Hybrid-stabilized silver nanoparticles and their biological impact on hospital infections, healing wounds, and wheat cultivation

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The structure and morphology of new biocide compositions based on silver nanoparticles and silica/polyacrylamide hybrids, as well as their diverse biological effects on traditional hospital infections and wound healing and winter wheat cultivation, were studied. The compositions showed a high bactericidal effect against bacteria of the genera *Staphylococcus* and *Pseudomonas*, and fungistatic effect against the yeast genus *Candida* and filamentous fungi of the genera *Aspergillus*, *Cladosporium*, *Penicillium*, *Exophiala*, and *Fusarium*. They also showed high activity in wound healing in rats and significantly changed the rate of development of winter wheat plants due to presowing treatment of seeds.

Introduction

Traditional biocide preparations for medicinal, industrial or everyday applications (antibiotics, disinfectants, preservatives, etc.) are based mainly on organic substances that are toxic for humans and the environment and can cause different allergic reactions, adverse effects and resistance [1-5]. The surface of the

body and especially wounds of different nature are the environments for the development of numerous bacteria, such as gram-positive *Staphylococcus spp.* (42.7 %) and gram-negative *Pseudomonas aeruginosa* (10.3 %) [6]; they can also be infected with yeast (*Candida albicans*) and filamentous fungi, as well as associations of microorganisms [7]. The

causative agents of hospital infections exhibit significant virulence and resistance to antibacterial drugs. Therefore, biocidal preparations of a new generation, including metal nanoparticles with broad antibacterial, antiviral and antifungal action, are of particular interest [8-10]. It is very important that microbial cells, as a rule, are less able to show resistance to these “nanobiocides” or “nanoantibiotics” [11]. In addition, such biocidal preparations are more tolerant to human cells [12]. Recently, nanoparticles of metals or their oxides have also become actively used in the practice of growing agricultural plants instead of the corresponding metal salts or chelates [13,14]. This allows the long-term supply of metal ions that stimulate plant growth and additionally have a biocidal effect on various plant pathogens [15].

Silver nanoparticles (AgNPs) are known to be one of the most active biocides among various nanometals [16,17]. Due to their excellent antimicrobial potential, AgNPs are among the most commercialized nanoparticles that are widely used as therapeutic agents, household goods, as well as components of textiles and cosmetics [18,19]. At the same time, the cyto- and genotoxicity of these nanoparticles *in vitro* and *in vivo*, as well as their distribution in living organisms after administration, are intensively investigated. The toxicity of AgNPs is the result of the combined action of nanoparticles and silver ions released

from the oxidized surface of the particles [20]. The internalization of AgNPs by mammalian cells occurs mainly by the mechanism of endocytosis [21]. The silver nanoparticles themselves and the Ag⁺-ions they release can interact with sulfur- and phosphorus-containing biopolymers, such as proteins and DNA, thus potentially causing cell damage [22,23]. The observed cytotoxicity of AgNPs is also expressed through the generation of reactive oxygen species (ROS) [24]. Increased oxidative stress can inhibit cell proliferative activity and induce genetically programmed cell death (apoptosis) or necrosis [24,25]. It was shown that the cytotoxicity level of AgNPs significantly depends on many factors, such as cell types [21,24], size and dose of nanoparticles [26,27], nature (and size) of the “corona” of nanoparticles [28-31], and temperature [21]. But of particular interest is the effect of gradual full functional recovery of cells (such as normal human lung fibroblasts) from the AgNP-induced stress, first demonstrated in the study [21].

Earlier we have shown that grafted polymer/inorganic hybrids (PIH), including silica nanoparticles (SiO₂) and grafted polyacrylamide chains (PAAm), are effective nanoreactors for *in situ* synthesis of silver nanoparticles in an aqueous medium [32]. They provide a high rate of AgNPs formation, high yield of nanoparticles and their long-term stabilization in solution. It was suggested that the strong retention of silver ions and then the

resulting AgNPs in hybrid matrices is due to the presence of silica “core” with a weakly negatively charged surface [32]. In addition, these hybrids contain nontoxic biocompatible components; therefore, it can be assumed that their compositions with AgNPs can be actively used in biomedicine. Such compositions may also be promising in agriculture because of the ability of PAAm chains in hybrids to decompose in the fields under atmospheric conditions [33].

There are numerous studies devoted to biocide effect of silver nanoparticles stabilized with synthetic and natural polymers [10,34-38]. At the same time, biocidal properties of AgNPs in a composition with silica/polyacrylamide hybrids have not been studied.

In the current work, we synthesized AgNPs in PIH solutions as templates, determined the morphology of the composition and size of nanoparticles, and then studied the biological impact of this composition on a number of bacterial and fungal cultures, which are traditional hospital infections. The ability of the composition to heal wounds and influence the growth of winter wheat plants has also been studied.

Experimental part

Synthesis and characterization of PIH.

We synthesized PIH by a free-radical grafting polymerization of acrylamide (AAm) “from” the surface of silica hydrosol, as in the study [32]. For this, we used Aerosil A-175

from “Orisil” (Ukraine) with a specific surface area of $1.82 \cdot 10^5 \text{ m}^2 \cdot \text{kg}^{-1}$, cerium (IV) ammonium nitrate (CAN) from “Aldrich” (USA) and AAm from “Merck” (Germany), which was recrystallized from chloroform. To prepare a silica hydrosol, the Aerosil suspension was stirred in deionized water with $C=100 \text{ kg} \cdot \text{m}^{-3}$ during 24 hours and then centrifuged twice at 6000 rpm. The concentration of silica sol in the supernatant was measured by the gravimetric method, and the average nanoparticle size (R_{SiO_2}) was determined by static light scattering, as in the study [39]. The weight ratios: $[\text{Ce}^{\text{IV}}]/[\text{SiO}_2]=0.2$ and $[\text{Ce}^{\text{IV}}]/[\text{AAm}]=7.7 \cdot 10^{-3}$, which determined the quantity and length of the grafted chains, and the concentration of $C_{\text{SiO}_2}=2.7 \text{ kg} \cdot \text{m}^{-3}$ were used. The reagents were mixed in an inert atmosphere at 293 K for 24 hours. The gel-like product was diluted, precipitated with acetone, re-dissolved in water and freeze-dried.

A detail structure of the hybrid was established by elemental analysis, dynamic thermogravimetric analysis (DTGA) and viscometry. Using elemental analysis data for C and N, the weight fraction of PAAm ($w_{\text{PAAm}}=0.852$).in PIH was determined. The weight part of water ($w_{\text{H}_2\text{O}}=0.053$) was found according to DTGA as weight loss by a hybrid sample before the destruction beginning. Based on these results, the weight fraction of SiO_2 ($w_{\text{SiO}_2}=0.095$) was calculated.

To determine the number and molecular weight of the grafted PAAm chains, the aqueous

PIH solution was kept for one week at pH=11.5 and room temperature. It is well-known that under such conditions complete degradation (dissolution) of silica nanoparticles occurs [40] and only a slight hydrolysis of PAAm acrylamide units can be observed [41,42]. The reaction mixture then was re-precipitated with acetone and dissolved in water. The degradation products were completely separated from the polymer chains by dialysis of the solution against deionized water, and the average molecular weight of the grafts (M_{vPAAm}) was determined using the method of viscometry.

The viscosity was measured in diluted aqueous solutions of PAAm ($C_{PAAm}=0.1-1.0 \text{ kg}\cdot\text{m}^{-3}$) at $T=303\pm 0.1 \text{ K}$ in the presence of NaNO_3 ($C_{\text{NaNO}_3}=1 \text{ M}$) as a low molecular weight electrolyte to suppress possible polyelectrolyte effect caused by partial hydrolyses of acrylamide units. We used the Ostvald-type viscometer with the water-salt expiration time $\tau_0=93.5 \text{ s}$. The resulting linear dependence of the reduced viscosity of polymer solution on its concentration was used to determine the intrinsic viscosity ($[\eta]$) and to calculate the viscosity-average molecular weight of PAAm using relation (1) [43]:

$$[\eta] = 3.73 \cdot 10^{-2} \cdot M_{vPAAm}^{0.66}, \quad (1)$$

where $[\eta]$ was expressed in $\text{cm}^3\cdot\text{g}^{-1}$.

The obtained characteristics made it possible to calculate the number of PAAm grafts (N) per one SiO_2 nanoparticle according

to the formula (2):

$$N = \frac{4\pi \cdot R_{\text{SiO}_2}^3 \cdot \rho \cdot w_{PAAm} \cdot N_A}{3 \cdot w_{\text{SiO}_2} \cdot M_{vPAAm}} \quad (2)$$

Here $\rho=2.1 \text{ g}\cdot\text{cm}^{-3}$ is the density of silica, the product $4/3\pi \cdot R_{\text{SiO}_2}^3 \cdot \rho$ is the weight of one silica nanoparticle, N_A is the Avogadro number. The results of calculations are shown in Table 1.

Table 1. The main parameters of the hybrid

Sample	R_{SiO_2} , nm	M_{vPAAm} , kDa	N
PIH	7.7	1588	14

In situ synthesis of silver nanoparticles.

To obtain a stable composition of AgNPs/PIH, we performed *in situ* reduction of silver nitrate with sodium borohydride in a hybrid aqueous solution, as in the study [32]. An eight-fold molar excess of NaBH_4 was used to achieve the complete conversion of Ag^+ -ions to zero-valent state at $C_{\text{AgNO}_3}=1.82 \cdot 10^{-2} \text{ kg}\cdot\text{m}^{-3}$ [44]. Hybrid solution with $C_{\text{PIH}}=2.0 \text{ kg}\cdot\text{m}^{-3}$ was mixed with AgNO_3 and kept in a dark box for 30 minutes. Then a reducing agent was added and a yellow color appeared, reflecting the occurrence of AgNPs. Earlier, we showed that the formation of nanoparticles in this system was fully completed after 24 hours [32]. To separate the reaction by-products, we re-precipitated the AgNPs/PIH composition by ethanol and re-dissolved it in water.

The formation of AgNPs was confirmed by UV-Vis spectroscopy. The extinction spectrum of an aqueous mixture of AgNO_3 with

PIH was recorded in a quartz cell $l=1$ cm long in the region of 200-1000 nm using a Cary 50 Scan UV-Vis spectrometer from “Varian” (USA).

Additional confirmation of the appearance of AgNPs in the composition was obtained using wide-angle X-ray scattering (WAXS). For this purpose, the composition was cast from aqueous solutions ($C_{\text{PIH}}=2.0$ and $C_{\text{AgNO}_3}=3.64 \text{ kg}\cdot\text{m}^{-3}$) into a Teflon mold placed in a dark box, and then dried in air and in a vacuum desiccator. The WAXS profile was obtained in a 2 mm thick cell using a DRON-2.0 X-ray diffractometer with Ni filter in a primary beam. Monochromatic Cu- K_α radiation with $\lambda=0.154$ nm, filtered by Ni, was provided by an IRIS M7 generator (at an operating voltage of 30 kV and a current of 30 mA). The scattering intensities were measured with a scintillation detector scanning with a step of 0.2° in the range of scattering angles $2\theta=3-45^\circ$. Diffraction curves were reduced to equal intensities of the primary beam and to equal scattering volume according to the technique described in studies [32,45].

Studies of the composition morphology.

The morphology of pure PIH and its composition with AgNPs in aqueous solutions was determined using transmission electron microscopy (TEM). Corresponding TEM images were obtained on a JEM-I230 instrument (JEOL, Japan) operating at an accelerating voltage of 90 kV. Small drops ($\sim 1\cdot 10^{-4} \text{ cm}^3$) of solutions of a pure hybrid and composition in deionized water ($C_{\text{PIH}}=1 \text{ kg}\cdot\text{m}^{-3}$) were applied to

copper grids coated with Formvar films and carbon; they were then dried in air for $\sim 1-2$ min and in a vacuum desiccator for 24 hours.

Biological experiments.

Standard microbiological agar nutrient media were used to characterize the effect of the AgNPs/PIH composition on the growth of bacterial and fungal cultures. The bacteria *Staphylococcus aureus* ATCC 25923, *Escherichia coli*, and *Pseudomonas aeruginosa* ATCC 27853, yeasts *Candida albicans* and filamentous fungi, such as *Aspergillus ustus* 165 FCKU, *Cladosporium shpaerospermum* 312 FCKU, *Fusarium oxysporum* 150 FCKU, and black yeast-like fungus *Exophiala alcalophila* 304 FCKU from the collection of microscopic fungi of Taras Shevchenko National University of Kiev (international acronym of collection is FCKU), were used as test cultures. In these experiments, we used both the initial composition and fragments of sterile cotton fabric (30x30 mm) treated with this biocidal preparation.

The effect of AgNPs/PIH composition on test cultures was studied by diffusion in agar (disc method for bacteria and agar-hole method for filamentous fungi), assessing the presence or absence of a zone of inhibition of the growth of microorganisms around the disc or well of the agar and its diameter. In control experiments, an aqueous solution of the well-known biocidal preparation of benzalconium chloride ($C=30 \text{ kg m}^{-3}$) and fragments of cotton fabric moistened

with sterile distilled water were used. The density of colony forming units (CFU) of microorganisms, such as cells or spores, in various experiments corresponded to 10^6 – 10^8 CFU·cm⁻³. The cell content in the bacterial suspension ($1.5 \cdot 10^8$ CFU·cm⁻³) was determined using a densitometer of Vitek-2 microbiological analyzer (Bio Merieux, France). The number of spores of filamentous fungi in suspension ($1 \cdot 10^6$ CFU·cm⁻³) was determined using a Goryaev camera.

To examine the ability of AgNPs/PIH composition to heal wounds, 4 groups of rats of 10 each were used. Skin 10x10 mm in size was removed on the back of each rat, and the resulting wounds obtained were treated with equal volumes of various preparations. The exception was the 1-st control group, in which wound healing occurred naturally without any treatment. We measured the wound area of rats at different time intervals using not only the AgNPs/PIH composition ($C_{\text{AgNPs}} = 1.2 \cdot 10^{-2}$ kg·m⁻³) but also the commercial preparation Carbopol, which promotes wound healing, and an aqueous solution of eumelanin ($C_{\text{EM}} = 1$ kg·m⁻³) produced by black yeasts *Pseudonadsoniella brunnea* 470 FCKU [46]. The last preparation, EM, was able to increase the body's defenses [47] and, therefore, accelerate wound healing. Conducting these experiments, we followed the International Guiding Principles for Scientific Research Involving Animals (CIOMS, Geneva, 1985) and Directive 2010/63/EU of the

European Parliament and of the Council of 22 September 2010 as to protection of animals used for scientific purposes [48].

In the final biological experiments, winter wheat seeds belonging to the Arctis variety were treated with the AgNPs/PIH composition immediately before sowing. For this purpose, the original composition (1:1) with $C_{\text{AgNPs}} = 1.2 \cdot 10^{-2}$ kg·m⁻³ and diluted 10 times (1:10) was used. Two 50 kg portions of winter wheat seeds were treated with 250 cm³ of every composition. The treated seeds were planted and grown on the fields of “Kernel” Research Center during 2018 year.

Results and discussion

The fact of the AgNP formation in an aqueous AgNO₃/PIH mixture at the addition of a reductant was confirmed by the appearance of a surface plasmon resonance band (SPRB) in the UV-Vis spectrum (**Figure 1**).

Figure 1. The extinction spectrum of the AgNO₃/PIH composition 90 min after the introduction of NaBH₄. $C_{\text{PIH}} = 2.0$ kg·m⁻³; $C_{\text{AgNO}_3} = 1.82 \cdot 10^{-2}$ kg·m⁻³; $T = 20$ °C.

The monomodal form of SFRP and its position in the spectrum ($\lambda_{\text{max}} = 400$ nm) indicated the

formation of spherical AgNPs with a size much smaller than the wavelength of light ($d < 30$ nm) [49,50]. Kinetic features of this process were discussed in detail earlier [32].

The presence of crystalline AgNPs in the dried composition was established by WAXS. The results (**Figure 2**) showed that the bulk structure of the compositions contained a polymere amorphous phase, which was displayed in WAXS profiles by two diffuse overlapping maxima at $2\theta \sim 15^\circ$ and 21° , and crystalline Ag nanoparticles, which was confirmed by the presence of an intense crystalline silver peak (111) at $2\theta \sim 38^\circ$ [51].

Figure 2. The intensity of wide-angle X-ray scattering vs the scattering angle for the AgNPs/PIH composition. $T = 20^\circ\text{C}$.

The appearance of this peak indicated the presence of crystalline AgNPs with a tetragonal face-centered cubic lattice [50].

Morphology of PIH in aqueous medium is shown in **Figure 3 (a, b)**. The main elements of its structure are small nanoparticles with

Figure 3. TEM images of: (a, b) pure PIH and (c) the composition of AgNPs/PIH in aqueous solution. $C_{\text{PIH}} = 1 \text{ kg}\cdot\text{m}^{-3}$, $C_{\text{AgNPs}} = 1.156 \cdot 10^{-2} \text{ kg}\cdot\text{m}^{-3}$, $T = 20^\circ\text{C}$.

a size of $d=6-18$ nm. TEM images demonstrate both individual nanoparticles and their fractal aggregates of various shapes and sizes. The reason for such developed aggregation of nanoparticles in an aqueous solution can be explained by the nature of the grafted chains in PIH. Indeed, it is known that PAAm forms various types of hydrogen bonds in an aqueous medium: along each polymer chain and between adjacent polymeric chains [52]. Being grafted to SiO_2 nanoparticles, PAAm chains also form a system of hydrogen bonds with the silica surface [53]. Partial adsorption of PAAm chains on the SiO_2 surface and their intra- and intermolecular interactions in the grafted layer lead to a relatively compact structure of PIH nanoparticles.

The situation changes dramatically after *in situ* synthesis of AgNPs in PIH solution. Significant changes in the morphology of PIH in the composition with AgNPs are observed (**Figure 3 c**). Hybrid nanoparticles containing one or more AgNPs swelled up to $d\sim 10-40$ nm and significantly separated from each other. This meant that the synthesis of AgNPs occurred previously in the grafted layer; therefore, PAAm chains were partially desorbed from the SiO_2 surface and swelled in solution. Thus, the AgNPs/PIH composition can be represented as a set of individual particles of the following structure (**Figure 4**). A size of spherical AgNPs in the composition varied in the range $d=3-9$ nm (**Figure 3 c**).

Figure 4. Schematic structure of individual particles of the AgNPs/PIH composition.

A series of microbiological tests showed that aqueous solutions of the AgNPs/PIH composition exhibit biocidal and biostatic effects of different degrees with respect to the gram-positive and gram-negative bacteria, yeast and filamentous fungi used. Examples of the impact of the initial composition and its dilutions 10 (1:10) and 100 (1:100) times on the bacterial cultures of *Staphylococcus aureus* and *Pseudomonas aeruginosa* are presented in **Figures 5** and **6**.

The biocidal effect of the composition with $C_{\text{AgNPs}}=1.2\cdot 10^{-2} \text{ kg}\cdot\text{m}^{-3}$ and its diluted solutions (1:10 and 1:100) was detected in relation to both of the above bacterial cultures. Indeed, zones of growth inhibition after 24 hours corresponded to 7, 10-12 and 11 mm, respectively, in the case of *Staphylococcus aureus* (**Figure 5 a, b**) and 9-10, 11-13 and 11-12 mm in the case of *Pseudomonas aeruginosa* (**Figure 6 a**). The biocidal effect persisted for the next 3-5 days (**Figure 5 b, d** and **Figure 6 b**), that is, it had a long-lasting character.

Figure 5. Zones of growth inhibition of *Staphylococcus aureus* under the influence of the initial and diluted compositions after (a, b) 24 hours and (c, d) 4 days. (a, c) Top view and (b, d) bottom are shown. T=35 °C.

Figure 6. Zones of growth inhibition of *Pseudomonas aeruginosa* (bottom view) under the influence of the initial and diluted compositions after (a) 24 hours and (b) 4 days. T=35 °C.

The effect of the composition with variable concentration on the reproduction of two types of filamentous fungi is depicted in **Figures 7 and 8**. The studied composition had fungicidal impact on test cultures of *Cladosporium sphaerospermum* and *Fusarium oxysporum* during the first 3-5 days of cultivation (**Figure 7 a**, **Figure 8 b, c**).

Figure 7. Zones of growth inhibition of *Cladosporium sphaerospermum* (top view) under the influence of (a) the initial composition and diluted 100 times (b) after (a) 5 and (b) 9 days. T=28 °C.

For these cultures, zones of growth inhibition were 14-15 and 12-13 mm, respectively.

Figure 8. Zones of growth inhibition of *Fusarium oxysporum* under the influence of (a) the initial composition and diluted (b, c) 10 and (d, e) 100 times that were observed for (b, c) 5 and (d, e) 18 days. Top view (a) and bottom (b-e) are shown. T=28 °C.

At the same time, the same composition demonstrated only fungistatic effect in relation to filamentous *Aspergillus ustus* fungi (these data are not shown). It should be noted that the fungicidal and fungistatic action of the composition on *Fusarium oxysporum* fungi was stable and prolonged, since it manifested itself as zones of inhibition and further growth retardation for 30 days.

In experiments with test cultures of *Candida albicans* yeast, only the biostatic action of the composition with AgNPs was recorded. Here we observed only zones of growth retardation in which the growth rate of the yeasts decreased.

It is also important to note that the biocidal and biostatic effect of the composition diluted 10 and 100 times turned out to be brighter practically in almost all experiments.

The results of similar microbiological experiments with pieces of cotton fabric that were treated with compositions with different AgNP content are shown in **Figures 9-14**. In these cases, aqueous solutions of the original composition with $C_{\text{AgNP}}=1.2 \cdot 10^{-2} \text{ kg} \cdot \text{m}^{-3}$ and its three dilutions: 1:10, 1:100, and even 1:1000 were used. Moreover, two additional types of fungi, namely *Penicillium variable* and *Exophiala alcalophila*, were used as test cultures. Three examples of the biocidal effect of the composition on the bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* are shown in **Figure 9**.

Figure 9. Zones of growth inhibition of bacteria (a, c-e) *Staphylococcus aureus* and (b) *Pseudomonas aeruginosa* under the action of a composition diluted (b) 10, (a) 100 and (c) 1000 times, (d) benzalkonium chloride with $C=30 \text{ kg m}^{-3}$ and (e) sterile water after 3 days. $T=35^\circ \text{C}$.

Zones of growth inhibition of 2.7-3.3 mm remained around the tissues treated with compositions in which the concentration of AgNPs varied in a very wide range from $C_{\text{AgNPs}}=1.2 \cdot 10^{-2}$ to $1.2 \cdot 10^{-5} \text{ kg} \cdot \text{m}^{-3}$ (**Figure 9 a-c**). Similar zones were 9-11 mm in the case of cotton fabric treated with the well-known biocidal agent benzalkonium chloride with a significantly higher concentration ($C=30 \text{ kg m}^{-3}$) of the active substance (**Figure 9 d**). In contrast, zones of growth inhibition of microorganisms

were absent in the control variant (**Figure 9 e**), when the fabric fragments were moistened with sterile water only.

The following examples relate to the effect of AgNPs/PIH compositions on different fungi. The width of the growth inhibition zones appearing around tissue fragments varied from 2 to 6.5 mm for dark pigmented fungi, such as *Cladosporium sphaerospermum* (**Figure 10 a, b**) and *Exophiala alcalophila* (**Figure 11**).

Figure 10. Zones of growth inhibition of fungi (a-c) *Cladosporium sphaerospermum* and (d) *Candida albicans* under the action of a composition diluted (d) 10, (a) 100 and (b) 1000 times, and (c) sterile water after 3 days. T=28 (a-c) and 35 °C (d).

The effect of the composition on the yeast-like fungi of *Candida albicans* was expressed in the formation of a large zone of growth inhibition (14-27 mm) after 2 days. But this zone decreased to 2.5–7 mm after 3 days (**Figure 10 d**). In the control variant (**Figure 10 c**), the cotton fabric was completely overgrown

with mycelium of fungal test cultures.

Figure 11. Zones of growth inhibition of *Exophiala alcalophila* fungus under the action of a composition diluted (a) 10, (b) 100 and (c) 1000 times after 3 days. T=28 °C.

The growth inhibition zones were 3-6 mm, 2-4 mm, and 1.5-2 mm wide for light-pigmented filamentous fungi *Fusarium oxysporum*, *Penicillium variable* and *Aspergillus ustus* (**Figures 12 a, 13, 14**, respectively). But the same zones were equal to 4-7 mm for *Fusarium oxysporum* when cotton fabric was treated with a biocide agent benzalkonium chloride having significantly higher concentration ($C=30 \text{ kg}\cdot\text{m}^{-3}$) of active substance (**Figure 12 b**). Finally, the zones of growth inhibition of *Fusarium oxysporum* turned out to be fully absent in the control test (**Figure 12 c**), in which the fabric fragment was moistened with sterile water.

It should also be noted that in these

experiments, the formation of only separate colonies of the tested cultures of microscopic fungi was observed in zones of growth inhibition (Figures 10 a, b; 11, 13, 14).

Figure 12. Zones of growth inhibition of *Fusarium oxysporum* fungus under the effect of (a) a composition diluted 10 times, (b) benzalkonium chloride with $C=30 \text{ kg m}^{-3}$ and (c) sterile water after 3 days. $T=28^\circ\text{C}$.

Figure 13. Zones of growth inhibition of *Penicillium variable* fungus under the action of a composition diluted (a) 10, (b) 100 and (c) 1000 times after 3 days. $T=28^\circ\text{C}$.

The described action of the AgNPs/PIH composition, with which the pieces of cotton fabric were processed, was evaluated as fungistatic. The test culture of *Candida albicans* was found to be the most sensitive to the effects of the composition in these experiments, while the culture of *Aspergillus ustus* turned out to be the most stable. It is also important to emphasize that the biostatic effect of the AgNPs/PIH composition, diluted 10 and 100 times, turned out to be more vivid in almost all tests, both using the disc method and using cotton fabric.

Figure 14. Zones of growth inhibition of *Aspergillus ustus* fungus under the action of a composition diluted (a) 10, (b) 100 and (c) 1000 times after 3 days. $T=28^\circ\text{C}$.

The results of these experiments confirmed the stable and long-lasting bactericide and fungistatic action of the AgNPs/PIH composition applied to cotton fabric in a very wide concentration range. This opens up

prospects for its successful application as a new biocidal preparation for the treatment and long-term disinfection of wound dressings and hygienic materials such as bandages, gauze, wipes, plasters and so on.

An important aspect of the use of biocidal preparations for the impregnation of dressings is their interaction with open wounds and the possible impact on the rate of wound healing. There are relatively few studies devoted to this subject; in addition, their results are often contradictory. For example, a significant reduction in the healing time and an increase in bacterial clearance from infected wounds was observed in a study [54] using wound dressings soaked with AgNPs compared to silver sulfadiazine solution. But in another study [55], wound dressings loaded with AgNPs increased the healing time of superficial burn wounds but showed no difference in the healing of deep burn wounds, compared with silver sulfadiazine.

To clarify this aspect in our case, we studied the healing process of open wounds (10x10 mm) in rats that were treated with some agents, primarily the composition of AgNPs/PIH (in the 2nd group of rats), as well as Carbopol and EM for a comparison (in the 3rd and 4th groups of animals). The 1st group of rats was control. Changes in time in the area of the wounds of rats are presented in **Table 2**.

We analyzed these data from different points of view. In the case of comparing the total number of days during which complete

Table 2. Effect of different agents on wound healing

Days	The average wound area (n=10), mm ²			
	1st group	2nd group	3rd group	4th group
	Control	AgNPs/PIH	Carbopol	EM
0	100 _{±3}	100 _{±4}	100 _{±3}	100 _{±3}
3	153 _{±21}	100 _{±1}	129 _{±14}	187 _{±36}
6	111 _{±5}	50 _{±1}	112 _{±5}	127 _{±12}
9	99 _{±4}	37 _{±1}	76 _{±3}	71 _{±7}
14	54 _{±1}	6 _{<1}	71 _{±3}	36 _{±3}
19	23 _{±1}	5 _{<1}	54 _{±1}	0
22	0	3 _{<1}	30 _{<1}	
25		0	0	

healing of the wounds took place, the best result (~19 days) was observed in the 4th group of rats, for the treatment of which an EM solution was used. This time was 4 days shorter compared to the time of complete wound healing in the 1st control group of animals. The time of complete wound healing in the 2nd and 3rd groups of rats when using the AgNPs/PIH composition and the Carbopol preparation was approximately the same (~25 days) and was higher than in the control group (~22 days).

But the picture will be different if we compare the healing processes of wounds that were treated with various preparations. From such point of view, the effect of EM on open wounds in the 4th group of rats was the most severe among all the investigated agents, because this drug caused the strongest

additional inflammation of the wounds during first 3 days, which led to a 2-fold increase in the area of the wounds. More clearly, this fact is evident from **Figure 15**. This effect was even 1.2 times higher than inflammation of wounds that were not treated with any agents (in the control group).

Figure 15. The dynamics of reducing the average area of wounds in rats under the influence of various drugs.

The drug Carbopol also caused mild inflammation of the wounds, which was expressed in an increase in S area by 29 % during the first 3 days in the 3rd group of rats. On the contrary, the effect of the AgNPs/PIH composition was the most tolerant of open wounds (in the 2nd group of rats), since it did not cause any inflammation of the wounds during the first 3 days and contributed to their rapid healing over the next 11 days (**Figure 15**). As a result, the condition of wounds treated with our composition on the 14th day was the best ($S=6 \text{ mm}^2$) compared to wounds treated with other drugs ($S=36$ and 71 mm^2 for EM and Carbopol, respectively) or not treated at all

($S=54 \text{ mm}^2$).

The next important question is the preparation efficacy, which is determined by the concentration of the active substance in the solution used to treat wounds. From this point of view, the AgNPs/PIH composition was more effective in the wound healing process than the EM solution. This is confirmed by a significantly lower concentration (more than 83 times) of active AgNPs compared to EM in solution.

Thus, the developed biocide composition has shown high efficiency in wound healing. It did not cause any inflammation of the wounds during the first 3 days, and then acted quickly and confidently, leaving only small traces ($\sim 6 \text{ mm}^2$) from the original wounds (100 mm^2) on day 14. In addition, the positive effect of this composition takes place at a very low concentration of active silver nanoparticles.

In the last decade, the achievements of nanotechnologies have been actively introduced in agriculture, in particular, in the practice of growing agricultural plants [56]. One of the promising directions in this area is the synthesis and delivery of biogenic metal nanoparticles (Co, Cu, Fe, Mn, Mg, Zn, etc.) or their oxides into plants to stimulate their growth and combat plant diseases [57]. The ions of biogenic metals in small doses contribute to the growth and development of plants, but in large doses they poison both plants and the environment [58]. Therefore, the fruitful idea appeared to use

stabilized aqueous dispersions or powders of metal nanoparticles instead of their salts or chelates for seed treatment and/or foliar treatment of growing plants [13,14].

Metal nanoparticles have less toxicity, but exhibit a complex effect: as small particles with a developed surface that interact with various cells and components of plants, as sources of active metal ions with controlled release over time and as biocidal agents that protect plants and soil from pathogenic bacteria, fungi and viruses. At the same time, the mechanism and effectiveness of the action of metallic nanoparticles on specific plants remain poorly understood.

There are only few publications devoted to the effect of AgNPs on various agricultural plants such as rice, corn, beans, pumpkin, spring wheat, peas, and radish [14]. Especially few studies describe such investigations conducted in the fields under real atmospheric conditions [57]. These studies have clearly shown that AgNPs can have different and significant effects on morphology, physiology, biochemistry and yield of plants. The final effect depends on the size, properties and concentration of nanoparticles, the methodology of their introduction (by treating the seeds or leaves of plants or through the soil), as well as on the nature of plants [14].

In the present work, we carried out pre-sowing treatment of winter wheat seeds with the developed AgNPs/PIH composition (**Figure 3**)

with the initial concentration (1:1) and diluted 10 times (1:10). Each portion of the treated seeds of 50 kg was sown on 0.4 acres of field. For this, a total of 250 cm³ of composition with $C_{\text{AgNPs}} = 1.2 \cdot 10^{-2} \text{ kg} \cdot \text{m}^{-3}$ was required. Also for comparison, two plots of the same field of 0.4 acres each were sown with untreated winter wheat seeds. All mentioned areas of this field are shown in **Figure 16 a**.

Figure 16. Photos show: (a) two experimental (near) and control (remote) sections of the winter wheat field and (b) several separate plants from these areas.

First of all, a significant difference was observed in the color of winter wheat plants (yellow compared to green) grown from untreated seeds or treated with a diluted composition of 1:10 (Control 1 and Control 2 areas and the left near area in **Figure 16 a**) and seeds treated with the original composition 1:1 (the right near area in **Figure 16 a**). At the same time, we observed the absence of a noticeable effect of the composition on the speed and degree of seed germination and the length of plant stems, while the size of spikelets in plants grown from seeds treated with an undiluted composition was slightly smaller (**Figure 16 b**). These interesting and vivid facts indicated an increase in the vegetation period of winter wheat plants under the influence of a composition with a sufficiently high concentration of AgNPs.

Conclusions

Thus, the obtained polymer/inorganic hybrid based on small silica “cores” and long polyacrylamide grafts formed compact particles of 6-18 nm in an aqueous medium due to partial adsorption of covalently-bound polymer chains on the surface of SiO₂ and additional binding of the nearest PAAm segments in the grafted layer. These particles demonstrated strong aggregation in solution due to the interaction between the grafted layers of various hybrid particles, which led to the appearance of fractal clusters of variable sizes and shapes. It was shown that the *in situ* synthesis of AgNPs in PIH solutions

developed mainly in the grafted PAAm chains and was accompanied by the destruction of clusters and swelling of hybrid nanoparticles. As a result, the final AgNPs/PIH composition can be represented as a set of discrete swollen PIH particles with a size of ~10-40 nm, containing one or several silver nanoparticles with a size of 3-9 nm.

A series of microbiological tests carried out using both the disc method and pieces of cotton fabric showed a stable and long-lasting bactericidal effect of the composition on bacteria of the genera *Staphylococcus* and *Pseudomonas*, as well as fungistatic effect on yeast of the genus *Candida* and filamentous fungi of the genera *Aspergillus*, *Cladosporium*, *Penicillium*, *Exophiala*, and *Fusarium* in a very wide range of concentrations. These properties have been successfully combined with the high efficacy of the composition in wound healing of rats. Indeed, its use did not cause additional inflammation of the wounds in the first 3 days, and subsequently contributed to their accelerated healing. In addition, a positive effect was achieved at very low concentrations of active AgNPs. All these results allow us to consider the AgNPs/PIH composition as a new promising biocidal preparation for the treatment and long-term disinfection of wound dressings and hygienic materials such as bandages, gauze, wipes, plasters and so on.

Our initial studies of the effect of the composition on the growth and development of

winter wheat (during pre-sowing seed treatment) allow us to record only the interesting fact of a significant extension of the vegetation period of this plant. But in order to establish the causes of this phenomenon and identify the positive and possible negative aspects of its practical application, further research is needed.

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