THE USE OF NANOTECHNOLOGY AS MODERN TOOLS TO TREAT INFECTIONS CAUSED BY MULTIRESISTANT BACTERIA STRAINS

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ABSTRACT

This work focuses on development of antimicrobial complex substances, suitable to cover vascular implants with the secondary use in transplantation surgery. This work also presents a comparison of the effects of used nanomaterials against ordinary substances. The formation of complexes took place between silver nanoparticles, silver ions and the polymer substances (hyaluronic acid, collagen and chitosan). The ability of complex formation of these substances was studied using electrochemistry and spectrophotometry. Bactericidal effect of these compounds was determined by growth-curve methods and inhibition zones on a bacterial culture *Staphylococcus aureus*. The viability of eukaryotic cells in straight confrontation with tested substances was observed using the MTT test. According to the data obtained, the complex of silver nanoparticles with chitosan was evaluated as the best substance to ensure antimicrobial behaviour of vascular implants.

Key words: microbiology, spectrophotometry, electrochemistry, nanotechnology, resistant microorganisms

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INTRODUCTION

In the field of transplant surgery is high rate of the postoperative complications caused by resistant strains of bacteria [1]. These problems lead to complex re-operations, health complications in the most serious cases to death [2]. Infection is caused by weakening of the immune system as a result of implantation of foreign bodies in the human body [3].

The bacteria *Staphylococcus aureus* causes serious infectious diseases, despite the high level of knowledge of medicine and antibiotic drugs [4]. Antibiotic drugs indirectly cause resistance of *Staphylococcus aureus*. But even this fact does not prevent overuse of antibiotics by people and even some physician. The consequences of stress create conditions for bacterial strains, when the vast majority of leads to their death [5]. However, there are individual cells that survive and reproduce and they already have developed a resistance to that antibiotic drug. This new property of bacteria is further transmitted to future colonies. The development of bacterial resistance is a natural phenomenon, but careless handling of antibiotics accelerates this process [6].

The solution of this problem is the search for compounds with the same result of action, but by a different mechanism of inhibition of bacterial growth, especially resistant *Staphylococcus aureus* [7]. Possible solutions offer the use of metals. The antibacterial effects have been known for centuries and also the use of the metal nanoparticles is possible. Nanoparticles, due to their small size can be more efficient, they enter into the body easily and at the same time have a greater active area [8]. A disadvantage of the use of metals in the human body is the negative effect on the metabolic pathways.

Alternatives to ensure the safety of the body is the substance that is completely biodegradable and biocompatible with the human body [9]. These properties have many polymers, which also exhibit high antimicrobial activity. The polymeric substances are capable in its structure to bind other substances, particularly metals and form complexes with them. Forming a complex compound obtained possessing a high antimicrobial effect which is also biocompatible and biodegradable for the human body. This complex substances offer useful properties to fight against resistant strains of bacteria and they are suitable material for the development of surface of vascular implants to ensure the safety and smooth adoption of the human body.

MATERIAL AND METHODS

AgNPs preparation

Silver nanoparticles (AgNPs) were prepared according method by Khan. $Na_2HPO_4 . 7H_2O$ (0.134 g) was dissolved in ACS water (25 ml). In solution of $Na_2HPO_4 . 7H_2O$ solution of $AgNO_3$ (0.085 g in 25 ml ACS water) was added in permanent stirring. Creation of AgNPs happened immediately. AgNPs were stored in cold (4 °C and darkness). Prepared AgNPs were in size range of 10 – 100 nm.

Electrochemical analysis

Electrochemical determination was performed using differential pulse voltammetry (DPV). CHInstruments (Austin, USA) device using three-electrode system was employed for analysis. Carbon paste electrode was used as a working electrode, Ag/AgCl/3M KCl as a reference electrode and platinum counter electrode. The working electrode was filled by a paste made up of expanded carbon powder (0.1 g) and mineral oil (300 µl). Mixture was rubbed for 25 minutes in agate bowl. Samples were measured in 0.2 M acetate buffer (pH 5). Parameters: initial potential of -0.2 V ultimate potential of 0.5 V, the pulse period of 0.05 s, the sensitivity (A/W) 1e-5, the amplitude of 50 mV. Sample was composed of 1950 µl acetate buffer, 50 µl of $AgNO_3$ or AgNPs (100 µM).

During the analysis 20 µl of polymer substance (HA, KOL or CHIT) was added. Pure acetate buffer was analysed as a reference

Spectropfotometric analysis

Spectrophotometric determination was performed by spectrophotometer SPECORD 210 (Analytik Jena, Germany). The range of wavelengths was 200 – 700 nm. To run the experiment, high purity silicon cells (Hellma Essex, UK) with absorption path 1 cm were used. Samples consisted of combination of $AgNO_3$ or AgNPs with HA, KOL, or CHIT and demineralized water (100 µM). Reference measurement was performed for demineralized water. At first the sample of demineralized water and $AgNO_3$ or AgNPs was made and analysed and after that polymer substances were added with increasing concentration for 20 µM every 5 minutes. Test was run to concentration range (0 - 160 µM). Data obtained in this experiment were transported to the diagram representing creation of complex between $AgNO_3$ or AgNPs with HA, KOL or CHIT.

RESULT AND DISCUSSION

The results obtained by electrochemical analysis using DPV method leads to investigation of creation of complex between metal particles and polymeric substances. The results obtained from spectrophotometric analysis proved the creation of complex substances in combination ($AgNO_3$+HA, $AgNO_3$+KOL, $AgNO_3$+CHIT, AgNPs+HA, AgNPs+KOL and AgNPs+CHIT) is happening. Two analytical methods using different principle were utilized to prove and confirm creation of complexes. Creation of complexes was proved in all examined combinations.

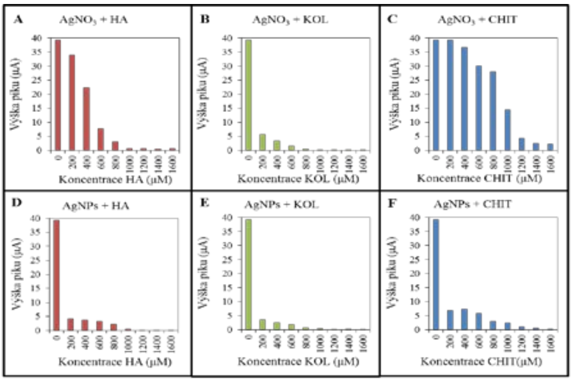

Fig. 1: Monitoring of complex creation using differential pulse voltametry. Interaction of $AgNO_3$ and hyaluronic acid (0-1600 µM) (A). Interaction of $AgNO_3$ and collagen (0-1600 µM) (B). Interaction of $AgNO_3$ and chitosan (0-1600 µM) (C). Interaction of AgNPs and hyaluronic acid (0-1600 µM) (D). Interaction of AgNPs and collagen (0-1600 µM) (E). Interaction of AgNPs and chitosan (0-1600 µM) (F).

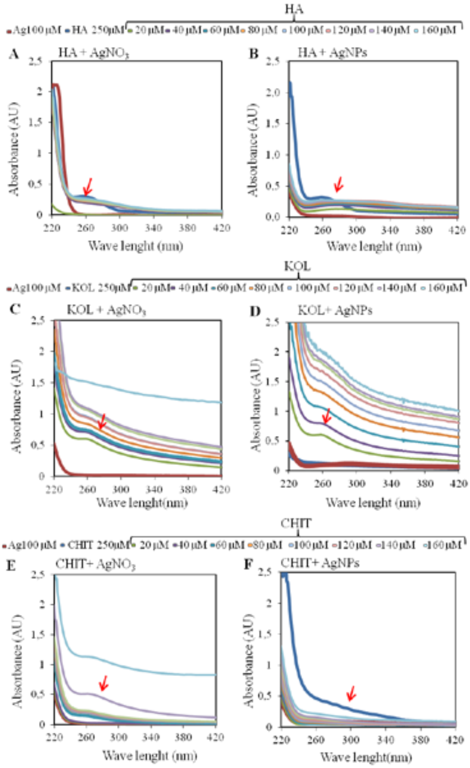

Fig. 2: Characterisation of complex using spectrophotometry. Interaction of $AgNO_3$ and hyaluronic acid (0-160 µM) (A) creation of complex detected in λ=260 nm. Interaction of $AgNO_3$ and collagen (0-160 µM), creation of complex in λ=270 nm (B). Interaction of $AgNO_3$ and chitosan (0-160 µM) (C), creation of complex in λ=268 nm. Interaction of AgNPs and hyaluronic acid (0-160 µM) (D), creation of complex in λ=254 nm. Interaction of AgNPs and collagen (0-160 µM) (E), creation of complex in λ=279 nm. Interaction of AgNPs and chitosan (0-160 µM) (F), creation of complex in λ=295 nm.

CONCLUSIONS

Creation of complexes of $AgNO_3$ with polymer substances was determined using electrochemical and spectrophotometric methods. Tests independently proved that creation of complexes occurred in all combinations. Creation of complexes in all combination was also confirmed in interaction between AgNPs with polymer substances. Complexes will be studied for its antibacterial and human-toxic properties. Complexes could be excellent choice for covering vascular grafts.

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