The effect of silver ions and silver nanoparticles on *Staphylococcus aureus*

D. Chudobova[1], D. Maskova[1], L. Nejdl[1], P. Kopel[1,2], M. A. Merlos Rodrigo[1], V. Adam[1,2] and R. Kizek[1,2]

[1] DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY, Faculty of Agronomy, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic, European Union
[2] CENTRAL EUROPEAN INSTITUTE OF TECHNOLOGY, Brno University of Technology, Technicka 3058/10, CZ-616 00 Brno, Czech Republic, European Union

Bacterial infections can cause serious health issues in treatment of tumour diseases, as they are one of the leading causes of dead in the developed countries. *Staphylococcus aureus* belong to the bacterial strains causing major health issues. Moreover, this strain is well adaptable to the environment. It is not surprising that formation of antibiotics resistance and creation of multi-resistant strains was found. Therefore, looking for new approaches for eliminating of the bacterial threat is still topic. Advanced metal-based nanomaterials seem to have promising properties for this purpose. The aim of this study was to determine the antimicrobial effect of different concentrations of silver nanoparticles (200 nm) on gram-positive bacteria *S. aureus*. Studying of the development of bacteria in the hostile conditions is also interesting from the point of view of very basic questions "what is live" and "are we alone". Many bacteria have the property to adapt over time to an environment modified with heavy metals. Recently, potential changes in sugar phosphate skeleton of DNA were described and discussed due to the effect of arsenic. Primarily, we utilized Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry for basic characterization of bacterial strain of *S. aureus*. Then, we used two types of methods for determination of silver nanoparticles inhibition effect as (i) method based on the growth curves and (ii) measurements of inhibition zones. The applied silver nanoparticles also generated oxidative stress revealed by spectrophotometric assays. We confirmed that silver nanoparticles had considerable inhibition of the growth of *S. aureus*.

Keywords nanomaterial; metal; inhibition; mass spectrometry; spectrometry; bacterial resistance

1. Introduction

Bacterial infections represent one of the most serious complications in the vascular surgery [1]. Increasing use of artificial vascular implants is the main cause of these complications [2]. Infections cause the death of 1 – 10 % of patients [3,4], who underwent transplantation of a blood vessel [5-7]. Pathogenic bacteria *S. aureus*, especially methicillin-resistant *S. aureus* (MRSA) that is responsible for more than one half of all infections in vascular surgery [8,9], is considered to be the most important cause of infections [8-11]. Resistance to antibiotics occurs in a wide range of nosocomial pathogens [12]. The emergence and spreading of multi-resistant strains of bacteria is based on the interactions of various factors, which include mutations in the genes encoding resistance to antibiotics (broaden, "improved" ability to resist to antimicrobial drugs), an exchange of genetic information between microorganisms, where genes for resistance are transferred to the new "hosts" bacteria, and the specific conditions of the hospital environment that facilitate the development and spreading of multi-resistant bacteria [13]. In order to prevent infections in vascular surgery substances with antibacterial effect are used. One of the possible solutions of this problem is to cover the surface of artificial vascular implant by antibiotics (rifampin, gentamicin, amikacin, vancomycin, levofloxacin), and to impregnate them by collagen or gelatine [14-16]. The use of antibiotics as antimicrobial agents has one fundamental disadvantage as the development of bacterial resistance to antibiotics used. Silver represents another compound with antimicrobial property that could reduce the incidence of infections in transplant surgery [17,18]. High toxicity of this element to prokaryotic cells has been established and is well known [19,20]. On the other hand, silver itself is almost nontoxic for eukaryotic cells (including animals). In addition, it promotes proliferation and creation of new cells (silver has found application in dermatology) and reduce probability of development of resistance, such as in the case of antibiotics [21]. Figure 1 describes mechanism of action of silver ions on bacteria. This effect is based on the penetration of silver ions the bacterial cell wall and the plasma membrane and inactivation of membrane-bound proteins by binding silver ions, and binding the bacterial DNA with subsequent disrupting the replication of DNA. In addition, silver ions impair the ability of ribosomes to translate messenger RNA into the form of essential proteins. Silver ions also activate cytochrome b [22,23].

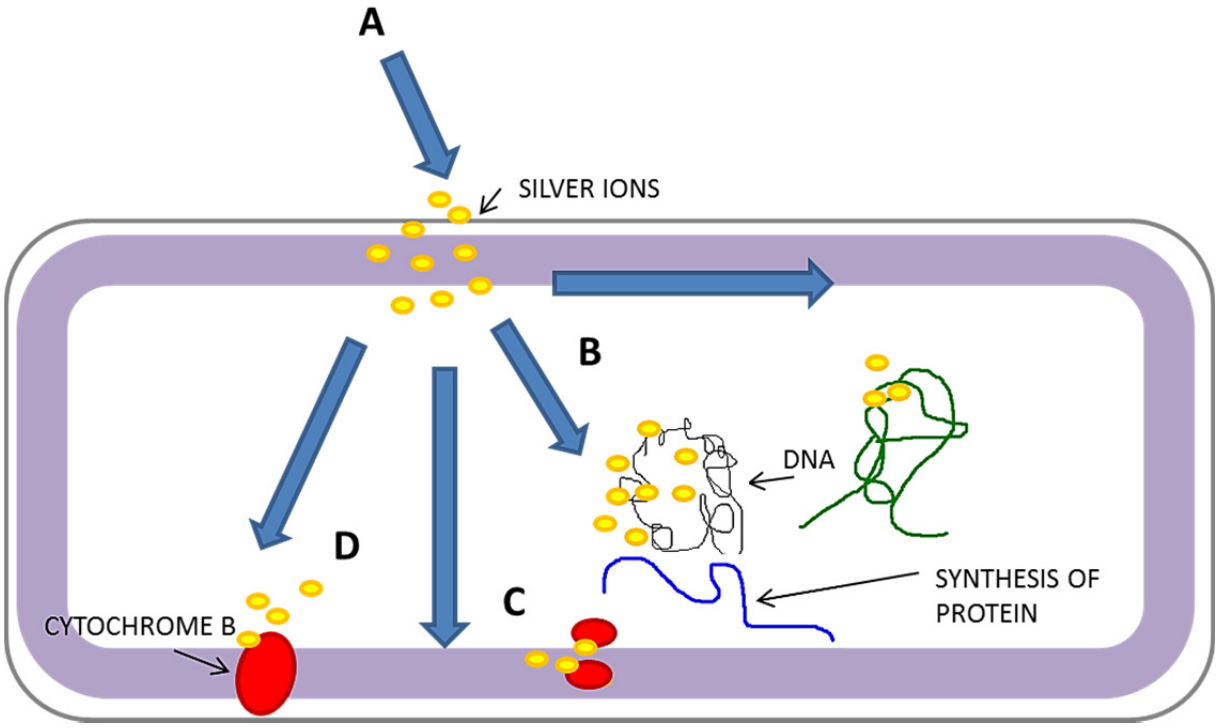

Fig. 1 Mechanisms of action of the silver ions on bacteria **a)** Silver ions penetrate the bacterial cell wall and bind to the phospholipid layer of the cytoplasmic membrane, **b)** Silver ions bind the bacterial DNA with subsequent disrupting DNA replication, **c)** Silver ions impair the ability of ribosomes to transcribe messenger RNA, **d)** Silver ions bind the sulfhydryl group of the cytochrome b. Adopted and modified according to [16].

2. Nanotechnologies and their application to prevent bacterial infections

2.1. Nanotechnologies

Nanotechnology is one of the most progressive branches of science today. The requirement is to modify nanoparticles to comply with our requirements in terms of size, shape, texture and distribution in organism. Metal nanoparticles of the order of sizes of 1-200 nm can be modified by applying layers of different compounds, such as biopolymers [24]. Nanoparticles prepared in this way show modified physical and chemical properties in the comparison with metal-based nanoparticles [25,26]. Due to these properties, the nanoparticles are used as catalysts, and electrochemical sensors; in addition, they have found many applications in biomedicine [27]. Silver nanoparticles are commonly used in the textile industry and medicine [28]. Silver ions affect process of the proliferation of cells, and silver ions have found application in dermatology to facilitate healing of wounds. In addition, antimicrobial properties of silver ions may be modified by a preparation of silver nanoparticles, which surface can be modified effectively. This nanomaterial is applicable in a wide variety of therapeutic uses [29]. In addition, silver nanoparticles or generally “nanosilver” show enhanced antimicrobial effect in comparison with silver ions [30]. Nanoparticles of dimension of at least 100 nm or less have unique physicochemical properties, such as high catalytic capability due to large surface, and the ability to generate reactive oxygen species [31]. Silver in the form of nanoparticles may therefore be more reactive due to its catalytic properties and becomes more toxic to bacteria than silver ions [32].

2.2. Preparation and characterization of silver phosphate nanoparticles

We prepared silver nanoparticles in accordance with method described by Khan et al. [33], where (di)sodium hydrogen phosphate heptahydrate was dissolved in ACS water and subsequently solution of silver nitrate in ACS water was added. The reaction proceeded immediately with the formation of yellow colloidal nanoparticles. This mixture was stirred for approximately one hour and then stored at 4 ° C in the dark. Formation of nanoparticles is very noticeable, as already mentioned, as a change in colour from colourless to yellow. The resulting nanoparticles were observed and viewed using SEM (scanning electron microscopy), where a compact structure of the nanoparticles was observed (Figs. 2a and 2b). However, also relatively large rifts in the structure of nanoparticles were evident. These structures can provide a space for the binding of different molecules, or eventually bacteria. Chosen microscopic technique shows a typical spherical structure of nanoparticles of the size of 80-350 nm; however, most of them show the diameter of 200-300 nm (more than 80%).

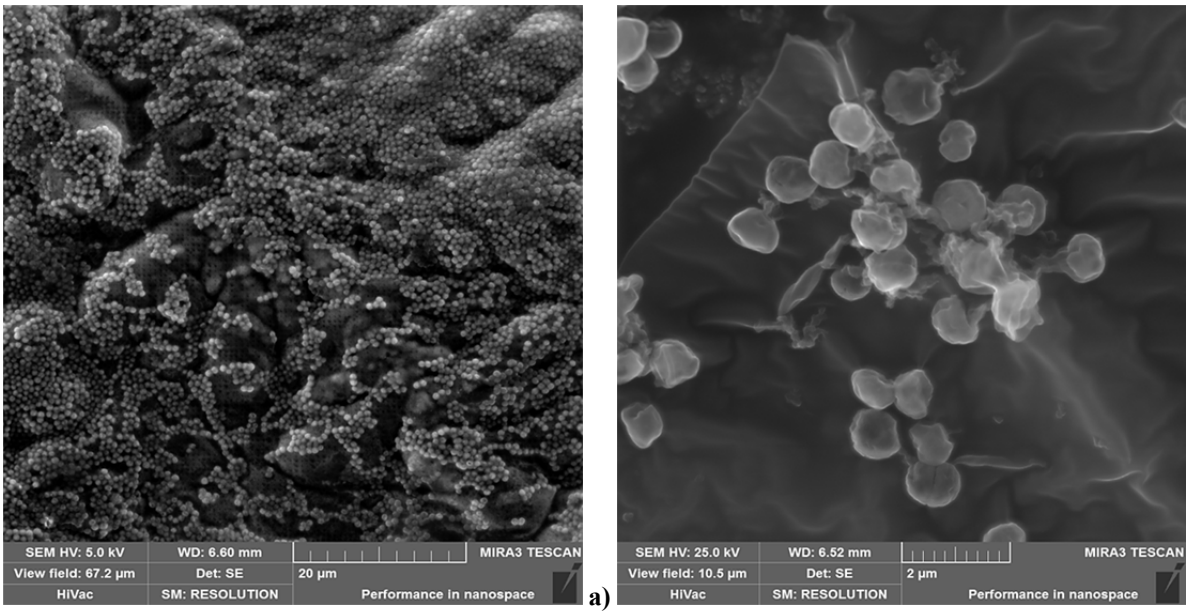

Fig. 2 AgNPs characterized by SEM micrograph: **a**) SEM HV: 5 kV, view field: 67.2 µm, WD: 6.60 mm, det: SE**, b)** SEM HV: 25 kV, view field: 10.5 µm, WD: 6.52 mm, det: SE.

2.3. Mass spectrometry for identification of bacteria

Matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF) technique was used to identify and to verify the purity of the bacterial culture of *S. aureus* (Figs. 3a, 3b, 3c and 3d). This technique combines the ionisation process and the TOF analyser for separating ions [34,35] and is commonly used to identify bacteria in clinical samples [36,37]. Bacterial culture of *S. aureus* was cultivated overnight and then centrifuged. The supernatant was discarded and the remaining pellet was resuspended in deionized water. Then ethanol was added into the sample, the sample was centrifuged and the pellet air-dried. Dried pellet was mixed with formic acid and acetonitrile, centrifuged and used for analysis by MALDI-TOF technique. The spectra were measured in the range from 2,000 to 20,000 Da. Spectra obtained were analysed using a software for analysis of samples that is provided by the manufacturer. Spectra with peaks out of these limits were not evaluated. Results in the range of 3.00 to 2.30 indicate the reliability of the results for tested bacterial culture. If the value falls below the limit, identification cannot be considered sufficient.

2.4. Antimicrobial activity of silver nanoparticles

It is generally known that silver ions inhibit the growth of microorganisms, and therefore, these ions represent a suitable material for incorporation into a variety of materials where the antimicrobial activity should be accentuated [38,39]. However, cytotoxic property of silver ions may reduce cell viability and proliferative activity of cells. Until recently, silver nanoparticles were commonly used in implanted materials to reduce the incidence of postoperative complications (infection), but these applications were commonly associated with a potential risk due to their toxicity [40]. Silver ions cause an inhibition of cell division; they interact with nucleic acids and thiol groups of amino acids and proteins. They are stored in structures similar to vacuoles in eukaryotic cells, vesicles, and in the cell walls in the form of "granules" (precipitates) [41]. For these reasons, silver ions are used to control the bacterial growth in a number of medical and non-medical applications [30,42-44]. The mechanism of action of silver ions is based on the inactivation of membrane proteins, interferences with the electron transport system, and inhibition of the respiratory enzymes to promote the creation of reactive oxygen species. Generally, increased concentration of silver ions leads to the increase in oxidative stress. Oxidative stress is one of the indicators that allow monitoring the toxic effects of heavy metals on microorganisms. This toxic effect is based on the binding of silver ions into the bacterial cell wall and plasma membrane, which leads to inhibition of the respiratory process of the bacteria [30,45,46].

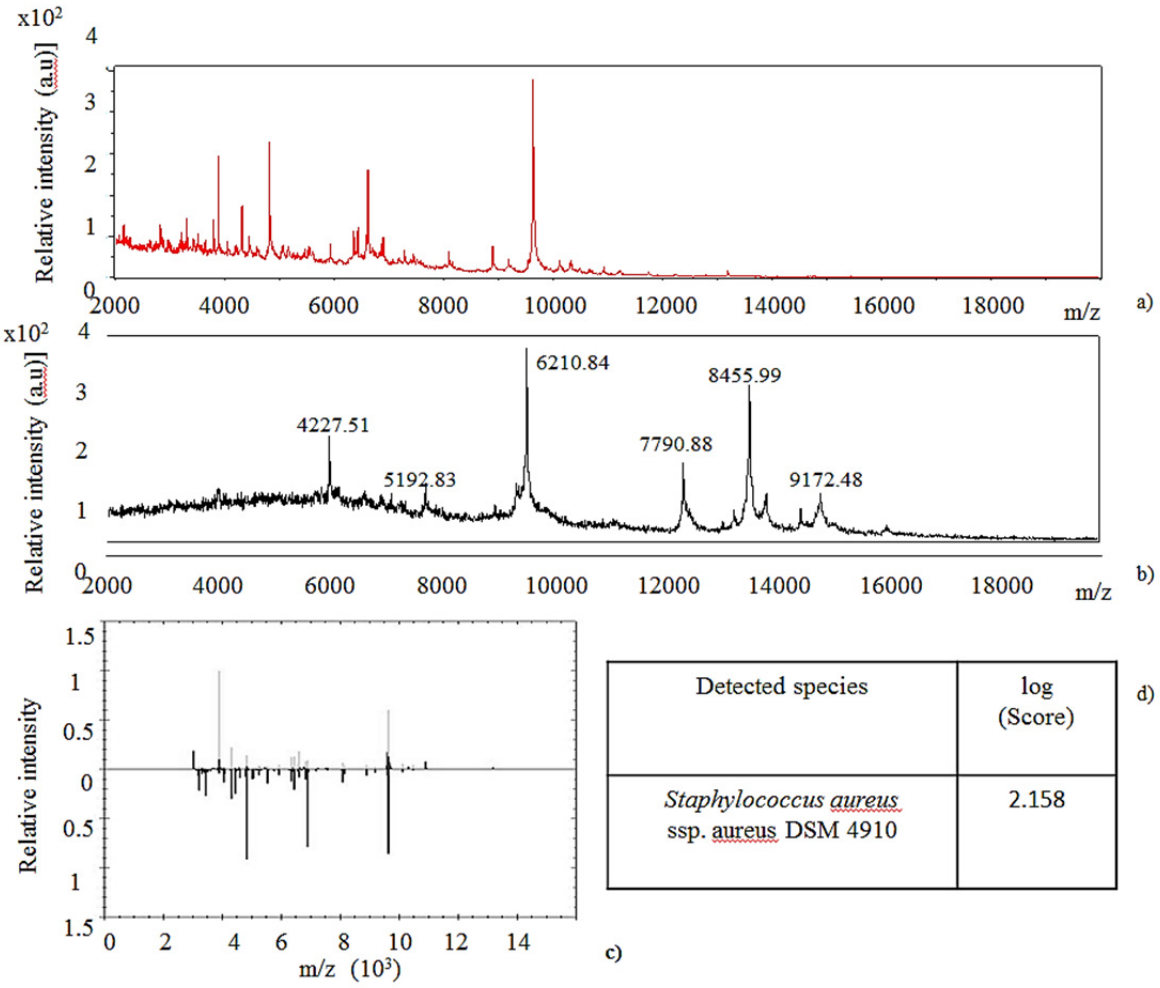

Fig. 3 a) MALDI/TOF mass spectra protein fingerprints for the identification of *S. aureus*. Data were collected in the *m/z* 2,000–20,000 range after processing 1 ml of *S. aureus* culture, **b)** MALDI/TOF mass spectra protein fingerprints for the identification of *S. aureus* with silver ions**, c)** comparison of spectra of biological samples and MSP library by MALDI Biotyper3 and **d)** species identification by MALDI Biotyper3 using HCCA as a matrix.

Bacteria have defence mechanisms that can effectively eliminate free radicals created, and thus eliminate the toxic effects of silver ions [22,47]. The excess of reactive oxygen species (ROS) allows monitoring the oxidative stress on the basis of the determination of antioxidant capacity. There are different methods that can be used to study oxidative stress. Spectrophotometric methods [48,49] belong to the most commonly used methods. Spectrophotometric methods, such as ABTS - 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), DPPH - 2,2-diphenyl-1-picrylhydrazyl, etc. to determine antioxidant capacity, or electrochemical methods using cyclic voltammetry on printed electrodes [50-53] are the most important methods in the study of oxidative stress. Results confirm the possibility of using these analytical techniques in microbiology to determine oxidative stress in bacterial cultures [54]. As it is shown in Figs. 4a and 4b, testing the antibacterial properties of silver nanoparticles was performed on a bacterial culture of *S. aureus* by measuring the size of the inhibition zones, where crosswise two squares of size 1x1 cm cut out from artificial vascular grafts and covered with the substance tested were placed into the Petri dish coated by bacterial culture. According to the size of the resulting zones of inhibition was then evaluated the antimicrobial activity of the tested components. The second method is the determination of the growth properties of the bacterial culture by the characterization of bacterial growth - construction of growth curves. This method can be used to determine the minimum inhibitory concentration and the maximal inhibitory concentration. The bacterial culture was mixed with the tested compounds (nanoparticles) and measured in half hour intervals at 37 °C for 24 hours. The resulting values of absorbance were then expressed by the growth curves individually for each concentration of silver nanoparticles.

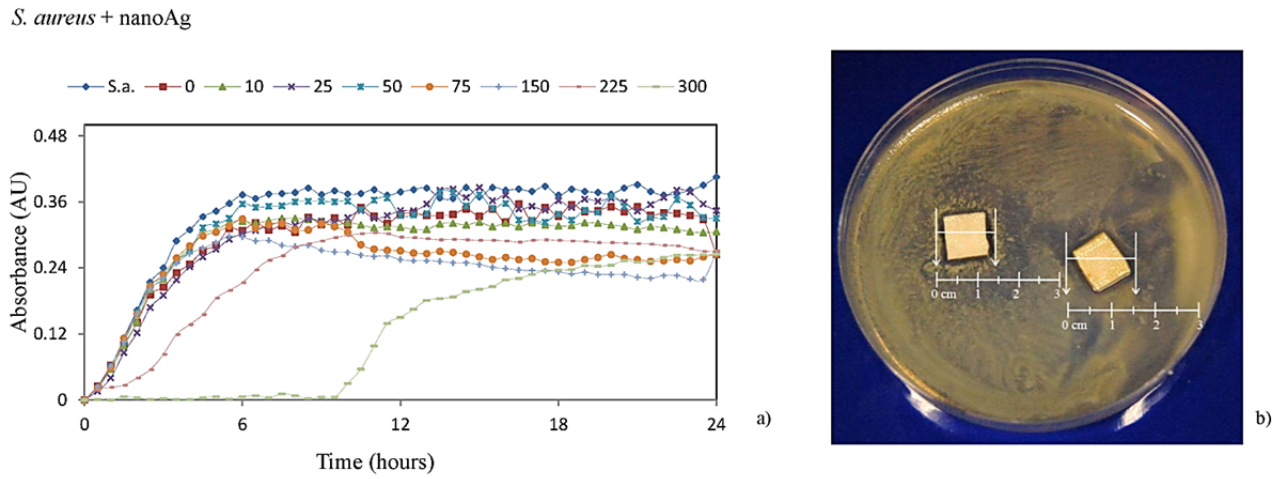

Fig. 4 Spectrophotometric analysis of the growth of *S. aureus* bacterial culture with silver nanoparticles in concentrations 0, 10, 25, 50, 75, 150, 225, and 300 μM: **a)** growth curves of silver phosphate nanoparticles on *S. aureus*, **b)** inhibition zones of silver phosphate nanoparticles on *S. aureus*.

It has been established that the complexes of silver ions or silver nanoparticles with chitosan show the highest antimicrobial properties. These results can be also used for further experiments with the possible application in vascular surgery, particularly to reduce the risk of bacterial infections, which represent a high risk of vascular grafts when implanted into the patient's body, in the future. The bacterial culture can be further assessed by its biochemical properties, which may naturally be influenced by the addition of silver ions or silver nanoparticles. The most common way to test biochemical properties of microorganisms is based on the principle of the interaction of the bacterial culture with 24 different compounds at the bottom of the microplate wells. After mixing with the microbial culture, apparent colour change as well as change in measured values of absorbance appears. The biochemical test allows carrying out forty examinations by the use of twenty four biochemical tests and visual as well as instrumental evaluation. Substances placed on the bottom microplates were the following: urease, arginine, ornithine, β-galactosidase, β-glucuronidase, β-glucosidase, phosphatase, esculin, N-acetyl-β-D-glucosamine, sucrose, mannitol, xylose, galactose, trehalose, maltose, mannose, lactose, sorbitol, ribose, fructose, cellobiose, arabinose, xylitol and raffinose. Based on the composition of bacterial cultures there are different metabolic changes of these substances and as a result changes in the colour in the wells appear. Colour comparative scale serves to evaluate the colour reactions. The result of the measurements were visible colour reactions caused from interactions of bacterial culture *S. aureus* in a complex with silver nanoparticles after application to the bottom of the wells of microplate. The complex of bacterial culture and silver nanoparticles showed the great biochemical activity in terms of all the substances in biochemical test, with all these ingredients the complex reacted and thus there are significant biochemical changes.

3.5. Increasing the antimicrobial effect of silver ions or silver nanoparticles on bacterial cultures

The application of ions of metals or their nanoparticles in combination with other substances with antimicrobial properties is the next way how to increase the antimicrobial activity [55,56]. It is therefore important to determine the effect of silver ions and silver nanoparticles in combination with compounds that also showing antibacterial effect. Hyaluronic acid (Fig. 5a) and chitosan (Fig. 5b) were the applied biopolymers with antimicrobial activity, which are characterized by biodegradability and biocompatibility with the human body. Hyaluronic acid is nonsulfated anionic glycosaminoglycan that is generally considered as an extracellular matrix that facilitates cell motility and proliferation [57,58]. Hyaluronic acid is present in almost all biological fluids and tissues [58]. Chitosan is a linear polysaccharide composed of β-(1-4)-D-glucosamine and N-acetyl-D-glucosamine moieties and is the most important derivative of chitin [59]. Chitosan is an effective material for biomedical applications, particularly in terms of the ability to heal wounds and antimicrobial and anti-inflammatory activities [60-62]. Chitosan can bind metals to form complexes with them [61,63,64]. These interactions are based on binding of metal ions to the amino groups of chitosan by chelating or complexation mechanisms [59]. Similarly, it has been demonstrated that hyaluronic acid is able to bind onto silver nanoparticles [65]. Application of a combination of different forms of silver and biopolymer materials to cover the vascular graft is therefore very suitable solution in the elimination of resistant strains of bacteria. Our work focuses on evaluation of antimicrobial activity of each component alone or in complexes using basic microbiological methods. Thus, choice of the best complex in terms of both antimicrobial activity and effectiveness of the application of this complex to the human belongs also to the objectives of this stud. Our results show the highest antimicrobial properties of complexes of silver ions or silver nanoparticles and chitosan (Figs. 6a, 6b, 6c and 6d), which can be used in the

future in vascular surgery, especially to reduce the risk of bacterial infections, which are a major threat during the implantation of artificial vascular grafts [66].

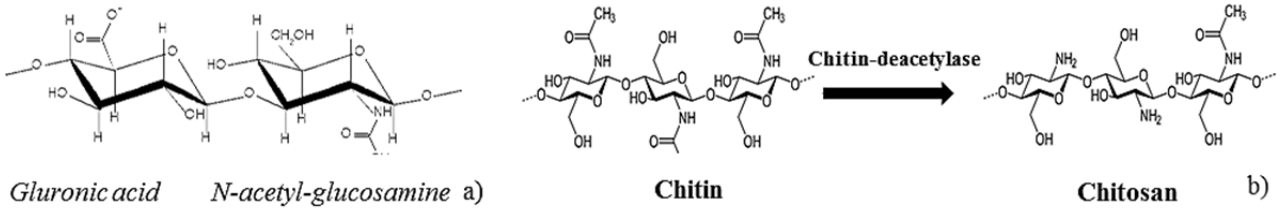

Fig. 5 Structure of polymers (a) Structure of hyaluronic acid, (b) The course of enzymatic deacetylation of chitin and subsequent development of chitosan

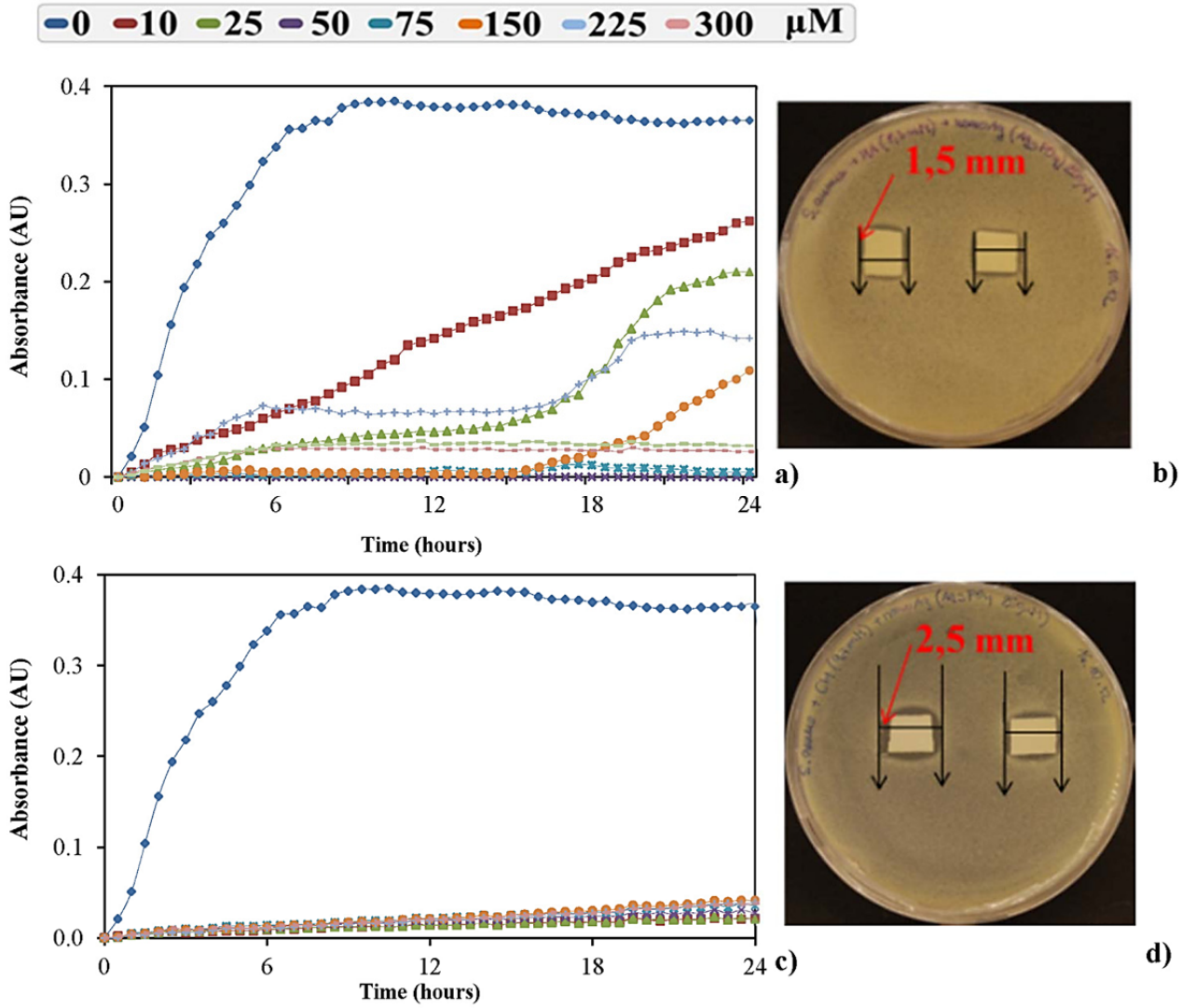

Fig. 6 Spectrophotometric analysis of the growth of *S. aureus* bacterial culture with silver nanoparticles in concentrations 0, 10, 25, 50, 75, 150, 225, and 300 µM and hyaluronic acid or chitosan: a) growth curves of silver phosphate nanoparticles in complex with hyaluronic acid on *S. aureus*, b) inhibition zones of silver phosphate nanoparticles in complex with hyaluronic acid on *S. aureus*, c) growth curves of silver phosphate nanoparticles in complex with chitosan on *S. aureus*, d) inhibition zones of silver phosphate nanoparticles in complex with chitosan on *S. aureus*.

Acknowledgments The support by TA CR NanoCeva TA01010088 is gratefully acknowledged. The authors wish to express their thanks to Assoc. Prof. Jindrich Kynicky for SEM photos and to Matej Sklenar for perfect technical assistance.

References

[1] Perl TM, Cullen JJ, Wenzel RP, Zimmerman MB, Pfaller MA, Sheppard D, Twombley J, French PP, Herwaldt LA and Mupirocin Risk Staphyloccocus A. Intranasal mupirocin to prevent postoperative staphylococcus aureus infections. *N. Engl. J. Med.* 2002;24:1871-1877.

[2] Ginalska G, Osinska M, Uryniak A, Urbanik-Sypniewska T, Belcarz A, Rzeski W and Wolski A. Antibacterial activity of gentamicin-bonded gelatin-sealed polyethylene terephthalate vascular prostheses. *Eur. J. Vasc. Endovasc. Surg.* 2005;4:419-424.

[3] Bandyk DF. Vascular surgical site infection: Risk factors and preventive measures. *Semin. Vasc. Surg.* 2008;3:119-123.

[4] Teebken OE, Bisdas T, Assadian O and Ricco JB. Recommendations for Reporting Treatment of Aortic Graft Infections. *Eur. J. Vasc. Endovasc. Surg.* 2012;2:174-181.

[5] Homer-Vanniasinkam S. Surgical site and vascular infections: treatment and prophylaxis. *Int. J. Infect. Dis.* 2007S17-S22.

[6] O'Brien R, Pocock N and Torella F. Wound infection after reconstructive arterial surgery of the lower limbs: Risk factors and consequences. *Surg. J. R. Coll. Surg. Edinb. Irel.* 2011;5:245-248.

[7] Tatterton MR and Homer-Vanniasinkam S. Infections in vascular surgery. *Injury-Int. J. Care Inj.* 2011S35-S41.

[8] Anderson DJ, Sexton DJ, Kanafani ZA, Auten G and Kaye KS. Severe surgical site infection in community hospitals: Epidemiology, key procedures, and the changing prevalence of methicillin-resistant staphylococcus aureus. *Infect. Control Hosp. Epidemiol.* 2007;9:1047-1053.

[9] Earnshaw JJ. Methicillin-resistant Staphylococcus aureus: Vascular surgeons should fight back. *Eur. J. Vasc. Endovasc. Surg.* 2002;4:283-286.

[10] Nasim A, Thompson MM, Naylor AR, Bell PRF and London NJM. The impact of MRSA on vascular surgery. *Eur. J. Vasc. Endovasc. Surg.* 2001;3:211-214.

[11] Young MH, Upchurch GR and Malani PN. Vascular Graft Infections. *Infect. Dis. Clin. North Am.* 2012;1:41-56.

[12] Tenover FC. Mechanisms of antimicrobial resistance in bacteria. *Am. J. Med.* 2006;6:S3-S10.

[13] Tenover FC. Development and spread of bacterial resistance to antimicrobial agents: An overview. *Clin. Infect. Dis.* 2001S108-S115.

[14] Driscoll AJ, Bhat N, Karron RA, O'Brien KL and Murdoch DR. Disk Diffusion Bioassays for the Detection of Antibiotic Activity in Body Fluids: Applications for the Pneumonia Etiology Research for Child Health Project. *Clin. Infect. Dis.* 2012S159-S164.

[15] Lew W and Moore W. Antibiotic-Impregnated Grafts for Aortic Reconstruction. *Semin. Vasc. Surg.* 2011;4:211-219.

[16] Ricco JB and Assadian O. Antimicrobial Silver Grafts for Prevention and Treatment of Vascular Graft Infection. *Semin. Vasc. Surg.* 2011;4:234-241.

[17] Green JBD, Fulghum T and Nordhaus MA. Review of immobilized antimicrobial agents and methods for testing. *Biointerphases*. 2011;4:MR13-MR28.

[18] Osinska-Jaroszuk M, Ginalska G, Belcarz A and Uryniak A. Vascular Prostheses with Covalently Bound Gentamicin and Amikacin Reveal Superior Antibacterial Properties than Silver-impregnated Ones - An In Vitro Study. *Eur. J. Vasc. Endovasc. Surg.* 2009;6:697-706.

[19] Rai MK, Deshmukh SD, Ingle AP and Gade AK. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. *J. Appl. Microbiol.* 2012;5:841-852.

[20] Unger C and Luck C. Inhibitory effects of silver ions on Legionella pneumophila grown on agar, intracellular in Acanthamoeba castellanii and in artificial biofilms. *J. Appl. Microbiol.* 2012;6:1212-1219.

[21] Xu HY, Qu F, Xu H, Lai WH, Wang YA, Aguilar ZP and Wei H. Role of reactive oxygen species in the antibacterial mechanism of silver nanoparticles on Escherichia coli O157:H7. *Biometals*. 2012;1:45-53.

[22] Park HJ, Kim JY, Kim J, Lee JH, Hahn JS, Gu MB and Yoon J. Silver-ion-mediated reactive oxygen species generation affecting bactericidal activity. *Water Res.* 2009;4:1027-1032.

[23] Kwakye-Awuah B, Williams C, Kenward MA and Radecka I. Antimicrobial action and efficiency of silver-loaded zeolite X. *J. Appl. Microbiol.* 2008;5:1516-1524.

[24] Khan MAM, Kumar S, Ahamed M, Alrokayan SA, Alsalhi MS, Alhoshan M and Aldwayyan AS. Structural and spectroscopic studies of thin film of silver nanoparticles. *Appl. Surf. Sci.* 2011;24:10607-10612.

[25] Banfi G, Degiorgio V and Ricard D. Nonlinear optical properties of semiconductor nanocrystals. *Adv. Phys.* 1998;3:447-510.

[26] Banyai L, Hu YZ, Lindberg M and Koch SW. 2-photon optical nonlinearities in semiconductor quantum dots. *J. De Phys.* 1988;C-2:225-228.

[27] Khlebtsov NG and Dykman LA. Optical properties and biomedical applications of plasmonic nanoparticles. *J. Quant. Spectrosc. Radiat. Transf.* 2010;1:1-35.

[28] Sharma BK, Gupta AK, Khare N, Dhawan SK and Gupta HC. Synthesis and characterization of polyaniline-ZnO composite and its dielectric behavior. *Synth. Met.* 2009;5-6:391-395.

[29] Shahzad MN and Ahmed N. Effectiveness of Aloe Vera Gel compared with 1% silver sulphadiazine cream as burn wound dressing in second degree burns. *J. Pak. Med. Assoc.* 2013;2:225-230.

[30] Li WR, Xie XB, Shi QS, Duan SS, Ouyang YS and Chen YB. Antibacterial effect of silver nanoparticles on Staphylococcus aureus. *Biometals*. 2011;1:135-141.

[31] Limbach LK, Wick P, Manser P, Grass RN, Bruinink A and Stark WJ. Exposure of engineered nanoparticles to human lung epithelial cells: Influence of chemical composition and catalytic activity on oxidative stress. *Environ. Sci. Technol.* 2007;11:4158-4163.

[32] Choi O, Deng KK, Kim NJ, Ross L, Surampalli RY and Hu ZQ. The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. *Water Res.* 2008;12:3066-3074.

[33] Khan A, Qamar M and Muneer M. Synthesis of highly active visible-light-driven colloidal silver orthophosphate. *Chem. Phys. Lett.* 201254-58.

[34] Murray PR. What Is New in Clinical Microbiology Microbial Identification by MALDI-TOF Mass Spectrometry A Paper from the 2011 William Beaumont Hospital Symposium on Molecular Pathology. *J. Mol. Diagn.* 2012;5:419-423.
[35] Welker M. Proteomics for routine identification of microorganisms. *Proteomics*. 2011;15:3143-3153.
[36] Jordana-Lluch E, Catala EM and Ruiz VA. Mass spectrometry in the clinical microbiology laboratory. *Enferm. Infec. Microbiol. Clin.* 2012;10:635-644.
[37] Kok J, Chen SCA, Dwyer DE and Iredell JR. Current status of matrix-assisted laser desorption ionisation-time of flight mass spectrometry in the clinical microbiology laboratory. *Pathology*. 2013;1:4-17.
[38] Martinez-Abad A, Sanchez G, Lagaron JM and Ocio MJ. On the different growth conditions affecting silver antimicrobial efficacy on Listeria monocytogenes and Salmonella enterica. *Int. J. Food Microbiol.* 2012;2:147-154.
[39] Percival SL, Thomas J, Linton S, Okel T, Corum L and Slone W. The antimicrobial efficacy of silver on antibiotic-resistant bacteria isolated from burn wounds. *Int. Wound J.* 2012;5:488-493.
[40] Liu S, Zhao JW, Ruan HJ, Wang W, Wu TY, Cui WG and Fan CY. Antibacterial and anti-adhesion effects of the silver nanoparticles-loaded poly(L-lactide) fibrous membrane. *Mater. Sci. Eng. C-Mater. Biol. Appl.* 2013;3:1176-1182.
[41] Furr JR, Russell AD, Turner TD and Andrews A. Antibacterial activity of actisorb-plus, actisorb and silver-nitrate *J. Hosp. Infect.* 1994;3:201-208.
[42] Jung WK, Kim SH, Koo HC, Shin S, Kim JM, Park YK, Hwang SY, Yang H and Park YH. Antifungal activity of the silver ion against contaminated fabric. *Mycoses*. 2007;4:265-269.
[43] Klasen HJ. Historical review of the use of silver in the treatment of burns. I. Early uses. *Burns*. 2000;2:117-130.
[44] Low WL, Martin C, Hill DJ and Kenward MA. Antimicrobial efficacy of silver ions in combination with tea tree oil against Pseudomonas aeruginosa, Staphylococcus aureus and Candida albicans. *Int. J. Antimicrob. Agents*. 2011;2:162-165.
[45] Blecher K and Friedman A. Nanotechnology and the Diagnosis of Dermatological Infectious Disease. *J. Drugs Dermatol.* 2012;7:846-851.
[46] Jung WK, Koo HC, Kim KW, Shin S, Kim SH and Park YH. Antibacterial activity and mechanism of action of the silver ion in Staphylococcus aureus and Escherichia coli. *Appl. Environ. Microbiol.* 2008;7:2171-2178.
[47] Lushchak VI. Oxidative stress and mechanisms of protection against it in bacteria. *Biochem.-Moscow*. 2001;5:476-489.
[48] Barriere C, Leroy-Setrin S and Talon R. Characterization of catalase and superoxide dismutase in Staphylococcus carnosus 833 strain. *J. Appl. Microbiol.* 2001;3:514-519.
[49] Bir F, Khireddine H, Touati A, Sidane D, Yala S and Oudadesse H. Electrochemical depositions of fluorohydroxyapatite doped by Cu2+, Zn2+, Ag+ on stainless steel substrates. *Appl. Surf. Sci.* 2012;18:7021-7030.
[50] Estevao MS, Carvalho LC, Ferreira LM, Fernandes E and Marques MMB. Analysis of the antioxidant activity of an indole library: cyclic voltammetry versus ROS scavenging activity. *Tetrahedron Lett.* 2011;1:101-106.
[51] Gomes A, Fernandes E, Garcia MBQ, Silva AMS, Pinto D, Santos CMM, Cavaleiro JAS and Lima J. Cyclic voltammetric analysis of 2-styrylchromones: Relationship with the antioxidant activity. *Bioorg. Med. Chem.* 2008;17:7939-7943.
[52] Nyska A and Kohen R. Oxidation of biological systems: Oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol. Pathol.* 2002;6:620-650.
[53] Chevion S and Chevion M Antioxidant status and human health - Use of cyclic voltammetry for the evaluation of the antioxidant capacity of plasma and of edible plants, in: C.C. Chiueh (Ed.), Reactive Oxygen Species: From Radiation to Molecular Biology: A Festschrift in Honor of Daniel L Gilbert, New York Acad Sciences, New York, 2000, pp. 308-325.
[54] Chudobova D, Dobes J, Nejdl L, Maskova D, Merlos Rodrigo MA, Ruttkay-Nedecky B, Krystofova O, Kynicky J, Konecna M, Pohanka M, Hubalek J, Zehnalek J, Klejdus B, Kizek R and Adam V. Oxidative Stress in Staphylococcus aureus Treated with Silver(I) Ions Revealed by Spectrometric and Voltammetric Assays. *Int. J. Electrochem. Sci.* 2013.
[55] Dowling DP, Betts AJ, Pope C, McConnell ML, Eloy R and Arnaud MN. Anti-bacterial silver coatings exhibiting enhanced activity through the addition of platinum. *Surf. Coat. Technol.* 2003637-640.
[56] Ruden S, Hilpert K, Berditsch M, Wadhwani P and Ulrich AS. Synergistic Interaction between Silver Nanoparticles and Membrane-Permeabilizing Antimicrobial Peptides. *Antimicrob. Agents Chemother.* 2009;8:3538-3540.
[57] Evanko SP and Wight TN. Intracellular localization of hyaluronan in proliferating cells. *J. Histochem. Cytochem.* 1999;10:1331-1341.
[58] Kogan G, Soltes L, Stern R and Gemeiner P. Hyaluronic acid: a natural biopolymer with a broad range of biomedical and industrial applications. *Biotechnol. Lett.* 2007;1:17-25.
[59] Guibal E. Interactions of metal ions with chitosan-based sorbents: a review. *Sep. Purif. Technol.* 2004;1:43-74.
[60] Pires NR, Cunha PLR, Maciel JS, Angelim AL, Melo VMM, de Paula RCM and Feitosa JPA. Sulfated chitosan as tear substitute with no antimicrobial activity. *Carbohydr. Polym.* 2013;1:92-99.
[61] Madhumathi K, Kumar PTS, Abhilash S, Sreeja V, Tamura H, Manzoor K, Nair SV and Jayakumar R. Development of novel chitin/nanosilver composite scaffolds for wound dressing applications. *J. Mater. Sci.-Mater. Med.* 2010;2:807-813.
[62] Jayakumar R, Menon D, Manzoor K, Nair SV and Tamura H. Biomedical applications of chitin and chitosan based nanomaterials-A short review. *Carbohydr. Polym.* 2010;2:227-232.
[63] Huang GQ, Sun YT, Xiao JX and Yang J. Complex coacervation of soybean protein isolate and chitosan. *Food Chem.* 2012;2:534-539.
[64] Lee SB, Kim YH, Chong MS and Lee YM. Preparation and characteristics of hybrid scaffolds composed of beta-chitin and collagen. *Biomaterials*. 2004;12:2309-2317.
[65] Abdel-Mohsen AM, Hrdina R, Burgert L, Krylova G, Abdel-Rahman RM, Krejcova A, Steinhart M and Benes L. Green synthesis of hyaluronan fibers with silver nanoparticles. *Carbohydr. Polym.* 2012;2:411-422.
[66] Chudobova D, Nejdl L, Gumulec J, Krystofova O, Merlos Rodrigo MA, Kynicky J, Ruttkay-Nedecky B, Kopel P, Adam V and Kizek R. Effect of silver(I) ions and silver phosphate nanoparticles in complexes with hyaluronic acid and chitosan on Staphylococcus aureus. *Int. J. Mol. Sci.* 2013.