Antibacterial properties of silver coated regenerated cellulose

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This work is focused on the study and characterisation of surface properties of regenerated cellulose after plasma modification, which significantly affects its physical and chemical properties such as wettability, surface chemistry, and surface morphology. Depending on the selected parameters, such as the plasma exposure time of the substrate, its aging and the sputtering time with silver, the modified material was studied in different analytical methods. The ablation of the substrate was determined gravimetrically. Changes in surface roughness were detected by atomic force microscopy and chemical changes were studied with X-ray spectroscopy (XPS). Silver nanolayers were sputtered on the activated substrate and the antibacterial properties of these layers were studied. It has been determined the aging period for surface-treated under different exposure time, gravimetric analysis has shown almost linear mass loss with plasma treatment time, the surface roughness slightly decreases with the action of plasma. XPS analysis revealed that the oxygen content increased due to the higher reactivity of the surface of the modified cellulose. Antibacterial tests have shown that the silver layer sputtered on plasma activated regenerated cellulose significantly reduces the colonies of both *Escherichia coli* and *Staphylococcus epidermidis.*

1. Introduction: The antibacterial surface can be considered as the surface of any material in the form of foil, granule or another specific type that can be used to limit the growth and proliferation of bacteria [1]. Bacteria adaptation on the surface and its adhesion with subsequent multiplication on the surfaces of substrate materials can lead to a wide range of health problems, where expensive cleaning processes have to be applied [2]. Bacterial may exist in several environments, such as water-cooled air conditioning systems [3] and have also been considered as the key factor in the phenomenon of bio corrosion, when cells attached to e.g. metallic surfaces lead to the corrosion of the material through the formation of 'pits' [4]. Cellulose is considered as promising material for biomedical applications in various forms, mostly as wound treatments [5]. Antibacterial activity of silver (Ag) nanoparticles introduced on the surface in the form of solution spraying onto cellulose [6], also processes as immersion of biopolymer into nanoparticle solution [7] or temperature-responsive release of antibacterial agents [8] have been introduced recently with promising results. For antibacterial studies several forms of cellulose have been tested, such as nanocomposite fibre membrane [9], cellulose acetate membranes with nanodiamond particles [10], antibacterial composites of Ag nanoparticles with metal–organic frameworks and carboxymethylated fibres [11] or bacterial cellulose composites with Ag nanoparticles and antibiotics [12]. Different approaches for surface treatment may influence the adhesion between metal and biopolymer, such as plasma [13], laser [14] or ultraviolet treatment [15].

Herein, we present a simple but effective approach for construction on antibacterial cellulose surface based on combination of plasma exposure followed by simple sputtering of thin Ag nanolayer. This technique can be also realised in large-fabrication scale in industry and was confirmed to be successful for inhibition of both Gram-positive and Gram-negative bacteria, even after very short time of interaction.

2. Experimental: For experiments, we used regenerated cellulose foils (thickness 90 µm, density 1.44 g cm^{-3}) supplied by Goodfellow Ltd. The samples were modified in diode plasma discharge on Balzers SCD 050 device for 10–240 s, using DC Ar plasma (gas purity was 99.997%, power 8 W). Process parameters were: Ar flow 0.3 l s^{-1}, Ar pressure 10 Pa, electrode area 48 cm^2, the inter-electrode distance of 50 mm, chamber volume 1000 cm^3. The gold layers were deposited from a gold target (99.999%) by means of diode sputtering technique (BAL-TEC SCD 050 device). Typical sputtering conditions were: room temperature, time 30 and 150 s, total argon pressure of about 5 Pa, electrode distance of 50 mm and current of 20 mA.

The contact angle was determined by goniometry using the static water drop method. The measurements of the advancing water contact angle (error 5%) were performed using glycerol and distilled water on 6 different positions using the Surface Energy Evaluation System (SEE System, Advex Instruments, Czech Republic). By automatic pipette, the water drop of volume (8.0 + − 0.2) ml was deposited on the polymer's surface and the consequent photo was evaluated.

Surface morphology was examined with atomic force microscope Dimension ICON (Bruker Corp.), ScanAsyst mode in the air was used for determination. Silicon tip on Nitride Lever SCANASYST-AIR with spring constant 0.4 N/m was used.

The presence of oxygen and carbon in the modified cellulose surface layer was proved by X-ray photoelectron spectroscopy. An Omicron Nanotechnology ESCAProbeP spectrometer was used. The X-ray source was monochromated at 1486.7 eV and the measurement was performed with the step of 0.05 eV. Mean mass loss of the ablated surface layer after the plasma ablation was measured using a Mettler Toledo UMX2 microbalance.

The antimicrobial activity of the prepared cellulose samples was determined by a drop test using Gram-negative bacteria *Escherichia coli* (*E. coli*, DBM 3138) and Gram-positive bacteria *Staphylococcus epidermidis* (*S. epidermidis*, DBM 2179). The bacterial strain of *E. coli* was diluted with fresh phosphate-buffered saline (PBS) buffer at a concentration of 2×10^4 cells per ml. The bacterial strain of *S. epidermidis* was diluted with fresh PBS buffer at a concentration of 4×10^4 cells per ml. One set of tubes with 2 ml PBS and *E. coli* and one set of tubes with 2 ml PBS and *S. epidermidis* were left without addition as controls. Other cellulose samples were prepared in the form of 1 cm^2 sample which was sonicated and allowed to contact the bacteria for 2 h at room temperature. The plates were incubated for 24 h at room temperature for *E. coli* and 37° C for *S. epidermidis*. The number of colonies of both cultures was evaluated by colony forming units, each sample being performed in triplicate. Control cells were incubated only in saline.

3. Results and discussion: The surface wettability of plasma modified regenerated cellulose was examined by goniometry. The dependence of glycerol contact angle on aging time for plasma power 8 W and exposure times 10–80 s are introduced in Fig. 1, for exposure times 120–240 s are introduced in Fig. 2. Two liquids were used for contact angle determination: water and glycerol; however, we introduce the glycerol contact angle dependence only from space reasons. It is evident that after plasma exposure the aging time for stabilisation depends on the exposure time. During the aging process, the contact angle of glycerol increases, the surface chemistry is changed due to the reorientation of oxygen containing groups into the polymer bulk [16]. However, there is no obvious correlation between an increase of exposure time of cellulose and the contact angle, which is determined as stable, for an 'aged' surface. It is obvious that for short exposure time of 10 s the stabilised value can be considered as ~50°, but for an exposure of 20 s the stabilised value is above 70°. One can conclude that the major difference can be observed for short exposure times, after which a periodic ablation takes place (will be discussed in the next section), thus influencing the surface chemistry and wettability significantly. The surface is stabilised for shorter exposure times after 168 h and for longer sputtering times (120–240 s) after 336 h.

The dependence of mass loss on exposure time is introduced in Fig. 3*a*. The gravimetric method revealed changes in weight of material which depended on modification time, as expected, however, the linear dependence confirmed our presumption of periodic changes of surface chemistry thus influencing the surface wettability. Since the ablation of cellulose material is closely connected also with surface chemistry changes (altered surface layer is modified with exposure time), we have shown in Fig. 3*b* the graph representing oxygen concentration for selected exposure times determined on cellulose. Even the pristine cellulose has 'natural' oxygen in its structure, it is evident that during plasma exposure the oxygen concentration increases, as is evident from the inset of Fig. 3*b*, the same effect was observed earlier for cellulose fibres [17]. This conclusion is very important also from the point of consequent metallisation.

Plasma modification induced the decrease of surface roughness of plasma modified cellulose, as is introduced in Figs. 4*a–d*. We have chosen two atomic force microscopy (AFM) squares for pristine and plasma-treated cellulose. Bottom images were acquired as

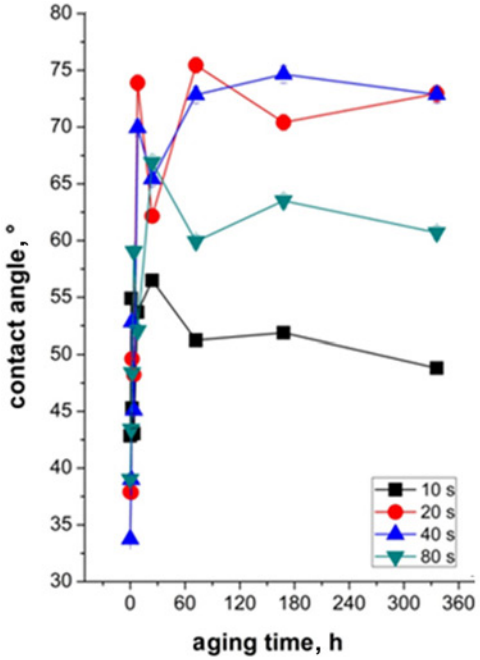

Fig. 1 *Dependence of the glycerol contact angle on aging time measured on cellulose plasma treated at discharge power 8 W and exposure times 10–80 s*

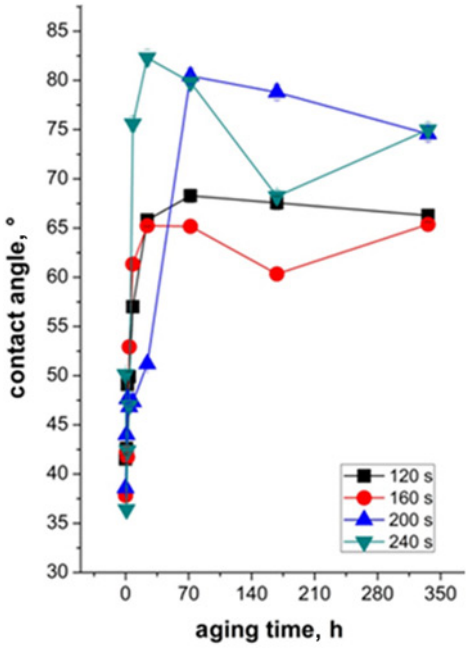

Fig. 2 *Dependence of the glycerol contact angle on aging time measured on cellulose plasma treated at discharge power 8 W and exposure times 120–240 s*

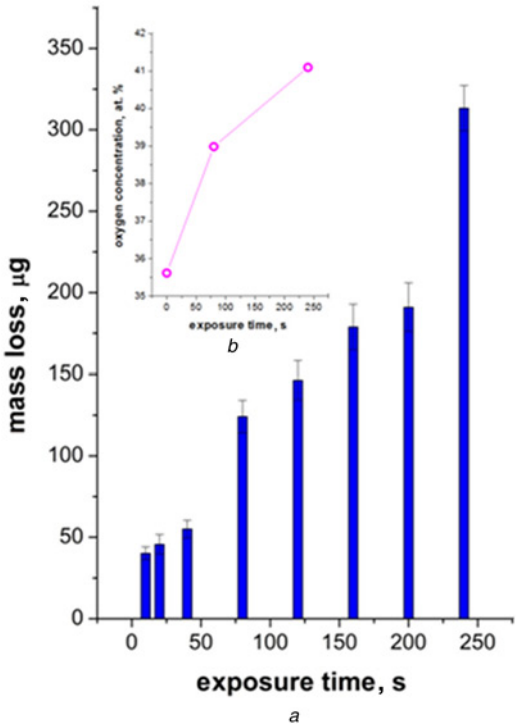

Fig. 3 *Mass loss and surface chemistry changes for plasma-treated cellulose*
a Dependence of the mass loss of the cellulose layer ablated by the plasma discharge with power 8 W on the plasma exposure time
b Dependence of oxygen concentration on plasma exposure time for selected modified samples

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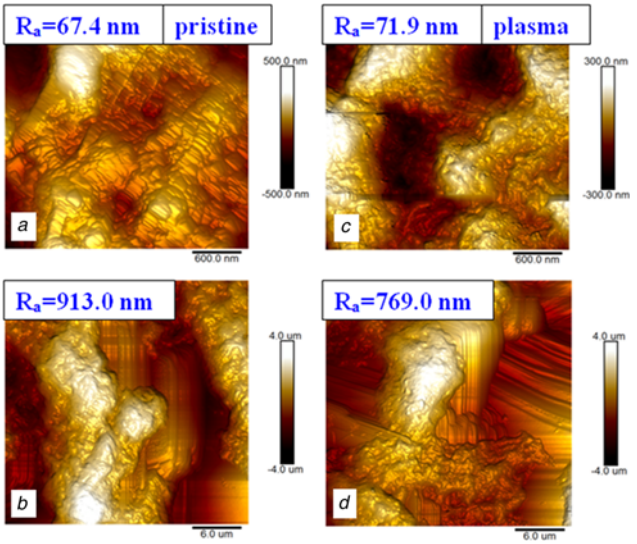

Fig. 4 *Surface morphology and roughness for pristine and plasma modified cellulose samples, R_a represents average surface roughness*
a Surface morphology of pristine cellulose, inspected square 3×3 μm^2
b Surface morphology of pristine cellulose, inspected square 30×30 μm^2
c Surface morphology of plasma-treated cellulose (8 W and 240 s), inspected square 3×3 μm^2
d Surface morphology of plasma-treated cellulose (8 W and 240 s), inspected square 30×30 μm^2

30×30 μm^2, the details of both samples are introduced in detail 3×3 μm^2. It is evident that the impact of accelerated argon ions induces morphology changes on the cellulose foil, see Figs. 4*a* and *c*. The disruption of macromolecular chains leads to the appearance of smaller surface fragments, which are further removed from the cellulose surface due to ablation, as was discussed previously. We present a detailed study of the argon plasma influence on changes in surface morphology, which is for cellulose biopolymer very difficult to achieve by AFM. Also, one may object that the roughness increases for plasma-treated foils, as it is introduced in Figs. 4*a* and *c*. However, we have to keep in mind that such a small scan is introduced only to show the details of surface morphology, and should not be noted as a representative sample scan. For a larger surface scan, the decrease of surface roughness is apparent, as introduced in Figs. 4*b* and *d*, where the ablation effect due to plasma exposure takes place. This effect influences the amorphous phase significantly, the crystalline phase (introduced as line structures with lower height between the amorphous clusters), and thus decreases the surface roughness.

E. coli and *S. epidermidis* were tested in this work. The regenerated cellulose foils were used as unmodified, modified with two different exposure times (8 W – 40 s, 240 s) and consequently metallised. We have previously tested this simple approach both for Gram-positive and Gram-negative bacteria with an excellent result [7]. Figs. 5*a* and 6*a* show a significant decrease in *E. coli* and *S. epidermidis* bacteria on samples that have been plasma modified and sputtered with Ag for 30 and 150 s. For *S. epidermidis*, the decline in the number of bacterial colonies is evident even with Ag sputtering for shorter time, but this trend is not so obvious for *E. coli*. One can see also for *E. coli* that plasma modification of the material without Ag sputtering has no significant effect on the growth of bacteria compared to the pristine cellulose. A small decrease of *S. epidermidis* colonies with increasing modification time even without sputtered Ag was observed. Corresponding photos of the colonies for selected samples are introduced in Figs. 5*b* and 6*b*. It is evident that the simple combination of Ag sputtering and plasma treatment results in construction of effective antibacterial surface both for Gram-positive and Gram-negative bacteria.

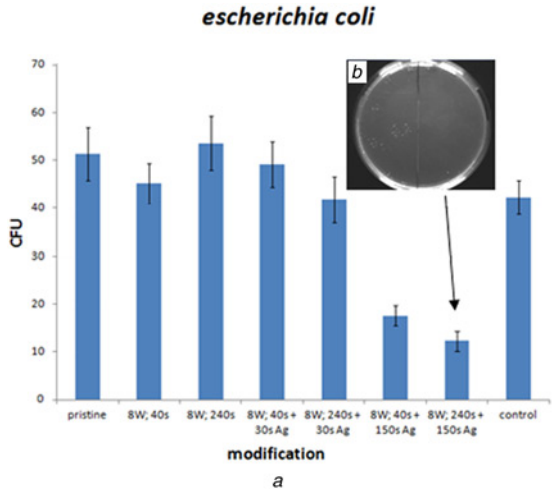

Fig. 5 *Antibacterial test results for E. coli on pristine and treated cellulose*
a Amount of *E. coli* bacteria in dependence on surface modification of cellulose foil
b Photograph of the colonies for plasma modified sample with Ag nanolayer (150 s)

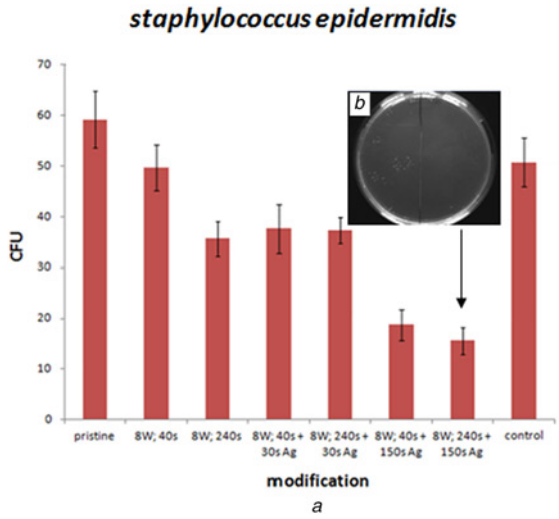

Fig. 6 *Antibacterial test results for S. epidermidis on pristine and treated cellulose*
a Amount of *S. epidermidis* bacteria in dependence on surface modification of cellulose foil
b Photograph of the colonies for plasma modified sample with Ag nanolayer (150 s)

The mechanism of the antibacterial activity of nanostructured Ag has not been fully clarified yet. However, it has been found that the mechanism consists of two synergistic processes: direct contact of the bacterial cell with metallic Ag on the one side and on the other side release of Ag^+ ions into the surrounding medium and its subsequent interaction with the cell, depending on the specific case that prevails. Morphology, roughness, and hydrophilicity/hydrophobicity of the surface of the material play a major role in the direct effect, and each of these parameters affects Gram-negative and Gram-positive bacterial strains differently. Ag ions interact with four major components of the bacterial cell (cell wall, plasma membrane, bacterial DNA, and proteins (e.g. specific enzymes involved in vital cellular processes such as the electron transport chain) [18, 19]. In our case, it can be seen that the antibacterial activity of *E. coli* is caused only by the release of Ag. In *S. epidermidis*, however, slight antibacterial effects were observed even after plasma modification and 30 s of Ag sputtering,

which resulted in a change in morphology and deposition of a thin layer of Ag.

Ions also cause degradation of the peptidoglycan cell wall and cell lysis (cell death), preventing further bacterial proliferation. Subsequently, they penetrate into the inner part of the cell where they bind on the basis of DNA. Chromosomal aberrations caused by Ag^+ are also a frequent phenomenon. In the interior of the cell, ions can further cause mitochondrial dysfunction and ribosome denaturation, thereby inhibiting protein synthesis and degrading the plasma membrane. This multi-stage mechanism of antibacterial action is a major factor that results in low bacterial resistance to Ag [18, 19].

In the case of Ag/polymer nanostructured composites, a significant effect of released Ag^+ ions on the resulting antibacterial effect was found. The concentration of released Ag ions must then reach the minimum inhibitory concentration, the minimum concentration at which bacteria are inhibited. The release rate depends on the chemical form of Ag, particle/cluster size, surface functionalisation, and crystallinity. Last but not least, the temperature and nature of the surrounding medium (the presence of salts or biomolecules) are key factors for the release [20].

4. Conclusion: We have determined the aging time for plasma-treated regenerated cellulose for different times of exposure and power 8 W to be 336 h. The gravimetric study of the effect of plasma exposure revealed the decrease of cellulose weight due to the ablation to be almost 1.2% for plasma power 8 W and 240 s. During plasma exposure the oxygen concentration on cellulose surface increases, which is very important for consequent Ag metallisation. Plasma modification induced the decrease of surface roughness of plasma modified cellulose; the structure of amorphous cellulose is disrupted. The simple combination of Ag sputtering and plasma treatment results in the construction of effective antibacterial surface both for Gram-positive and Gram-negative bacteria.

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