CHT, Germany) was used as a wetting agent.

2.1.2 Viscose non-woven treatment

Firstly, the solutions of binder and silver nano-particles were prepared. The composition of the solution is shown in **Table 1**.

Table 1: The composition of the treatment solutions
Tabela 1: Sestava obdelovalne raztopine

sample	5 g/L iSys Ag	5 g/L iSys MTX	5 g/L iSys LTX	0,7 g/L Kollasol
S1	✓	✓		
S2	✓		✓	
S3	✓		✓	✓

10 g of viscose non-woven was impregnated in 300 mL of solution for 1 h at room temperature. After treatment, the viscose was wrung-out with a foulard (Werner Mathis Ag, Switzerland), dried in an oven in a stretched state at 80 °C, and also condensed for 1 min at 150 °C (Werner Mathis Ag, Switzerland).

2.2 Methods

2.2.1 Inductively-coupled plasma using mass spectroscopy (ICP/MS)

The concentrations of Ag on the viscose samples were determined by inductively-coupled plasma using mass spectroscopy (ICP/MS).

7 mL of HNO_3 (s.p.) and 1 mL of H_2O_2 (s.p.) were added to 100 mg of sample. The microwave system Milestone Ethos® touch control with a segmented rotor for high pressures (HPR – 1000/10S) was used for digestion of the samples. The sample digestion was performed over two steps: step 1 – sample was heated to 200 °C (1000 W) for 10 min, step 2 – the temperature was kept stable at 200 °C (1000 W) for 10 min. After digestion the sample was diluted to 50 mL with 1% HNO_3. Further dilutions were automatically performed with 1% HNO_3 to adjust the measuring ranges (10 / 50 / 100 µg/L) of the Ag solutions.

A Perkin Elmer® Sciex Elan DRC-e was used for ICP/MS analysis. The instrumental and acquisition parameters were: RF power 1125 W, lens voltage 12,75 V, argon gas flow: nebulizer 0,95 L/min, auxiliary 1,20 L/min, plasma 15 L/min, dwell time 50 ms and number of sweeps 20. Three replicates of the samples were measured using the peak-hopping measuring mode (one point per peak) at an integration time of 1000 ms. The reference element (Rh) at a concentration of 10 µg/L was used as internal standard.

2.2.2 Atomic absorption spectroscopy (AAS)

Ag concentration in milli-Q solution after the Ag release from viscose samples was determined using atomic absorption spectroscopy (Perkin-Elmer AAnalyst 600). An atomic absorption spectrometer equipped with an air-acetylene burner and silver hollow cathode lamp operating at 5 mA was used for determining 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 thr flow-rate of air: 8 L/min.

A stock standard solution of silver (500 mg/L) was prepared by dissolving the appropriate amount of silver nitrate ($AgNO_3$) in water with 1 % (v/v) HNO_3. The working standard solutions were prepared by dilution.

2.2.3 In vitro silver release

In vitro release studies were performed using static diffusion cells, according to Franz[16]. The receptor compartment had a mean volume of 7,5 mL, filled with milli-Q water, and its temperature was maintained at 37 °C by stirring of termostated water surrounding the cell.

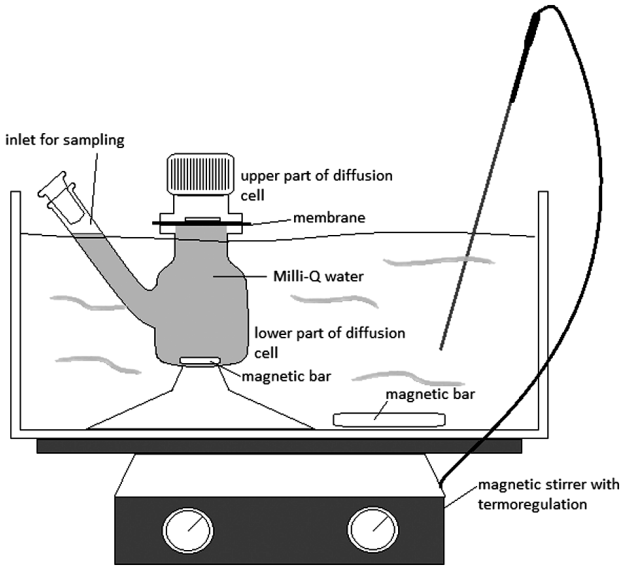


Figure 1: Thermoregulated system of Franz diffusion cell
Slika 1: Termoregulacijski sistem s Frazevo difuzijsko celico

This temperature value was chosen in order to reproduce the human temperature under normal conditions. The Ag release was determined after 24 h. The thermoregulation system of the Franz diffusion cell, is presented in **Figure 1**.

2.2.4 Tensiometry

The hydrophilic/hydrophobic character was studied by contact angle measurements between the polymer material samples and water. The Powder contact angle method was used as developed for determining the wetting properties of porous materials. The samples were cut into 2 × 5 cm rectangular pieces and suspended in a special sample holder of a Krüss K12 processor Tensiometer. Immediately before measurement, the container with the liquid (n-heptane; water) was raised until the sample edge touched the liquid surface.

The change in samples' 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[17]:

$$\cos\theta = \frac{m^2}{t} \cdot \frac{\eta}{\rho^2 \cdot \gamma \cdot c} \quad (1)$$

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 more detailed description of this experimental method, see Persin et al.[18] and Fras Zemljic et al.[19,20].

2.2.5 Reduction of bacteria

The antimicrobial effect of viscose non-woven loaded with AgNPs on gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and on gram-positive bacteria *Staphylococcus aureus* and *Enterococcus faecalis* was investigated by modified AATCC 100-1999 standard method. These bacteria were chosen as being the most frequently isolated micro-organisms from skin wounds in humans[21–23].

Circular swatches (4,8 cm in diameter) of viscose samples were put into a 70 mL container and inoculated with 1.0 mL of inoculums containing 1–2 · 10^5 colony forming units of bacteria. After incubation at 37 °C for 24 h, the bacteria were eluted from the swathes 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. Afterwards the number of bacteria was counted and the reduction of bacteria, *R*, was calculated as follows:

$$R = \frac{B - A}{b} \times 100\ (\%) \quad (2)$$

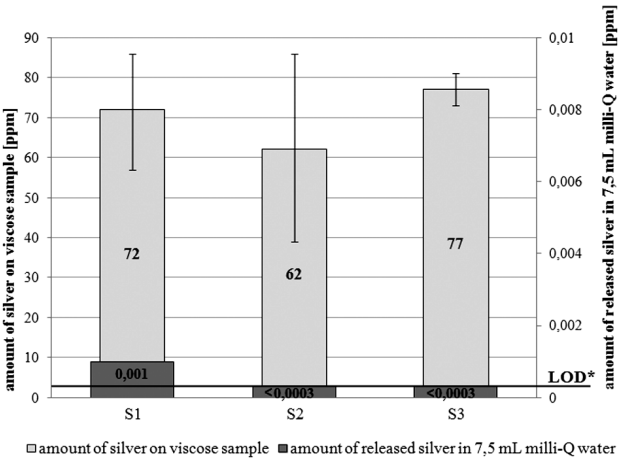
where *A* is the number of bacteria recovered from the inoculated swatch of the viscose sample in the plastic container incubated over the desired contact period (24 h) and *B* is the number of bacteria recovered from the inoculated swatch of the viscose sample in the container immediately after inoculation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

3 RESULTS

The amount of silver on the viscose samples and amount of silver in the milli-Q solutions after Ag release from the samples, are shown in **Figure 2**.

Although the amount of silver in the treated solutions was the same for all samples, the concentrations of Ag on the treated fibres differed. The highest measured concentration of silver was detected for sample S3.

Independent of the binding procedure (using different sol-gel systems) the amount of released silver was very low. All the measured concentrations were at or below the detection limit (LOD) of AAS.



*LOD: less than 0,05 mg /L (or 0,0003 ppm Ag in 7,5 mL of the milli-Q solution)

Figure 2: The amount of silver on the viscose samples, and amount of silver in the milli-Q solutions after Ag release from the samples

Slika 2: Vsebnost srebra na viskozni kopreni in vsebnost srebra v milli-Q raztopini po sproščanju srebra iz vzorcev

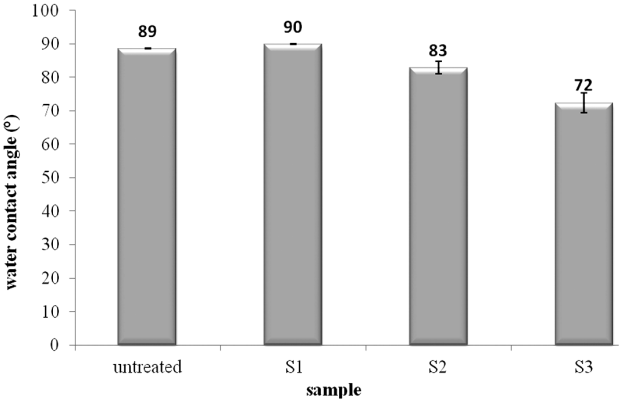


Figure 3: Water contact angle

Slika 3: Stični kot med vodo in vzorcem

The water contact angles for the untreated sample and with the silver treated samples, are shown in **Figure 3**.

The contact angle of sample S1 was higher than that of the untreated one. An improvement in the hydrophilic properties can be observed for both the other samples. The contact angle for sample S3 decreased by 19 %.

The results of antimicrobial activity for the untreated and with silver-treated viscose samples, are presented in **Table 2**.

Table 2: Reduction, *R*, of bacteria determinate according to the AATCC 100-1999 standard method

Tabela 2: Redukcija, *R*, bakterij, določena po AATCC100 – 1999 standardni metodi

sample	Reduction, *R* (%)			
	Staphylococcus aureus (G+)	*Escherichia coli* (G-)	*Pseudomonas aeruginosa* (G-)	*Enterococcus faecalis* (G+)
untreated	No reduction	No reduction	No reduction	77
S1	100	100	98,8	100
S2	100	100	100	100
S3	In regard to the concentration of silver on this sample, the same antimicrobial activity as for other samples can be predicted.			

As expected, no reduction of the bacteria *S. aureus*, *E. Coli* and *P. aeruginosa* was found for the untreated viscose sample. The reduction of *E. faecalis* was 77 %. All the silver-treated samples showed excellent antimicrobial activity. Sample S3 was not tested. In regard to the concentration of silver on this sample, the same antimicrobial activity as for other samples can be predicted.

4 DISCUSSION

Existing studies of the binding of silver to fibrous materials[12–14] using a sol-gel procedure show that the selection of this method is a good solution for the development of materials intended for the treatment of open wounds. Sol-gel systems vary significantly in their chemical composition. Differently chemically modified sol-gels therefore act differently and can thermally and chemically change or improve the material to which they were bound. Thus, a sol-gel system that will provide the desired properties can be chosen with regard to the intended purpose of the material. For example, in order to achieve hydrophilicity of a material intended for wound dressings for faster wound healing, sol-gel precursors that are completely hydrolysed can be chosen. Sol-gels containing derivates with long alkyl chains, phenol rings or fluorine[13] work the opposite way (are hydrophobic). Commercial products that give textile materials such properties are already available on the market.

In our research commercial sol-gel systems produced by CHT (Germany) were used. Our aim was to observe how differently chemically modified sol-gel systems affect the quantity of the adsorbed silver on the material, and its release from the material. Dispersion of AgCl nanoparticles (iSys Ag) was used as the source of silver nanoparticles in all samples. The following binding agents were used for binding Ag particles: iSys MTX, a sol-gel system with a hydrophobic character, in the S1 sample, and iSys LTX, a sol-gel system with a hydrophilic character, in the S2 and S3 samples. Kollasol CDO, described by the producer as a hydrophilic silicone surface-active substance mixed with higher alcohols, was also added to the S3 sample.

The selection of the sol-gel has a different influence on the silver adsorption on viscose and, consequently, the amount of Ag on the material, as is evident from **Figure 2**. Discrepancies in the measured concentrations of silver on viscose are, of course, also possible because of measurement errors, but an increase in adsorption of AgCl nanoparticles is expected when using a more hydrophilic sol-gel system. It is clear that the addition of a surfactant (Kollasol CDO) to a hydrophilic cross-linking agent, iSys LTX, in the treatment of the S3 sample increased the concentration of the bound Ag by 6.5 %. The measurement results of silver released also show better adsorption of AgCl nanoparticles when using a hydrophilic sol-gel system. The hydrophilic sol-gel system in combination with a surfactant affects the formation of a three-dimensional network that is created from SiO_4 tetrahedrons[24,25] and captures the AgCl nanoparticles in its structure, as well as causes a slight release of silver from viscose (S2 and S3 samples).

It is known that AAS in ICP/MS methods enable the detection of Ag without high measuring error, what is not the case in presented results. The explanation for this we can find in the low solubility of the commercial viscose non-woven due to the commercial coatings residues (on the basic of different polysaccharides). The commercial viscose samples were not soluble good enough in HNO_3. We founded out that the use of the H_2SO_4 and HNO_3 mixture give us a clear solution of dissolved commercial viscose, nevertheless the AAS detected Ag was below 1 ppm. Another used dissolution procedure (dissolving of the commercial viscose non-woven in microwave under the high pressure in the solution of HNO_3 and H_2O_2) leads to a clear viscose solution, but again the measuring error of ICP/MS detected Ag have been too high. Contrary to described viscose dissolution and connected Ag detection problems, our preliminary studies showed that the pure viscose (without surface coating) could be dissolved very well in the heated HNO_3, contrary to commercial viscose non-woven used in this study. The AAS detected Ag, of well dissolved viscose, without measuring error could be obtained.

The hydrophilic/hydrophobic properties of the samples were determined by measuring the contact angles between water and differently treated viscose non-

wovens. The results shown in **Figure 3** confirm that the use of iSys MTX in the S1 sample leads to an increase in the hydrophobic character compared to the untreated sample, while the use of the cross-linking agent iSys LTX increases the hydrophilic properties of cellulose non-wovens by 8 %. These properties are further improved by 19 % with the addition of the wetting agent Kollasol CDO to iSys LTX in the S3 sample. The hydrophilic properties of fibrous materials can also be provided by other methods as the non-equilibrium gaseous plasma treatment that represents a quick technique which could be performed after silver deposition using sol-gel procedure[26–30]. Finally, these must be taken into account in our future development of materials which could be used in wound dressing.

According to the literature, antimicrobial activity of silver is related to its concentration and its release rate[1]; therefore, the antimicrobial activity of our samples on the bacteria most commonly found in wounds[21–23] was also examined. It was established that silver concentrations ranging from 62 ppm to 77 ppm are sufficient to give a viscose non-woven excellent antimicrobial properties, regardless of the strength of its adsorption to viscose and its release, contrary to the literature reports which show that even the application of modern reliable techniques for destruction of bacteria like *S. aureus* and *E. coli* shows limited sterilization efficiencies[31–35]. Furthermore, M. Gorjanc et al. studied similar effects on textiles and never found 100 % bacteria reduction[26]. Contrary to this report S. Strnad at al. found out that even pure viscose shows antimicrobial activity on some microorganisms[36], but the appearance is not completely explained. It can be seen that it is very difficult to compare different antimicrobial material activities reported in the literature. The main reason for that are testing procedures used in different studies and reported in the literature (testing procedures as ASTM E2149-01, AATCC 100-1999 which are not direct comparable…) as well as the differences in studied and modified materials which differ in their supra-molecular structure and chemistry.

Franz diffusion cells in combination with AAS were used to monitor the release of Ag from viscose non-woven. We believe the use of Franz cells serves as a good representation of realistic conditions inside a wound and therefore an excellent method for monitoring the release of other drugs[40]. We also believe that the AAS method is a good choice to determine the concentration of the silver released. Although silver concentrations that can acceptably enter the body have not been determined in the literature, we believe that detection limit of AAS is so low that those viscose non-woven samples with a released silver concentration below the detection limit can be seen as potential wound dressings for open wound treatment.

5 CONCLUSION

In our research, the greatest attention was paid to the study of the influence of differently chemically modified sol-gel systems on the adsorption of AgCl nanoparticles to viscose non-woven and the release of Ag from viscose non-woven. It was established that, when compared to a hydrophobic sol-gel system, a combination of a hydrophilic sol-gel system and a surfactant improves the hydrophilic properties of the carrier material (19%), increases the concentration of bound Ag (6.5%) and reduces the release of Ag from the non-woven to a concentration detectable below the AAS detection limit. With regard to the excellent antimicrobial properties of treated materials, it can be concluded that the selection of the right sol-gel system combination, which ensures the best hydrophilic properties of the materials and the strongest binding of silver to it, can be seen as a good starting point in the preparation of safe wound dressings with positive effects on the wound healing process.

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