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ORIGINAL PAPER

Antimicrobial effect of oxidized cellulose salts

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Abstract Antimicrobial properties of oxidized cellulose and its salts in linters (-L) and microsphere (-M) form (OKCEL® H-L, OKCEL® Zn-M, OKCEL® ZnNa-L, OKCEL® ZnNa-M and OKCEL® Ag-L) were tested by a dilution method against a spectrum of microbial strains: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Bacillus licheniformis*, *Aspergillus niger*, *Penicillium chrysogenum*, *Rhizopus oryzae*, *Scopulariopsis brevicaulis*, *Candida albicans* and *Candida tropicalis*. OKCEL® Ag-L exhibited antimicrobial activity in the range 0.1–3.5% w/v against all the bacteria and fungi involved in this study. Strong inhibition by OKCEL® ZnNa-M was observed for *Staphylococcus epidermidis*, *Bacillus licheniformis*, *Rhizopus oryzae*, *Candida albicans* and *Candida tropicalis* in the range 0.5–2.0% w/v. Antimicrobial effects of oxidized cellulose and its salts in textile form were investigated by a diffusion and dilution method against the spectrum of above-cited microbial strains extended by *Clostridium perfringens*. Generally, OKCEL® Ag-T, OKCEL® Zn-T and OKCEL® H-T showed high antimicrobial activity against populations of *Pseudomonas aeruginosa*, *Bacillus licheniformis* and *Staphylococcus epidermidis*. OKCEL® Zn-T was the only sample suppressing the growth of species.

Keywords Antimicrobial properties · Minimal inhibition concentration · Oxidized cellulose · Silver salts · Zinc salts

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Introduction

Oxidized cellulose (6-carboxycellulose) containing 3–25% of carboxyl group represents compatible and absorbable polymer capable of biodegradation in the human organism. Oxidized cellulose (OC) and its salts exhibited hemostatic and antimicrobial activity as well as the ability to bind pharmaceuticals and other substances. The antimicrobial effect of 6-carboxycellulose depended on the number of carboxyl group in which ionogenic H^+ and OH^- are directly responsible for their antimicrobial action [1]. Adhesion of bacterial cells to OC and the antimicrobial activity can be induced by incorporation of other functional groups on the surface of such a skeletal structure [6]. These modified structures may also serve as a binding center for pharmaceuticals, peptides, etc.

A recent study by Spangler et al. [15] showed the antimicrobial activity of oxidized cellulose against a variety of pathogens in vitro. Coagulase-positive staphylococci, *Pseudomonas aeruginosa* and *Acinetobacter calcoaceticus* were also sensitive to OC samples, where the mode of antimicrobial action has been linked to the acidity (2.5 pH in vitro) of OC samples [11]. In order to neutralize oxidized cellulose and prepare oxidized cellulose salts, the addition of alkalic salt of weak acid could be applied. The physical form of OC salts is the most important characteristic for a majority

of applications. The L-type (i.e., linters) represents the OC salts retained its microfiber structure common in native cellulose (manufactured by Aliachem a. s., Synthesia, Czech Republic), whereas, the M-type has a microsphere structure. For practical purposes, a textile material (T-type) was fabricated from various OC salts by this company.

Various inorganic ions incorporated into the structure of OC may act as an additional antimicrobial component in OC salts. Since silver ions exhibited antimicrobial activity with relatively low toxicity against human cells, many medical instruments and bandages are made from material containing silver ions [10]. The antimicrobial effect of Ag^+ is well documented in concentrations that ranged from 10^{-9} to 10^{-6} M against a broad spectrum of organisms that included Gram-positive and Gram-negative bacteria, fungi and viruses [12]. Although the zinc ions (10^{-7}–10^{-5} M) are necessary for the optimal growth of microorganisms in vitro, the higher concentrations of Zn^{2+} have lethal effects [16]. Calcium salts of oxidized cellulose are predominantly used as hemostatics. There are commercial products for hemorrhage and madescent wounds treatment such as Traumacel® (Bioser a.s., Czech Republic), Spofax® (Zentiva a.s., Czech Republic), Systogen® (Infusia a.s., Czech Republic), etc. Aliachem, a.s., Synthesia is one of the companies producing material on the basis of oxidized cellulose for medicinal and technological applications (OKCEL®).

The objective of this study was to evaluate antimicrobial activity of oxidized cellulose salts both powder and textile forms against a broad spectrum of microorganisms.

Materials and methods

Microbial culture preparation

Five bacterial, two yeast and four fungal strains were used: *Escherichia coli* CCM 3954, *Pseudomonas aeruginosa* CCM 3955, *Bacillus licheniformis* CCM 2145, *Staphylococcus epidermidis* CCM 2124, *Clostridium perfringens* CCM 4435, *Candida albicans* CCM 8180, *Candida tropicalis* CCM 8223, *Aspergillus niger* CCM 8189, *Penicillium chrysogenum* CCM 8034, *Rhizopus oryzae* CCM 8075, *Scopulariopsis brevicaulis* CCM F-388. All the strains were provided by Czech Collection of Microorganisms (CCM), Brno, Czech Republic.

A suspension of bacterial and yeast strain was prepared from freshly grown colonies on nutrient agar no. 2 or blood agar (for bacteria) and malt agar (for yeasts) after 24–48 h incubation at optimum temperature. The concentration of cells was adjusted to 10^8 cfu/ml using the 0.5 McFarland turbidity scale and serially diluted to initial inoculum of 10^6 cfu/ml. Suspensions of fungal spores were prepared from 7-day cultures grown on Malt agar's slant at 24 °C. The density of spores was adjusted to 10^6 spores/ml using Bürker cell counting chamber. All the nutrient media used throughout the study were purchased from HiMedia, India.

Tested samples

Three types of OC salts were used in this study, i.e., linter (-L), microspherical (-M) and textile (-T). Linter and microspherical types were used as a powder. For practical purposes, textile samples were made from various OC salts. All the samples were kept in refrigerator prior to use. Chemical and physical characteristics of OC salts are summarized in Table 1.

Evaluation of antimicrobial activity of L- and M-type of OC salts

A simple dilution method was used for determining the antimicrobial activity of OC salts. Concentration range (0.1–3.5% w/v) of each OC salt was prepared by diluting appropriate amount of OC salt in 10 ml of buffer peptone water or Brain Heart Infusion broth (for bacteria), Fluid Thioglycollate Medium (for *C. perfringens*) and Malt extract broth base (for yeasts and fungi). An aliquot (1 ml) of the microbial suspension (density 10^4 cfu/ml) was added to each tube containing the dressing. Prior to incubation, 0.1 ml of suspension was streaked on the surface of solid nutrient media (Nutrient medium No. 2 for bacteria, Tryptone

Table 1 Chemical and physical characteristics of OKCEL® salt samples used in the study

OKCEL® salt	pH[a]	Concentration in % (w/w)				
		COOH	Ag	Mg	Na	Zn
Linter type						
OKCEL®-H	3.93	19.30				
OKCEL®-Mg-L	2.62	20.60		0.91		
OKCEL®-Zn-L	3.20	20.50				5.50
OKCEL®-Ag-L	4.40	18.10	0.57			
OKCEL®-ZnNa-L	6.60	0.00			2.67	8.76
Microsphere type						
OKCEL®-Zn-M[b]	4.51	19.60				13.50
OKCEL®-Zn-M	4.32	16.40				10.90
OKCEL®-ZnNa-M	4.70	0.80			3.11	9.21
Textile type						
OKCEL®-H-T	3.90	19.20				
OKCEL®-Mg-T	4.60	2.70		3.58		
OKCEL®-Zn-T	3.50	17.50				2.92
OKCEL®-Ag-T	4.30	16.9	0.58			

[a] pH of 1% solution

[b] Sample was stored at ambient temperature exposed to daylight for 1 year

Sulfite Neomycine agar for *C. perfringens* and Malt agar for yeasts and fungi) in order to determine the initial concentration. Then tubes were incubated at optimum conditions for each microbe and subsequently an aliquot of 0.1 ml was sampled from each tube at specific time intervals (2nd and 7th day) on the surface of appropriate solidified media. After incubation period (24–48 h, 37 °C for bacteria; 2–4 day, 24 °C for yeasts and fungi), the grown colonies were counted. The minimal inhibitory concentration (MIC) was defined as the lowest concentration (in % w/v) where no growth of microorganism was observed after 7 days of incubation. Each sample was examined in duplicate. Negative control (without OC salt) was also included.

Evaluation of antimicrobial activity of -T type of OC salts

The antimicrobial effect of OC salts in textile form was performed using dilution and two diffusion methods. The dilution method was applied as described above. A textile sample (1 g) was added into the tube containing 1.0 ml of bacterial suspension and 9 ml of appropriate nutrient medium (working concentration 9.0% w/v). Diffusion methods were followed: firstly, suspension (0.1 ml, 10^6 cfu/ml) of each microorganism was streaked on the agar medium and the square (25 cm^2) of each textile sample (i.e., T-type) was deposited on the surface. The growth/no growth pattern and the zone of inhibition were determined after appropriate incubation period. Secondly, a square of oxidized cellulose salt (T-type) (25 cm^2) was aseptically placed on the surface of the agar medium; thereafter 0.1 ml inoculum (10^6 cfu/ml) was directly added on the textile sample and incubated under appropriate conditions. The growth/no growth pattern was determined. The experiment was repeated twice with negative control as well.

Results and discussion

Evaluation of antimicrobial activity of L- and M-type of OC salts

OKCEL® Ag-L and ZnNa-M were most effective against the tested microorganisms. OKCEL® Ag-L exhibited antimicrobial effect in the range from 0.1 to 3.5% w/v against all the bacteria and fungi involved in this study. After 2 days of incubation, fungi and *R. oryzae* cell counts decreased about two and three log ranks, respectively. No growth of bacteria was observed after 2 days of incubation, and interestingly, decreases of cell numbers were noted immediately after inoculation (1–2 logs). The minimal inhibitory concentration (MIC) for bacteria was lower than those for fungi (Table 2). However, the appearance of the liquid media had changed due to reduction of silver ions during incubation of bacteria in the presence of OKCEL® Ag-L. It presents self-protection mechanism of bacteria [9] since silver in its elemental form was purely absorbed by mammal and bacterial cells [7]. No silver ion reduction was observed when fungi and yeasts grew in the presence of OKCEL® Ag-L. In addition, this sample did not inhibit the growth of yeasts. This is in contradiction to several studies. Wells et al. [18] found that Ag^+ inhibited *C. albicans* by suppression of phosphomanose isomerase, a key enzyme for synthesis of their cell walls. The solution of $AgNO_3$ also inhibited the growth of *C. albicans*; however, Ag^+ formed electrochemically showed enhanced inhibitory activity [13]. On the other hand, the resistance of *Bacillus* spp., *P. aeruginosa*, *Salmonella typhimurium* and *Enterobacter cloacae* towards Ag^+ was documented [3, 19]. Moreover, a frequent exposition to elevated Ag^+ concentration also decreased the sensitivity of bacteria to silver ions [8]. An inability of OKCEL® Ag-L at the tested concentrations to suppress the growth of *Candida* species used in the study remained unclear. We can only speculate that the chemical or physical state of Ag^+ ions presented in oxidized cellulose samples plays a certain role in the antimicrobial activity against yeasts. In our previous work, textile samples impregnated with 0.66% (w/w) of silver ions ($AgNO_3$ was used) inhibited the growth of *C. albicans*, but no inhibition was observed when the colloid form of silver was tested [17].

OKCEL® ZnNa-M did not show antimicrobial activity against *P. aeruginosa* and partly inhibited the growth of *E. coli*, *P. chrysogenum*, *S. brevicaulis* and *A. niger* in the range of 0.5–3.5% w/v. Strong inhibition was observed in

Table 2 MIC values for two oxidized cellulose salts

Microorganism testing	MIC[a] (% w/v)	
	OKCEL® Ag-L	OKCEL® ZnNa-M
Pseudomonas aeruginosa	1.0	N
Staphylococcus epidermidis	1.3	2.0
Bacillus licheniformis	1.0	1.5
Escherichia coli	0.5	n[c]
Penicillium chrysogenum	2.0	n[c]
Scopulariopsis brevicaulis	3.5	n[c]
Aspergillus niger	2.5	n[c]
Rhizopus oryzae	3.5	1.0
Candida albicans	N[b]	1.0
Candida tropicalis	N[b]	0.5

[a] Minimal inhibitory concentration (the lowest concentration of antimicrobials resulting in no growth observed after 7 days incubation)

[b] The cell count increased during incubation in the range of 0.5–3.5% (w/v) after 7 days incubation

[c] The cell count decreased during incubation in the range of 0.5–3.5% (w/v) but bacterial cells were still detected after 7 days incubation

Table 3 The viable count (cfu/ml) of microorganisms after 0, 2nd and 7th day of incubation in the presence of oxidized cellulose and its salts in textile form (-T) in the concentration 9.0% (w/v) determined by dilution method

MO	OKCEL® H-T			OKCEL® Ag-T			OKCEL® Zn-T			OKCEL® Mg-T		
	Sampling time (days)			Sampling time (days)			Sampling time (days)			Sampling time (days)		
	0	2	7	0	2	7	0	2	7	0	2	7
PA[a]	<10	<10	<10	30	<10	<10	<10	<10	<10	1.8×10^3	$>10^4$	$>10^4$
SE[b]	4.6×10^2	10	<10	10	<10	<10	9.8×10^2	<10	<10	1.6×10^3	3.3×10^3	$>10^4$
BL[c]	<10	<10	<10	<10	<10	<10	2.1×10^2	<10	<10	3.4×10^3	$>10^4$	$>10^4$
PC[d]	1.9×10^3	1.1×10^3	1.1×10^2	3.0×10^3	8.5×10^2	4.6×10^3	3.0×10^3	5.3×10^2	10^2	2.1×10^2	1.3×10^2	$>10^4$
SB[e]	2.5×10^3	1.8×10^3	8.5×10^2	3.1×10^3	6.2×10^2	6.0×10^2	2.0×10^3	1.3×10^3	8.9×10^2	2.3×10^3	$>10^4$	$>10^4$
AN[f]	4.5×10^3	6.0×10^2	$>10^4$	5.3×10^3	1.4×10^3	$>10^4$	4.0×10^3	1.6×10^3	3.0×10^2	4.6×10^3	$>10^4$	$>10^4$
RO[g]	1.3×10^2	2.0×10^2	$>10^4$	2.0×10^2	30	50	1.5×10^2	20	<10	2.2×10^2	2.0×10^2	$>10^4$
CA[h]	6.1×10^2	2.5×10^3	$>10^4$	5.9×10^2	8.4×10^3	$>10^4$	8.1×10^2	<10	<10	7.8×10^2	$>10^4$	$>10^4$
CT[i]	9.9×10^2	5.4×10^3	$>10^4$	9.1×10^2	1.2×10^2	$>10^4$	9.6×10^2	<10	<10	1.3×10^2	$>10^4$	$>10^4$

[a] *Pseudomonas aeruginosa*

[b] *Staphylococcus epidermidis*

[c] *Bacillus licheniformis*

[d] *Penicillium chrysogenum*

[e] *Scopulariopsis brevicaulis*

[f] *Aspergillus niger*

[g] *Rhizopus oryzae*

[h] *Candida albicans*

[i] *Candida tropicalis*

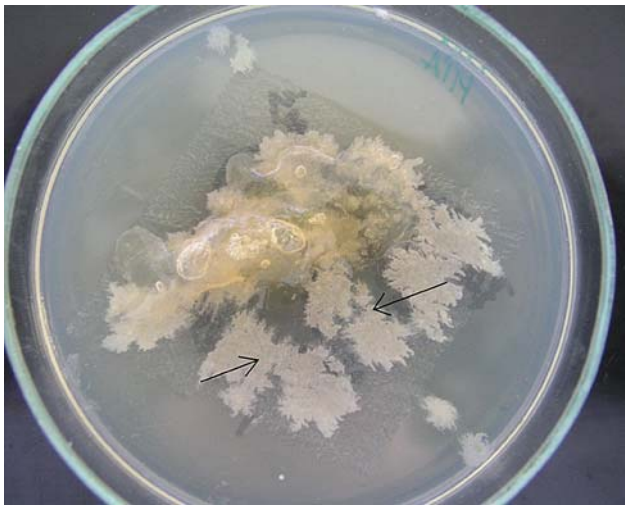

Fig. 1 The utilization of OKCEL® Mg-T sample by *Bacillus licheniformis* after 7 days incubation at 30 °C in Nutrient agar no. 2. An *arrow* indicates the decomposed sample

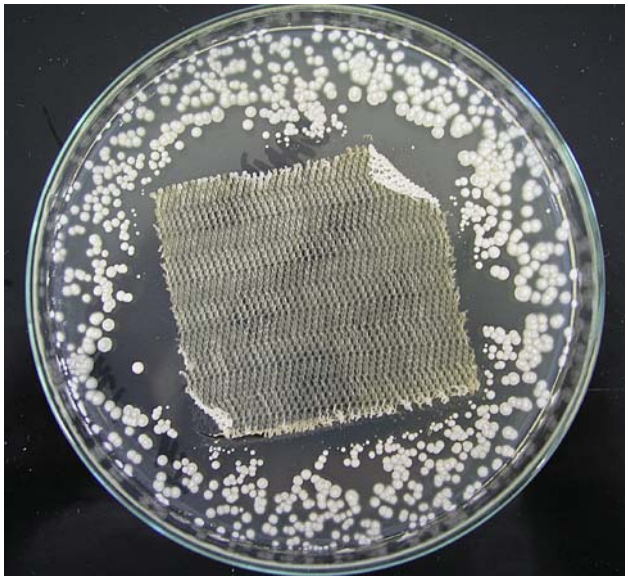

Fig. 2 The inhibition of *Candida tropicalis* by OKCEL® Zn-T after 7 days incubation at 24 °C on Malt agar plate

S. epidermidis, *B. licheniformis*, *R. oryzae*, *C. albicans* and *C. tropicalis* with MIC values that ranged from 0.5 to 2.0% w/v (Tab. 2). Our results are consistent with those reported by Södberg et al. [14] and Atmaca et al. [2] who concluded that Gram-positive bacteria were more sensitive to Zn^{2+} compared to Gram-negative bacteria.

Evaluation of antimicrobial activity of T-type of OC salts

The antimicrobial activities of cellulose salts in textile form against microorganisms determined by the dilution method are presented in Table 3. *P. aeruginosa*, *B. licheniformis* and *S. epidermidis* were most sensitive microorganisms to OKCEL® H-T, OKCEL® Ag-T, OKCEL® Zn-T with either no cells or 2–3 log decrease detected immediately after inoculation using the dilution method. The bactericidal effect of silver ions incorporated into the medicinal bandages predominantly against Gram-negative bacteria has been reported [5]. Moreover, these OKCEL® samples also successfully inhibited two Gram-positive microorganisms in our study. The sample with OKCEL® Mg-T did not show antimicrobial activity, and additionally the cellulose material was utilized by microorganisms after 7 days of incubation (Fig. 1). The lack of inhibitory action of OKCEL® Mg-T should be related to the low content of carboxyl groups (2.70% w/w) compared to OKCEL® H-T (19.20% w/w). Interestingly, the growth of *C. albicans* and *C. tropicalis* has been observed in the presence of OKCEL® Ag-T, similar to the activity of the linter form of oxidized cellulose salt with silver ions as mentioned earlier. On the other hand, textile samples made from OKCEL® Zn-T showed strong antimicrobial activity against both *Candida* species with no colonies detected after 2 days of incubation at 24 °C.

The zone of inhibition and growth/no growth of microorganisms on/under the textile samples was determined by the diffusion method. OKCEL® Ag-T inhibited the growth of bacteria in the presence of the zone of inhibition, whereas fungi and yeasts showed resistance with the exception of *R. oryzae* and *Sc. brevicaulis*. The inhibitory activity of OKCEL® Ag-T against the tested bacteria was accompanied by the reduction of silver ions resulting in a formation of brown pigment around the textile samples. In addition, OKCEL® Zn-T also showed enhanced antimicrobial activity against *R. oryzae*, *C. albicans* and *C. tropicalis* in the presence of the zone of inhibition and no growth on/under the textile samples as well (Fig. 2). Contradictory effects of silver and zinc OC salts against *C. albicans* and *C. tropicalis* have not been explained by comparing the contents of carboxyl groups or ions in samples.

A comparison of assays for determination of antimicrobial activity of textile form of OC and its salts

The antimicrobial efficiency of textile impregnated by OKCEL® substances is based upon the liberation rate of active substance into solution. The dilution method yields quantitative results about reduction rate of microorganisms; however, this procedure should not be recommended for textile samples since the textile material itself could be utilized by microorganisms. Moreover, the dilution method is more time consuming (7–14 days), needs intensive work and more laboratory equipments so it is mostly applied in research [4]. The diffusion method answers the question if the particular microorganism is sensitive or resistant to

antimicrobial compounds. However, the lack of standards similar to those determining the antibiotic resistance makes this method inaccurate. Nevertheless if no growth occurred on, under and around the textile sample placed on the surface of nutrient agar media, this may be recognized as one with good antimicrobial properties for further practical application. Moreover, the diffusion method is simple for laboring and gives real information about the function of oxidized cellulose in textile form.

Conclusion

The results of this study revealed that the acidic form of oxidized cellulose with zinc (OKCEL® Zn-T) has a great potential to be used as a dressing material in medicinal practices against bacteria and *Candida* species. Although OKCEL® Ag-T showed antimicrobial activity against the tested bacteria, a reduction of silver ion occurred accompanied by changes of surroundings to brown and/or black colors in contact with some bacteria. Interestingly, the OC salts with silver ions did not inhibit the growth of *C. albicans* and *C. tropicalis* in our research, probably due to the adverse chemical or physical state of silver ions in oxidized cellulose samples. This phenomenon needs to be resolved in further study.

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References

1. Abaev IK, Kaputskii VE, Adarchenko AA, Sobeshchukh OP (1986) Mechanism of the antibacterial action of monocarboxycellulose and other ion-exchange derivatives of cellulose. Antibiot Med Biotechnol 31:624–628
2. Atmaca S, Gül K, Çiçek R (1998) The effect of zinc on microbial growth. Tr J Med Sci 28:595–597
3. Cason JS, Jackson DM, Lowbury EJ, Ricketts CR (1966) Antiseptic and aseptic prophylaxis for burns: use of silver nitrate and of isolators. Br Med J 2:1288–1294
4. Ferrari MJ, Turnidge JD (2003) Antibacterial agents and suspectibility test methods. ASM Press, Washington, pp 1037–1196
5. Ip M, Lui SL, Poon VK, Lung I, Burd A (2006) Antimicrobial activities of silver dressings: an in vitro comparison. J Med Microbiol 55:59–63
6. Kumar V (2004) Regenerated cellulose and oxidized cellulose membranes as potential biodegradable platforms for drug delivery and tissue engineering. University of Iowa Research Foundation (Iowa City, IA), United States Patent no. 6.800.753. 5. 10. 2004
7. Lansdown AB (2002) The role of silver. J Eur Tissue Rep Soc 9:108–111
8. Li XZ, Nikaido H, Williams KE (1997) Silver-resistant mutants of *Escherichia coli* display active efflux of Ag^+ and are deficient in porins. J Bacteriol 179:6127–6132
9. Maillard JY, Denyer SP (2006) Focus on silver. EWMA J 6:5–7
10. Percival SL, Bowler PG, Russell D (2005) Bacterial resistance to silver in wound care. J Hosp Infect 60:1–7
11. Pernet M (1983) Antibacterial effect of oxidized regenerated cellulose. Ann Chir 37:700–701
12. Russell AD, Hugo WB (1994) Antimicrobial activity and action of silver. Prog Med Chem 31:351–370
13. Simonetti N, Simonetti G, Bougnol F, Scalzo M (1992) Electrochemical Ag^+ for preservative use. Appl Environ Microbiol 58:3834–3836
14. Södeberg TA, Sunze B, Holm S, Elmro T, Hallmans G, Sjöberg S (1990) Antibacterial effect of zinc oxide in vitro. Scand J Plast Reconstr Hand Surg 24:193–197
15. Spangler D, Rothenburger S, Nguyen K, Jampani H, Weiss S, Bhende S (2003) In vitro antimicrobial activity of oxidized regenerated cellulose against antibiotic-resistant microorganisms. Surg Infect (Larchmt) 4:255–262
16. Sugarman B (1983) Zinc and infection. Rev Infect Dis 5:138–147
17. Valouchová V, Vytrasova J (2005) Antimicrobial activity of impregnated textiles. Diploma thesis, University of Pardubice, Pardubice
18. Wells TN, Scully P, Paravicini G, Proudfoot AE, Payton MA (1995) Mechanisms of irreversible inactivation of phosphomannose isomerases by silver ions and flamazine. Biochemistry 34:7896–7903
19. Woodward RL (1963) Review of the effectiveness of silver. J Am Water Works Assoc 55:881–886