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ORIGINAL ARTICLE

Nanocomposites with ornidazole—antibacterial and antiadhesive agents against Gram-positive and Gram-negative bacteria

I. Furtat[1] · M. Lupatsii[1] · T. Murlanova[1] · P. Vakuliuk[1] · A. Gaidai[1] · O. Biliayeva[2] · H. Sobczuk[3] · A. Golub[1]

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Abstract

Antimicrobial resistance of many microbial species can cause to thousands of deaths worldwide, in this regard new therapeutic strategies have to be invented. To address the question, we have prepared nanocomposites on the basis of pyrogenic silicon dioxide with ornidazole immobilized on the surface (ornidasil) and studied their antimicrobial properties and the therapeutic potential. It has also been shown, that in comparison with pure ornidazole the addition of ornidazole to nanocomposite composition can enhance the antimicrobial spectrum, including Gram-positive and Gram-negative bacteria. The most significant bactericidal effect has been reached after more than 24-h treatment with the nanocomposite. Antiadhesive properties of nanocomposite materials were studied using blood types OO+, AO+, BO+, AB+, the degree of bacterial adhesion was estimated using three indexes: average adhesion index, index of erythrocytes involvement, index of microbial adhesion. The effectiveness of the treatment with the nanocomposites obtained was studied on complicated wounds of various etiologies, in particular the wounds caused by diabetic foot syndrome.

Keywords Antimicrobial nanocomposites · Antiadhesive agents · Highly dispersed silica · Ornidazole · Slow-released drugs

Introduction

Nowdays, the emergence of strains of microorganisms resistant to antimicrobial compounds is one of the leading problems, because introduction of antibiotic compounds to clinical practice has rapidly increased the resistance of the majority of clinically significant strains of microorganisms to the above mentioned compounds (Poole 2002). Such a rapid increase is caused by several factors, the main ones are the overuse of antibiotics in clinical practice, inadequate use of antimicrobial compounds or their overdosage, and the intensive use of antibiotics in agriculture and industry (Ventola 2015).

Consequently, the increase in the number of infectious diseases caused by resistant strains of microorganisms and the difficulty of their treatment have not only stimulated the development of new generations of antibiotics, but also facilitated the search for alternative antibiotic compounds to which major microorganisms would not be able to develop resistance. Compared to traditional antibiotics, modern alternative medications must demonstrate not only antimicrobial activity, but also they have to be anti-adhesive, as it would allow them to prevent the spread of a disease as well as to eliminate the growth of microorganisms. One of the important examples of such compounds are inhibitors of adhesion (Ofek et al. 2003), because the blocking of adhesion disables the interaction of bacterial cells with the target cells of the macroorganism.

Considering that adhesion is an essential stage in the development of infectious diseases, the blocking process allows preventing the infection of a macroorganism and fails to form resistant microorganisms. The above mentioned properties of anti-adhesive compounds make them quite perspective antimicrobials (Krachler and Orth 2013). One of the main strategies in the development of adhesion inhibitors is the introduction of such compounds that can

✉ H. Sobczuk
h.sobczuk@gmail.com

[1] National University of Kyiv-Mohyla Academy, Kyiv, Ukraine

[2] Shupik National Medical Academy for Postgraduate Education, Kyiv, Ukraine

[3] Lublin University of Technology, Lublin, Poland

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compete with the ligands or receptors for binding the sites at the interface "bacterial cells—eukaryotic cells". This proces will enable them to interact either with bacterial or with eukaryotic cells. Selective binding of microbial ligands is considered to be more perspective for the use in medications or prophylactic therapy, because its influence on the target cells receptors, as well as on the processes in the tissues of the macroorganism, will be lower (Cegelski et al. 2008).

Numerous studies proved the possibility of using nanocomposite materials and also nanocomposites based on highly dispersed pyrogenic silica modified by different antimicrobial compounds, such as antimicrobial compounds, although nowadays the studies are more focused on using these nanocomposites as anti-adhesive compounds (Armentano et al. 2018). Such a tendency may be explained by the broad spectrum of nanocomposites' action, among which there are antimicrobial and anti-adhesive modes of their action due to the ability of nanocomposite materials to enter ligand–receptor interactions with adhesives located on the cellular surface of microorganisms (Gaidai et al. 2019). Moreover, nanocomposites can provide the target delivery of active compounds which makes it possible to prolong their action, enhance their effectiveness and broadens the activity spectrum which finally minimizes the damage to the healthy cells of a macroorganism (Wang et al. 2017).

Taking into account numerous advantages of nancomposites modified by antimicrobial compounds, the aim of our study was to develop nanocomposites with the use of highly dispersed pyrogenic silica matrices and immobilized antimicrobial agent ornidazole on their surface, and to characterize their antimicrobial activity on Gram-positive and Gram-negative bacteria, and also their anti-adhesive activity and to test these nanocomposites in the treatment of complicated purulent wounds.

Materials and methods

Materials

Pyrogenic silica with the surface area of 290 m^2/g (at low-temperature adsorption of nitrogen by the BET method) was used as a carrier for producing nanocomposite.

Ornidazole (1-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole), analytical reagent grade was used as a modifying substance (Fig. 1).

Preparation of nanocomposites and their surface modification

Nanocomposite materials were obtained by impregnation of ornidazole on the surface of nanosilica (Biliayeva et al. 2017a).

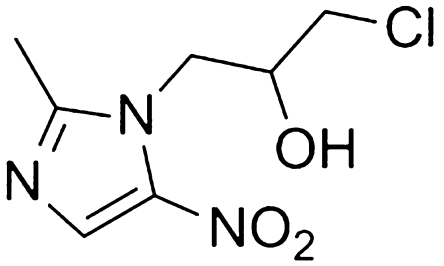

Fig. 1 The formula of ornidazole (1-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole (Hoffer and Grunberg 1974)

The method of ornidazole impregnation on the surface of highly dispersed silicon dioxide is quite simple. One of its features is a weak binding of the modifier on the surface of silica, which can lead to the continous release of the active component from the surface of the carrier into the wound. The formation of adsorption complexes of ornidazole molecules on the surface of the dispersed silica is due to the formation of hydrogen bonds between the surface silanol groups and the functional groups of ornidazole, mainly nitro and hydroxyl groups (Fig. 2). In addition, the formation of weak bonds between ornidazole and the surface of pyrogenic silica due to physical adsorption with the participation of dipole–dipole interactions and hydrogen bonds with other polar groups of ornidazole cannot be excluded (Hrebeniuk and Golub 2017).

The investigation of the nanocomposites obtained and the confirmation of of ornidazole present on the aerosil surface was performed by IR spectroscopy with the Fourier transform on an IFS-66 spectrometer (Bruker, Germany) using Opus 4.0 software.

The modification was carried out from alcohol and aqueous solutions of ornidazole in such a way that the final concentration of ornidazole in the dry nanocomposite was 2, 4 and 12 wt. %. The patented name of the nanocomposite produced by this technique is ornidasil (Biliayeva et al. 2016).

Estimation of antibacterial effectiveness of nanocomposites

The antibacterial effectiveness of the ornidasil action was studied according to the Gould method (Gould 1965) with the use of 24 h test cultures of Gram-positive bacteria (*Staphylococcus aureus* strain ATCC 25923) and Gram-negative (*Pseudomonas aeruginosa* strain ATCC 27853 and *Escherichia coli* ATCC 25922) (Furtat et al. 2004). Suspensions of bacterial cells standardized in McFarland units with concentration of 1.5×10^8 cells/ml (Bollela et al. 1999) were exposed to the nanocomposite for 2 and 24 h at the temperature of 37 °C and constant rotation. Thereafter, the suspensions of the nanocomposites exposed to different

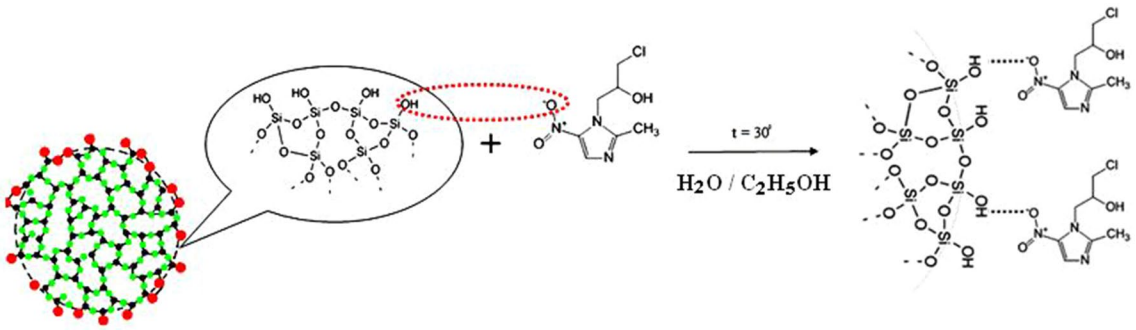

Fig. 2 Scheme for producing ornidasil

Table 1 The scale for the evaluation of drug efficacy

Concentration value *C* (CFU/ml)	The number of dead cells (%)
Between − 2.0 and −2.9	99.0
Between − 3.0 and − 3.9	99.9
Between − 4.0 and − 4.9	99.99
Between − 5.0 and − 5.9	99.999
Between − 6.0 and less	> 99.999

concentrations of ornidazole bacterial cells were cultured for 24 h on solid medium.

The number of the cells survived was estimated by counting the colony-forming units (CFU) on the surface of a solid nutrient medium. After that, the viability of the test cultures (survival index) was determined, using the formula: $C = \lg N^{t}/N_{tk}$, where N_t is the number of bacteria that has survived after the influence of the test compound, N_{tk}—the number of bacteria in the control experiment (without influence of the compound) for the same period of time. The effectiveness of the antibacterial action of nanocomposites was established according to Bryan's scale of numerical values (Table 1) (Bryan 1982), by calculating the index *C* in logarithmic equation which accounts for the number of bacterial cells which survived after the nanocmoposites action, according to which the compounds are considered effective only if the number of test cultures cells was reduced is below 4 log CFU/ml. Standardized suspensions of bacteria cells at the concentration of 1.5×10^8 cells/ml which did not contact nanocomposites were used as a negative control. Suspensions of the test cultures cells after the contact with 0.025% Ornigil® solution were used as a positive control.

Determination of the anti-adhesive effect of nanocomposites

To assess the ability of the bacterial cultures cells tested to adhere to human red blood cells we used different types of heparinized human blood (OO+, AO+, BO+, AB+). Erythrocyte's suspension was prepared as described in (Oborin et al. 2010). To separate erythrocytes from the blood plasma the sonication at 3000 spins/min was performed for 15 min, thereafter red blood cell were washed with the Hanks solution three times (Shipitsyna et al. 2014). To estimate the cell number, the above mentioned erythrocytes were diluted with the Hanks solution in the ratio 1:25 and the number of red blood cells was counted using a hemocytometer in five large squares, located diagonally at the magnification of (10 × 8). The number of erythrocytes in 1 ml of the solution was counted according to the formula:

$$X = \frac{A \times 4000 \times B}{80}$$

After the estimation of red blood cell count the solution was further diluted by the Hanks solution to reach the concentration of 8 M cells/ml. The final standardized erythrocyte solution prepared was used to determine the adhesive activity of the intact bacterial cell cultures (control) and of the ones treated with nanocomposites. In the study the bacterial cell cultures grown within 24 h on the tryptic-soy agar (TSA) plates at 36 ± 1 °C and under aerobic conditions were used. The bacterial cell suspension was prepared as described above and was standardized in McFarland units (Bollela et al. 1999). The bacterial cells were incubated with nanocomposites for 2 or 24 h, respectively. After the sample studied incubation, the cell suspension was separated from each sample studied and was added to the standardized suspension of human red blood cells in the volume ratio 1:1 in the wells of a 96-round-shaped-well microtiter plate (Zakharova 2011). Each sample studied was tested 3–6 times repeatedly. Thereafter, bacterial cells were incubated with erythrocytes for 60 min at 37 °C under constant rotation. Later on, the blood smears were prepared, stained by Giemsa and analysed with a light microscope at the magnification of (10 × 100). The adhesive activity of bacterial cell cultures was evaluated using light microscopy by estimating the average adhesion index (AAI) that equals to the average number

of the bacterial cells attached to one red blood cell (counted on 50 erythrocytes). Furthermore, we estimated the coefficient of the erythrocyte involvement (CEI) which stands for the percentage of the erythrocytes with bacterial cells attached to their surface and index of microbial adhesion (IMA)—average number of bacterial cells attached to one of the erythrocytes involved into the adhesion (Minukhin et al. 2013). IMA was estimated according to the following formula:

$$\mathrm{IMA} = \mathrm{AAI} \times \frac{\mathrm{CEI}}{100}$$

The degree of adhesivity was evaluated according to the scale (Minukhin et al. 2013) where low adhesive strains are the ones with IMA between 1.75 and 2.49, middle-adhesive with IMA = 2.5–4.0 and high-adhesive in the case when IMA was > 4.0. If IMA ≤ 1.7, the strains are classified as the ones not capable of adhesion.

Considering the fact that the data obtained was not normally distributed, which was determined by the Shapiro–Wilk test (sample size under 50), the statistic analysis was conducted using the Kruskal–Wallis test ($p = 0.05$ and $p = 0.01$), the H criteria was calculated according to the formula (Sacks and Glantz 1983):

$$H = \frac{12 \times D}{N \times (N+1)} = \frac{12}{N \times (N+1)} \sum n_M (R_M - R)^2$$

N the total number of observations across all the groups, n_M the number of observations in group M, R_M the average rank of all observations in group M, R the average rank of observations across all the groups.

The therapeutic effect of nanocomposites on complicated wounds

The therapeutic effect of the synthesized nanocomposites on complicated wounds of different aetiology was studied on 77 patients with diabetic foot syndrome (DFS) operated at Surgery Department No. 2, Kyiv City Clinical Hospital No. 6. The distribution of the patients by gender was as follows: 33 (42.86%) of female patients, 44 (62.5%) of male patients (Biliayeva et al. 2017b). All the patients were divided into two groups: the first one (I) was the main experimental grouup, the second one (II) was the comparison group treated with traditional medications. Group I included 31 patients, where local treatment of complications from DFS after surgical intervention was carried out by the application of 2% ornidasil sorbent. The comparison group was also divided into two subgroups of IIA and IIB. The IIA subgroup included 27 patients and the application Gentaxan sorbent was used for the local treatment. The IIB subgroup included 19 patients and Levomecol ointment was used for the local treatment. These two last drugs for the comparison group are traditionally used in the treatment of DFS.

Results and discussion

Ornidazole relates to the third generation of nitroimidazoles, and it is active in the treatment of anaerobic bacteria and protozoa. Its high efficiency is characterized by a fast acting bactericidal effect, low suppressive concentration relative to certain groups of bacteria, mainly anaerobic, low toxicity and prolong antimicrobial effect. However, the main advantages of ornidazole are the inhibition of the formation of the resistant forms of microorganisms due to the presence of chloromethyl group in its molecule (Fig. 1) and a low frequency of undesirable effects (Hizarcioğlu et al. 2004).

It has been found that water molecules can destroy the adsorption complex of ornidazole on the surface of silica by embedding between the hydroxyl groups of the surface and the active centres of the drug molecule. The hydrated complex formed in such a way appeared to be more stable than the complex in which the direct interaction of the adsorbed molecule with the surface occurs, and water molecules form weak links with this molecule and are arranged farther away from the surface. Thus, the solvated ornidazole is transferred from the surface of silica to the solution in which it can demonstrate its inherent antimicrobial action. This means that due to the water excess the molecules of ornidazole adsorbed on the surface of silica are gradually washed off (Hrebeniuk and Golub 2017).

In the aerosil modified by ornidazole (Fig. 3), there is only a partial shift in the band of the valence vibrations of the OH-group from 3500 to 3400 cm^{-1} and one can only

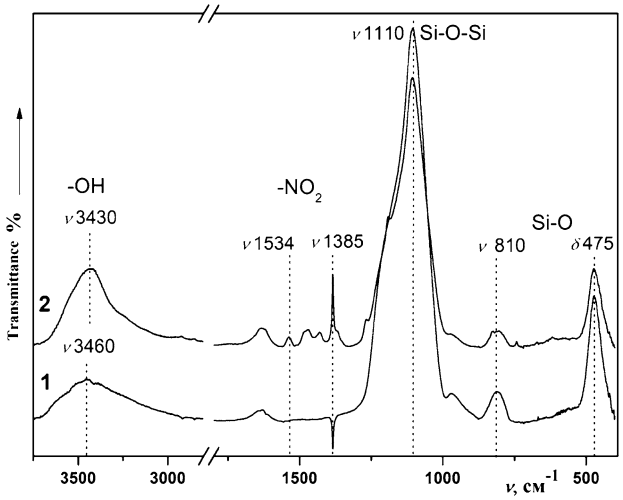

Fig. 3 IR absorption spectra of the nanocomposite based on pyrogenic silica and ornidazole

see the appearance of low-intensity absorption bands at 1570 cm^{-1} which corresponds to the asymmetric vibrations of the NO_2 group, which suggests that only physical adsorption takes place, and the shift of the oscillation band corresponding to the hydroxyl groups indicates that they are involved in the formation of hydrogen bonds of the ornidazole molecules adsorbed. This confirms the availability of ornidazole in the nanocomposite without any significant chemical interaction of the nanocomposite components with each other.

The concentrations of 2, 4 and 12%, which were selected for the impregnation with ornidazole, can be considered optimal because the content of the active substance was quite sufficient to show the antimicrobial activity (Herych et al. 2013), and there were still a lot of active centers on the surface of nanosilica, which were not occupied by the adsorbed molecules and they were able to adsorb some poisonous wound discharge of polypeptide nature (Jones 2006). It should also be mentioned that the nanocomposites with the content of the active ingredient of 2 and 4% proved to be more effective, than the ones with the increased content of ornidazole increased to 12%. The percentage of the release of the drug did not practically change, while the number of active adsorption centres capable to adsorbing the wound effluent decreased significantly (Murlanova et al. 2017). The results obtained confirm the correspondence of the experimental data to the previously done theoretical quantum-chemical calculations of the desorption process (Hrebeniuk and Golub 2017).

Considering that the main pathogens involved in the surgical infection of soft tissues are *Staphylococcus aureus* (43.5%), *Proteus mirabilis* (23.1%), *Escherichia coli* (12.6%), *Pseudomonas aeruginosa* (7.9%), *Staphylococcus epidermidis* (4.3%), *Enterobacter spp.* (3.8%), *Streptococcus pyogenes* (2.7%), and *Acinetobacter baumanii* (2.1%) (Biliayeva et al. 2017b), the antibacterial effectiveness of the ornidasil was tested using both Gram-positive (*Staphylococcus aureus* strain ATCC 25923) and Gram-negative (*Pseudomonas aeruginosa* strain ATCC 27853 and *Escherichia coli* strain ATCC 25922) bacteria. The estimation of antimicrobial activity was performed by the Gould method, since it allows the determination of the number of cells that survived after the action of various antimicrobial agents (Furtat et al. 2004).

The study of antibacterial activity of the nanocomposite (ornidasil 1%) showed its activity against the cells of Gram-positive bacteria *Staphylococcus aureus.* After a 2 h contact with the above-mentioned nanocomposite, 99.0% of staphylococcal cells were eliminated. However, the prolongation of the contact with the nanocomposite up to 24 h did not result in the increase of the antibacterial effect (Figs. 4 and 5).

It has also been found that in the case of a short-term action ornidasil was less effective towards the cells of Gram-negative bacteria. In particular, in contact with the cells within 2 h, les then 90% of the cells of the strains *Pseudomonas aeruginosa* and *Escherichia coli* were killed. In contrast in the case of a prolong contact up to 24 h, one hundred percent of the cell of the strains of Gram-negative test cultures of bacteria were found to be dead (Table 2). The increased efficiency of nanocomposite, in terms of a prolong contact with the test cultures may account for the continous release of the active substance, which ensures a much more effective action of the nanocomposite compared with that of the pure substance. Based on the data obtained, one can state that the highest bactericidal effect on Gram-negative bacteria was observed when the nanocomposite was applied longer than 24 h.

It has been shown that ornidazole in the composition of the pharmacopeial drug Ornigil® was less effective for the Gram-positive and Gram-negative bacteria studied than the ornidasil itself (Fig. 3, Table 2). According to the instruction, Ornigil® is known to be used as an antiprotozoal agent. However, we have detected its suppressive action as part of the nanocomposite for the bacterial test cultures. Thus, compared with the pure substance, the strengthening of the effect of ornidazole in the nanocomposites has been shown. Consequently, one can assume that the combination of the properties of the active substance and a nanocarrier can change the antimicrobial spectrum of ornidazole making it more effective and essential.

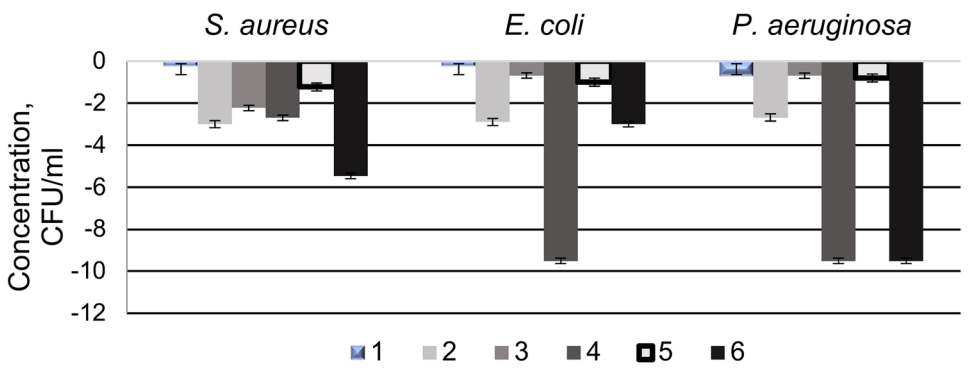

Fig. 4 Antibacterial activity of pure ornidazole and nanocomposites modified with ornidazole: within 2 h: 1—pure substance; 3—water form; 5—alcohol form; within 24 h: 2—pure substance; 4—water form; 6—alcohol form, the final concentration of the ornidazole in the nanocomposite is 1%

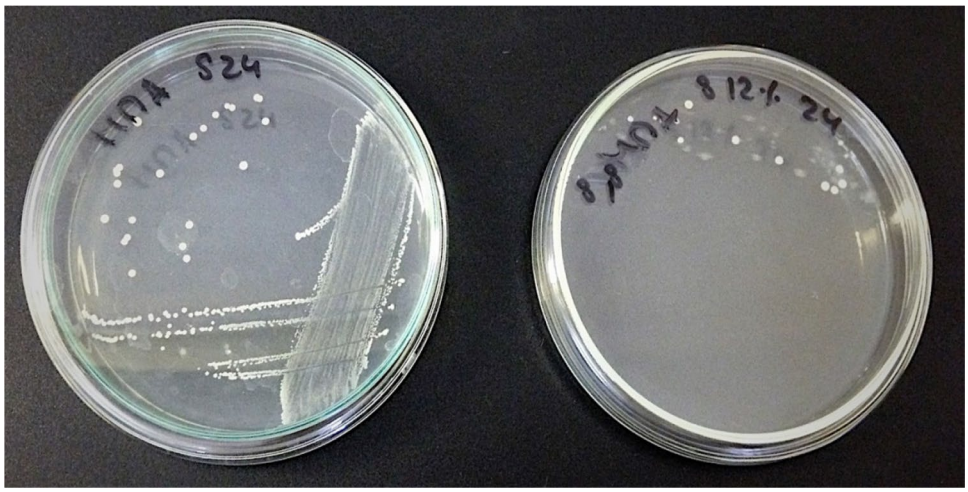

Fig. 5 Antibacterial effect of nanocomposites modified with ornidazole against *S. aureus* cells: 1—colonies of *S. aureus* before the treatment with nanocomposite; 2—after the treatment

Table 2 Antibacterial activity of nanocomposites

Test culture	The final concentration of the active substance (%)	Exposure time of the test cultures with the nanocomposite (h)			
		2		24	
		Concentration value (CFU/ml)	Number of killed cells (%)	Concentration value (CFU/ml)	Number of killed cells (%)
S. aureus	1	− 2.2	99.0	− 2.7	99.0
E. coli	1	− 0.7	< 90.0	No growth	100.0
P. aeruginosa	1	− 0.7	< 90.0	No growth	100.0

It is a well known fact that possessing the specific blood type is one of the characteristics of the human body which is indirectly linked to the probability of harboring specific bacterial species and as a consequence, suffering from infectious diseases caused by these specific bacteria (Blackwell et al. 2002; Cooling 2015). Therefore the ability of bacteria to adhere to the surface of the target cells in macroorganisms, such as erythrocytes, is determined by the blood type.

As a rule, bacterial adhesion to human red blood cells is studied using OO+ blood type, considering that glycophorins of the erythrocytes membranes are structurally identical to the glycocalyx of epithelial cells where the receptors of bacterial adhesins are located. On the one hand, the identity mentioned, and, on the other hand, the simplicity of erythrocyte preparation allows using these cells as a common model to study bacterial adhesive properties (Telesmanich et al. 2004). Moreover, the results of numerous studies (Cooling 2015; Berger et al. 1989; Spaan et al. 2015; Telen 2005) have identified the significant dependence of the risk of getting infected with certain diseases on a person's blood type. Taking into account the antigenic structure of red blood cells, and the fact that the composition of agglutinogens is unique for the erythrocytes of each blood type, that influences bacterial pathogenesis in the mammalian organism (Telen 2005), in our research the adhesive activity of the bacterial cultures was studied using OO+, AO+, BO+, and AB+ blood types.

Determination of the adhesive activity of the untreated bacterial cultures, which, thereafter, were used as a control group in comparison to the adhesivity of those after being treated with nanocomposites, allowed us to identify the tested strains as moderate- (IMA 2.51–4.0) and high-adhesive (IMA > 4.0), according to the scale described in the following studies (Minukhin et al. 2013). The index of microbial adhesion equaled 3.5 for *S. aureus* ATCC 25923 which corresponds to the findings of other authors (Shevchenko et al. 2017; Khrystian et al. 2018), according to whom the above mentioned index for collection's *S. aureus* strains varied between 2.44 and 3.5. In the case of Gram-negative bacteria the index of the microbial adhesion differed, the tested *E. coli* strain ATCC 25922 was classified as moderate-adhesive (IMA = 3.62) and *P. aeruginosa* strain ATCC 27853 as high-adhesive (IMA = 6.09). The results regarding *E. coli* strain appeared to be similar to the ones from other studies (Khrystian et al. 2018; Sukhodub et al. 2014) where IMA was between 3.48 and 3.5 for these bacteria.

In addition, we have found the dependence between the degree of the adhesivity of the tested bacterial cultures and the human blood types. The highest adhesive activity of the untreated *S. aureus* strain was observed in the case of the

attachment of OO+ and BO+ blood types to erythrocytes. In contrast, the lowest adhesivity rate has been registered for the attachment of AB+ blood type to the red blood cells. Our findings confirm the results obtained by Nurjadi et al. (2012) who stated that people with OO+ and AO+ blood types are more susceptible to staphylococcal colonization of nasal epithelium. Considering the above mentioned results, it can be assumed that the glycoprotein composition of erythrocytes may be one of the factors influencing the person's susceptibility to staphylococcal infections. However, the contact and attachment of staphylococcal cells to the blood cells is possible only in the case of pathogen's crossing the protective barriers, in particular, skin and mucosal layers.

As for the Gram-negative bacteria the highest adhesion rates have been observed for BO+ and AO+ blood type erythrocytes. The lowest IMA rates were observed by *E. coli* attachment to AB+ blood type erythrocytes and in the case of *P. aeruginosa*—to OO+ blood type cells. Our results regarding the cells of *E. coli* ATCC 25922 strain correspond to the study made by Ahmed et al. (2009) who observed higher risk of diarrhea, caused by *E. coli*, in children with AO+ blood type.

As we have already mentioned, the blocking of the bacterial adhesion to the cells of macroorganisms, such as erythrocytes, and abiotic surfaces is one of the mechanisms of the surface colonization prevention. These mechanisms are observed for the nanocomposites activity which is targeted towards primary stages of adhesion. Consequently, we have studied the potential of nanocomposites with ornidazole to bind with the adhesion sites on the surface of both Gram-positive and Gram-negative bacteria at the stage of primary adhesion (incubation for 2 h) and secondary irreversible adhesion (incubation for 24 h). It was confirmed that the nanocomposites tested were effective in the blocking of adhesion to red blood cells of all the bacterial strains tested. Reduced adhesivity of *S. aureus* cells to erythrocytes was observed at both studied time intervals (2 and 24 h). During a 2 h incubation a more effective blockage of adhesion was noticed when nanocomposites with the concentration of ornidazol 4% ($H = 14.03$, $p = 0.0002$) were used, and at a lower concentration of ornidazol (2%) the blockage was also significantly effective ($H = 11.06$, $p = 0.0007$). The prolongation of the treatment with nanocomposites (till 24 h) led to the increased effectivity at both concentrations of ornidazol: 2%—$H = 25.6$, and 4%—$H = 20.8$.

Furthermore, we have established the dependence between the efficiency of the nanocomposites anti-adhesive activity and the blood type of the erythrocytes to which the bacteria were able to adhere. As it was observed, a 2 h treatment with nanocomposite with 2% ornidazol caused the decrease in the adhesivity of *S. aureus* cells to the OO+ type red blood cells by 42%. The increase of ornidazol concentration to 4% was followed by the decrease in the bacterial adhesivity by 56%. The prolongation of the treatment till 24 h resulted in the increase in anti-adhesive activity for both concentrations of ornidazol tested. So, the decrease in staphylococcal adhesion was estimated as 84% (2% concentration of ornidazol) and as 75% (4% concentration) (Fig. 6). The observed anti-adhesive effect for OO+ blood type erythrocytes, could also be identified for AO+ type red blood cells, and the effectivity increased with the prolongation of the treatment. During a 2 h treatment the adhesive activity dropped by 41 and 43% at 2 and 4% concentration of ornidazol respectively, and the prolongation of the treatment till 24 h resulted in the decrease by 71 and 65%, respectively.

On the contrary, the study of BO+ and AB+ type red blood cells has not shown the dependence of staphylococcal adhesivity decreased upon the duration of the treatment with nanocomposites. During a 2 h treatment the adhesivity of *S. aureus* cells to BO+ blood type erythrocytes decreased by 60 and 33% respectively, when the concentrations of 2 and 4% ornidazol were used, for AB+ type erythrocytes, under the same conditions, the adhesivity dropped by 27 and 46%. The extension of the treatment to 24 h led to the decrease

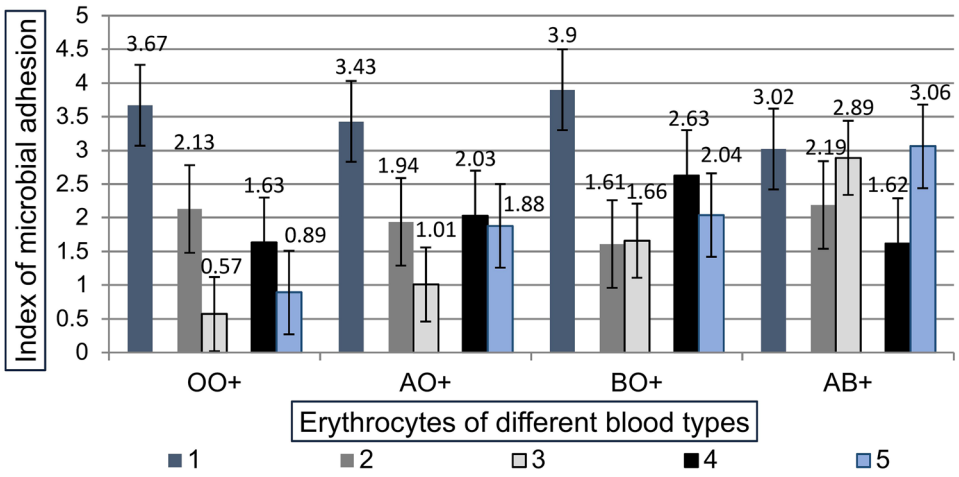

Fig.6 The effect of the treatment with nanocomposites with ornidazol on *S. aureus* cells adhesion: 1—untreated bacterial cells (control), 2—a 2 h treatment with nanocomposites (concentration of ornidazol 2%), 3—a 24 h treatment with nanocomposites (2% of ornidazol), 4—a 2 h treatment with nanocomposites (4% of ornidazol), 5—a 24 h treatment with nanocomposites (4% of ornidazol)

in staphylococcal adhesivity by 57 and 48%. In contrast, the treatment with 2% ornidazol during *S. aureus* adhesion to AB+ type red blood cells resulted in the decrease in adhesivity rate by 4% only, whereas increasing ornidazol concentration to 4% led to the absence of the inhibitory activity (Fig. 6).

With regard to Gram-negative bacteria, under the same conditions the significant inhibition of bacterial adhesion has not been observed.The treatment for 2 and 24 h against *E. coli* adhesive activity was more effective when using nanocomposite with 4%concentration of ornidazol ($H = 11.7$, $p = 0.0006$ by 2 h incubation and $H = 15.8$, $p = 0.00007$—24 h incubation). In case of the treatment with nanocomposites with 2% concentration of ornidazol the decrease in adhesivity has also been observed—$H = 6.6$, $p = 0.01$ during a 2 h treatment and $H = 13.3$, $p = 0.0003$ during a 24 h treatment. The decrease in *E. coli* ATCC 25922 adhesive activity to OO+ blood type erythrocytes was as high as 18% after a 2 h incubation and 50% after a 24 h incubation, respectively. The increase in ornidazol concentration to 4% strongly influenced the anti-adhesive activity of the nanocomposite, the adhesivity of *E. coli* decreased by 50% only after a 24 h treatment (Fig. 6).

At the same time, while studying *E. coli* adhesion to AO+ type red blood cells, the dependence between the effectivity of nanocomposites, concentration of ornidazol and the duration of the treatment has not been found. For instance, a 2 h treatment with nanocomposites resulted into the decrease in *E. coli* adhesivity by 41 and 45%, respectively when using 2 and 4% ornidazol, and by 41 and 48% after a 24 h treatment, respectively (Fig. 7). However, the study of the bacterial attachment to BO+ type red blood cells, revealed the dependence between the duration of the treatment with nanocomposites, concentration of ornidazol and the decrease in bacterial adhesive activity. A 2 h treatment led to the decrease in *E. coli* adhesivity by 15% when using lower ornidazol concentrations, while 4% ornidazol concentration resulted in the inhibition by 26%. The treatment of *E. coli* for 24 h with nanocomposites decreased the bacterial adhesive activity by 60% when using 2% ornidazol and by 69–4% ornidazol, respectively. Studying the adhesion to AB+ blood type erythrocytes showed the opposite—in particular, the reduction of anti-adhesive effectivity after the prolongation of the treatment and the increase of ornidazol concentration in nanocomposites. A 2 h treatment of nanocomposites with 2% ornidazol resulted in the decrease in *E. coli* adhesion by 45%, while for 4% ornidazol it decreased by 4% only. The treatment for 24 h had no effect on the bacterial adhesion, in comparison to the control.

Studying the adhesive activity of *P. aerugionsa* ATCC 27853 strain demonstrated that in comparison to other tested strains the blocking of the adhesion of nanocomposites with ornidazol was the most effective. While using 2 and 4% of concentrations of ornidazol in nanocomposites the decrease in adhesion to OO+ blood type erythrocytes was by 66 and 67% during a 2 h treatment and by 78 and 81% during 24 h, respectively (Fig. 7).

As for the BO+ type red blood cells, the anti-adhesive effect of the nanocomposite was observed only when the prolonged treatment with nanocomposite till 24 h and the increased concentrations of ornidazol till 4% were used. Adhesivity decreased by 53% after a 2 h treatment and by 60% after a 24 h, respectively. However, when the incubation with nanocomposites with 2% ornidazol was extended till 24 h, the increase in anti-adhesive activity was not observed: the adhesion inhibition was by 62% during a 2 h treatment and 57% during a 24 h incubation with nanocomposite (Fig. 8). The attachment of *P. aeruginosa* cells to AB+ blood type erythrocytes was inhibited more effectively when using lower concentration of ornidazol. For example, after a 2 h treatment adhesion of bacterial cells to AB+ type red blood cells decreased by

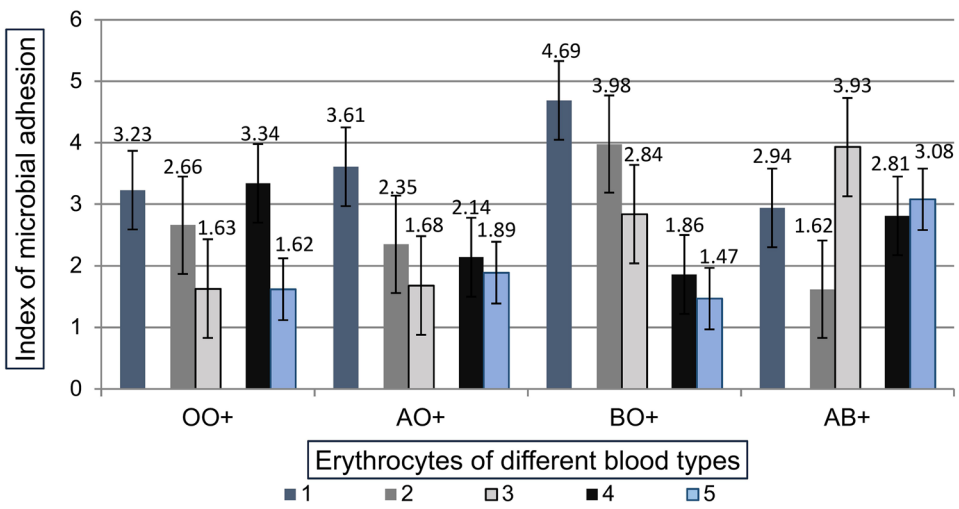

Fig. 7 The effect of the treatment with nanocomposites with ornidazol on *E. coli* cells adhesion: 1—untreated bacterial cells (control), 2—a 2 h treatment with nanocomposites (concentration of ornidazol 2%), 3—a 24 h treatment with nanocomposites (2% ornidazol), 4—a 2 h treatment with nanocomposites (4% of ornidazol), 5—a 24 h treatment with nanocomposites (4% ornidazol)

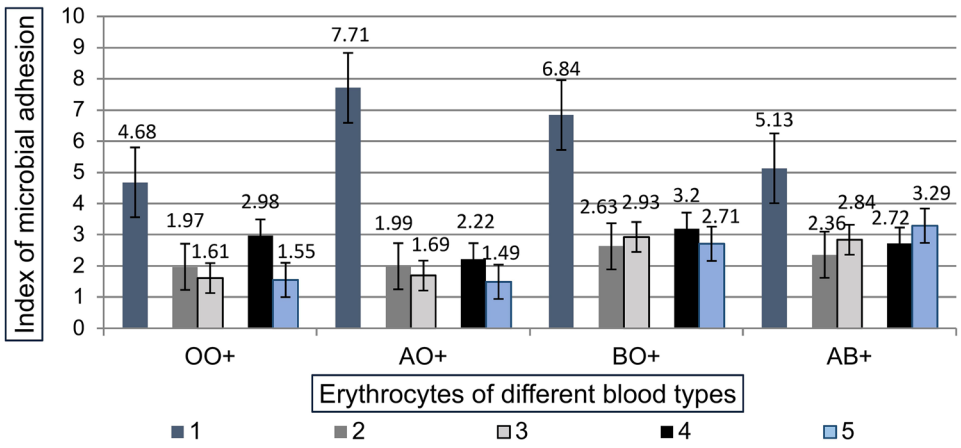

Fig. 8 The effect of the treatment with nanocomposites with ornidazol on *P. aeruginosa* cells adhesion: 1—untreated bacterial cells (control), 2—a 2 h treatment with nanocomposites (concentration of ornidazol 2%), 3—a 24 h treatment with nanocomposites (2% ornidazol), 4—a 2 h treatment with nanocomposites (4% of ornidazol), 5—a 24 h treatment with nanocomposites (4% ornidazol)

54% (the concentration of ornidazol 2%) and by 45% (the concentration 4%), after a 24 h treatment—47 and 36%, respectively.

In addition, in the study involving 77 patients with diabetic foot syndrome (after surgery), the therapeutic effect of ornidasil on the complicated wounds of different aetiologies and its efficacy compared with the conventionally used drugs Gentaxan and Levomekol were investigated.

Thus, according to clinical studies (Fig. 9), the relief of pain syndrome in case of ornidasil is 2.1 times quicker, the wound cleans up 1.7 times faster, the appearance of granulation is 2.5 times better, the marginal epithelization is 2.4 times more effective compared with the conventional methods of treatment with Gentaxan and Levomekol.

Conclusions

The research shows that nanocomposites with ornidazole are characterized as antimicrobial and antiadhesive agents against Gram-positive and Gram-negative bacteria, which proves that this type of nanocomposites can be prospective to inhibit the spread and development of infectious diseases. The prolonged treatment with nanocomposites resulted into higher antimicrobial effectivity.

Summarizing the obtained results of antiadhesiveactivity it can be stated that nanocomposites with alcohol form of ornidazol can be characterized as anti-adhesive agents against all the bacterial strains tested. As for *P. aeruginosa* and *S. aureus,* the blocking of adhesion appeared to be more effective when treating the nanocomposites with 2% ornidazol concentration for 24 h. The increase of ornidazol concentration till 4% in the nanocomposites resulted into a higher anti-adhesive effectivity of the nanocomposite

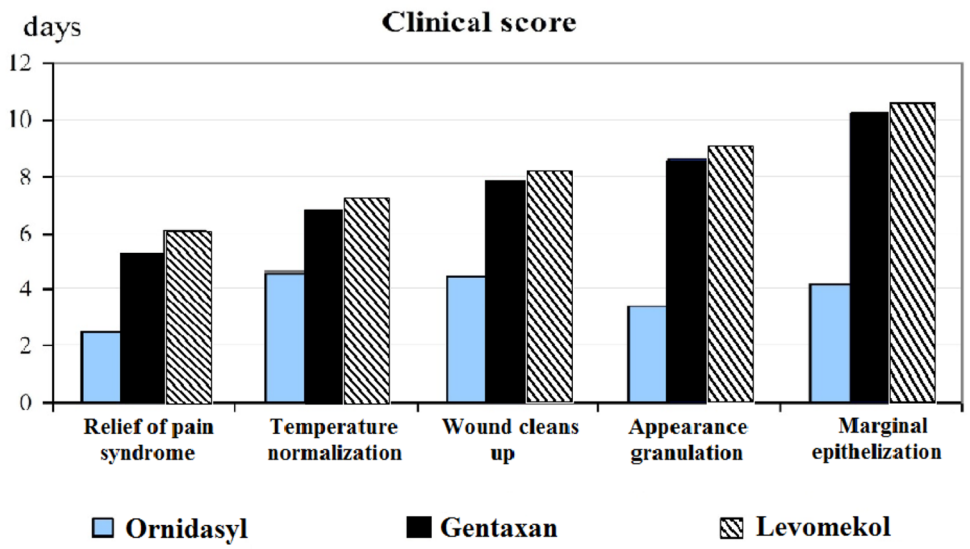

Fig. 9 The course of the wound process in the groups of clinical research

against *E. coli* and *S. aureus*, for a shorter treatment time. According to the results of the study, nanocomposite with ornidazol can be used as an adhesion inhibitor, however it should be noted that its effectivity may differ depending on the person's blood type.

The therapeutic effect of the synthesized nanocomposites studied on complicated wounds of different aetiologies and their efficacy have been shown in comparison with the traditionally used drugs.

The results obtained confirm that the synthesized nanocomposites based on pyrogenic silica with ornidazole immobilized on its surface are perspective as prolonged-action medicines for the delivery of ornidazole to the wound to prevent infectious processes, to burns, purulent wounds, diabetic foot syndrome and other wounds of complicated aetiology.

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Compliance with ethical standards

Conflict of interest On behalf of all the authors, the corresponding author states that there is no conflict of interest.

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