Bio-functionalization of phytogenic Ag and ZnO nanobactericides onto cellulose films for bactericidal activity against multiple drug resistant pathogens

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Abstract

The present study envisages synthesis of silver and zinc oxide nanobactericides from phytogenic source *Bupleurum aureum.* The synthesized nanobactericides were subjected to biophysical characterization and evaluated for bio-functionalization onto bacterial cellulose membrane which was synthesized by *Komagataeibacter xylinus* B-12068 culture strain. The synthesis was initially confirmed with UV -visible spectroscopy which conferred maximum absorbance at 415 nm for silver nanobactericides and 280 nm for zinc nanobactericides. The molecular binding and stabilization were predicted using FTIR which confirmed the presence of functional moieties. The XRD analysis revealed the crystalline nature and morphological characteristics was studied using TEM which confirmed the poly-dispersity of nanobactericides with the average size being 20-25 nm. The nanobactericides were tested against seven multi-drug resistant pathogens which were clinically isolated from patients suffering from myriad microbial infections. The tested pathogens were having antimicrobial resistant to ten different antibiotics and are reported to be members of ESKAPE pathogen group which are the major cause of drug-resistance. The nanobactericides displayed significant activity against test pathogens with silver nanobactericides showed the highest activity against *Escherichia coli* strain 55 with 24 mm zone of inhibition and zinc oxide nanobactericides displayed the highest activity against *Methicillin-resistant Staphylococcus aureus* with 20 mm inhibition zone. The nanobactericides embedded onto bacterial cellulose to develop nanobactericides incorporated films which were characterized using SEM along with physicochemical analysis. The bacterial films with nanobactericides were evaluated against test pathogens which showed activity against all the pathogens. The results obtained in the present investigation attributes towards growing scientific knowledge on drug resistance during the post drug-resistance era.

Keywords: *Bupleurum aureum*; Bio-functionalization; Silver nanobactericides; Zinc oxide nanobactericides; Multi-Drug resistance pathogens; Bacterial cellulose

1. Introduction

Implementation of nanotechnologies to develop nanobactericides has envisaged new possibilities to combat multi-drug resistant pathogens. The rapid expansion of multi-drug resistance mechanism among the microbial pathogens has created increasing demand for developing potent antimicrobial agents (Lobanovska and Pilla, 2017). The range of available antibiotics is reported to be ineffective towards combating drug-resistant pathogens (Ventola, 2015; Khan et al., 2016). The magnitude of resistance has exerted a huge impact on all forms of lives (Fair and Tor, 2014). Ever since the first antibiotic was discovered, there has been following of drug resistance which has resulted in the post antimicrobial resistance era (Ventola, 2015). Hence scientific communities are working towards the development of novel or new antimicrobial agents which can panacea antimicrobial resistance (Baker et al., 2013; Syed et al., 2016b). In order to develop new antimicrobial agents, rational strategies should be implemented which can act efficiently against a broad range of microbial pathogens (Reinhardt and Neundorf 2016; Navya and Daima, 2016). The recent technical advancement of nanomaterials can offer great potential as one of the alternatives in combating multi-drug resistant pathogens (Dakal et al., 2016). Nanobactericides are antibacterial agents at nano-scale bearing biocidal activity against wide range of pathogens owing to their unique physicochemical properties (King et al., 2018). Recent scientific studies demonstrated that bactericidal properties with a wide range of nanobactericides such as silver, gold, zinc, copper and platinum (Wang et al., 2017). The use of silver is well documented in traditional ancient records and before scientific knowledge could evolve it was one of the prime sources for curing microbial infections. The introduction of nano-sized silver has envisioned its multi-applicative properties (Yamada et al., 2014). Similarly, zinc is one of the essential element required by almost all forms of lives to carry out their metabolic activities (Osredkar and Sustar, 2011). Based on the scientific records, both silver and zinc can be one of the ideal sources for developing nanobactericides which can attract scientific attention in biomedical and pharmaceutical sectors owing to its biocidal activity at low concentration (Iravani, 2014). The synthesis of zinc and silver at the nanoscale is one of the interesting facets and various techniques are implemented. But most of these popular conventional methods including the chemical mode of synthesis are often bound with various implications such as generation of heat, use of toxic elements in synthesis protocol and require sophisticated facilities (Khan et al., 2017). In order to overcome these limitations, biogenic principle based production of nanobactericides is gaining importance (Baker et al., 2013). The biogenic sources include plants, microorganisms or their products which are able to synthesize different classes of nanobactericides with tunable applicative properties (Kavitha et al., 2013). The plant-mediated synthesis is regarded as simple and one-pot synthesis process in comparison with microbial mediated synthesis. The use of plants to synthesize nano-size particles can be attributed to phyto-remediation properties (Iravani, 2014). Plants constitute diverse classes of phyto-components ranging from phenol, terpenoids, alkaloids, polyphenols etc. these components act as reducing agents to synthesize nano-size particles with desirable properties (Patra et al., 2017). In recent times, there has been great interest in developing membranes bearing antimicrobial properties by incorporation of nanobactericides. Among of the different polymers evaluated for developing membranes, use of bacterial cellulose (BC) is regarded as one of the safe and efficient material. The BC is a network of cellulose fibrils with less than 100 nm which has resulted in unique properties thus forming one of the promising material. Scientific reports highlight the importance of BC due to its versatile properties, ease in large production via fermentation, inexpensive, generation in different shapes, absorptivity, elasticity, biocompatible, inert, hypoallergenity and mechanical properties (Torres et al., 2012; Moniri et al., 2017; Volova et al., 2018). The wide range of BC applications has already demonstrated significant progress in different industrial sectors such as textiles, paints, papers, cosmetics and pharmaceuticals. In recent years, BC has been one of the ideal choices among other polymers especially in designing novel biomaterials such as artificial skin substitutes for treatment of wounds, ulcers and burns (Volova et al., 2018). During such process, prevention of microbial infections is one of the top priority and bacterial cellulose lack antimicrobial potential (Hu and Hsieh, 2015; Heli et al.,2016). Development of nano-composites embedded with different types of nanobactericides is one of the interesting fields of nano-science which offers multi-applicative potentials (Faria-Tischer et al., 2016; Tsai et al., 2017). Based on this facts and consideration, BCF were prepared and tested against targeted multi-drug resistant pathogens. The isolation of drug-resistant pathogens were carried out in accordance with standard protocols at Krasnoyarsk Medical University. In the present investigation, seven drug resistant pathogens were selected which include both Gram +ve and Gram -ve Bacteria. The selection of pathogens was carried out based on the resistant to antibiotics and degree of infection. The selected pathogens were resistant to more than five different antibiotics.

2.Experimental

2.1.Plant processing

The plant materials (Stem and leaves) were collected from the abundant growing area of Krasnoyarsk region, Siberia, Russia. Plant materials were washed thoroughly under running tap water to remove the soil debris. The plant materials were chopped into small segments and 20 g of finely cut materials was added to one liter beaker containing 500 ml of sterile distilled water. The mixture was boiled for 30 minutes to obtained aqueous extract which was stored at 4^oC until further use.

2.2.Synthesis of silver and zinc oxide nanobactericides

The aqueous extract was subjected to synthesis of nanobactericides wherein, for the synthesis of silver nanobactericides, 1 mM silver nitrate was incubated with aqueous extract at ratio 7:3. The conversion of $Ag+$ to Ag^o was initially confirmed with a change in the color of reaction mixture and further confirmation was achieved with UV-Visible spectrophotometer. Samples were drawn every 5 minutes over period of 30 minutes and synthesis was monitored. Similarly, zinc oxide nanobactericides were synthesized by treating aqueous extract with 100mM zinc sulphate heptahydrate solution which was kept on magnetic stirrer and pH was adjusted with 1M NaOH. Samples were drawn at regular intervals as mentioned above and monitored using UV-Visible spectroscopy with spectral range between 100 to 800 nm.

2.3. Characterization of nanobactericides

The synthesized nanobactericides were subjected to characterization using various hyphenated techniques. The morphological structure and local elemental composition were determined via a high-resolution transmission electron microscope (HRTEM) JEOL JEM-2100 operating at an acceleration voltage of 200 kV. The HRTEM with an energy-dispersive spectrometer Oxford Inca x-sight. Selected area electron diffraction (SAED) was used to determine the crystal structure of nanoparticles. The possible role of aqueous extract as reducing agent was studied using FTIR spectroscopy. The crystalline nature was studied using X-ray diffractometer instrument operating at a voltage of 30 kV and average size was calculated based on Scherrer equation recorded spectra. $N= K \lambda / \beta \cos \theta$. Where K is the Scherrer constant with value from 0.9 to 1 (shape factor), where λ is the X-ray wavelength (1.5418 Å), β1/2 is the width of the XRD peak at half height and θ is the Bragg angle.

2.4.Production of bacterial cellulose films

The production of bacterial cellulose films was carried out according to the protocol described by Shidlovskiy et al., 2017. In brief, BCF were synthesized by *Komagataeibacter xylinus* B-12068 culture strain which was previously isolated from fermented tea (kombucha). The actively growing isolate was cultured in Hestrin-Schramm (HS) liquid medium and for 7 days at a temperature of 30°C under static conditions. Later after the incubation period, BCF were separated from bacterial cells and media component with the treatment of 1.0 M NaOH at 70 ^{o}C which was followed by repeated washing with deionized water. Then, BCF were placed in 0.5 % solution of hydrochloric acid for 24 hours to neutralize which was later rinsed with double distilled water. Then the films were stored until further use.

2.5. Bio-functionalization of nanobactericides onto BCF and their biophysical characterization

The synthesized nanobactericides were centrifuged and subjected to washing repeated for three times with sterile distilled water. The pellet was dissolved and pipette on the bacterial cellulose membranes until the complete membrane is immersed which was incubated at 37 ^{o}C for one hour on water bath. The obtained film was dried and subjected to biophysical characterization. The BCF were excised into small blocks (5×5 mm) which were subjected to morphological characteristics using scanning electron microscopy of ultrahigh resolution S-5500 (Hitachi, Япония, 2009). The sample was processed by placing onto the sample stage and sputter-coated with gold, using an Emitech K575X sputter coater (10 mA, 2×40 s). The sample was examined and the morphological characteristics were measured and recorded with image analysis program (Image Processing and Data Analysis) in Java. Mechanical properties of the BCF were investigated using an electromechanical tensile testing machine Instron 5565 (U.K.). Samples 75 mm long, 12 mm wide were prepared for studying physical and mechanical properties of the films.

2.6.Multi-drug resistant pathogens

The selected strains are reported to be multi-drug resistant strains bearing resistant mechanism for more than 5 different antibiotics. The test pathogens are *Acinetobacter baumannii* strain 210, *Acinetobacter baumannii* strain 211, *Pseudomonas aeruginosa* strain 55, *Pseudomonas aeruginosa* strain 40, *Klebsiella pneumoniae* strain 104, *Methicillin-resistant Staphylococcus aureus*, *Escherichia coli* strain 55.

2.7.Preparation of test bacterial suspension

The inoculum of test bacteria were prepared according to the protocol described by Teh et al., 2017 with slight modification. In brief, the actively growing test bacterial strains were inoculated into 10 ml sterile Mueller Hinton broth (MHB) and incubated overnight at 37 ^{o}C. The overnight test bacterial suspensions were adjusted to 0.5 McFarland Standard with sterile Mueller Hinton broth under aseptic conditions. The preparation of inoculum was carried as per the Clinical and Laboratory Standards Institute (CLSI) guidelines

2.8. Antimicrobial activity of Ag and ZnO nanobactericides

The synthesized nanobactericides were centrifuged at 15,000 rpm for 20 minutes. The obtained pellet was washed thrice with double distilled water and 5 mg/ml concentration was evaluated for antimicrobial activity via well diffusion assay and micro-broth dilution assay. In brief pre-warmed MHA (Mueller-Hinton agar) plates were seeded with test bacterial suspension ($1.5 x 10^{6}$ CFU/ml) and swabbed uniformly, later by using sterile cork borer agar was punched to obtained wells and 100 µl nanobactericides were added into each well and incubated at 37°C for 24 hours. After incubation, the zone of inhibition was measured and interpreted with different antibiotics.

2.9.Minimal inhibitory concentration

The minimal inhibitory concentration (MIC) was carried out according to the protocol described by Syed et al., 2016b. In brief, the plates were prepared under aseptic conditions and volume of 100 µL of test material (nanobactericides 1 mg/ml). The test material was pipette it out in the first row followed by addition of 50 µL of nutrient broth to all other wells. Further, serial dilutions were performed using a multichannel pipette and 10 µL of resazurin as growth indicator was seeded to each well. The final volume of the broth was adjusted with addition of 30 µL isosensitised broth to each well ensuring the final volume of the nutrient broth. Finally, 10 µL of bacterial suspension ($1.5 x 10^{6}$ CFU/ml) was added to each well. The plate was incubated at 37° C for 18 to 24 hours. The color change was then assessed visually from purple to pink or colorless. The lowest concentration at which color change occurred was taken as the MIC value (Sarker et al., 2007).

2.10. Antibacterial activity of silver and zinc oxide nanobactericidal cellulose films

The BCF embedded with nanobactericides were subjected to antibacterial activity according to the protocol described by Volova et al., 2018 with slight modification. In brief, small blocks of BCF with nanobactericides were excised under aseptic condition. The pre-warmed MHA (Mueller-Hinton agar) plates were seeded with ($1.5 x 10^{6}$ CFU/ml) which was swabbed uniformly. The prepared blocks of BCF were placed onto the swabbed media and incubated at 37° C for 24 hours. The activity was measured as zone of inhibition across the block in millimeters.

3. Results and Discussion

The results obtained in the present investigation attributes towards growing scientific knowledge on developing potent antimicrobial agents against drug resistant microbial pathogens. The scientific evidence of nanomaterials bearing antimicrobial agents are enormous which has led to implementation of nano-sized particles as effective tool against microbial infections (Beyth et al., 2015). Scanty reports are available on bio-functional nanomaterials embedded onto bacterial cellulose membranes and their evaluation against drug-resistant isolates. In the present investigation, clinical isolates were collected from the patients with severe microbial infection. The main theme of the present investigation was to select multiple drug resistant strains which are ineffective by ten broad-spectrum antibiotics. The selected strains were bearing resistance to ampicillin, cefoperazone, cefepime, chloramphenicol, imipenem, meropenem, gentamicin, tetracycline, tobramycin and vancomycin. In order to synthesize facile and green route for nanobactericides, Siberian plant *Bupleurum aureum* was selected as subject of interest owing to its traditional records and its current usage by local Siberian healers in curing various ailments which is being used since 2000 years. Studies demonstrate that, *Bupleurum aureum* is rich in phyto-constituents such as saponins, tannins, essential oils, vitamin C and alkaloids (Naboka et al., 2014). Furthermore, *Bupleurum aureum* is reported to bear multiple health benefits for instance anti-tumor, anti-viral, immuno-modulator, improves metabolic process (Nyobe et al.,2012). Even though Siberia constitute one of the rich biodiversity, less exploratory studies have been carried out with its medicinal plants. In the present study, *Bupleurum aureum* medicinal plant was processed to obtained aqueous extract which was evaluated for the reduction of metal salts to synthesized silver and zinc nanobactericides. The synthesis was initially confirmed with change in color of the reaction mixture and further confirmation was studied using UV-Visible spectroscopy which displayed maximum absorption peaks between 100 to 800 nm. The maximum absorbance of silver nanobactericides was observed at 415 nm and zinc oxide nanobactericides was obtained at 280 nm (Fig.1) respectively. The UV-visible spectroscopy is one of the ideal and sensitive tool to predict the formation of silver and zinc nanomaterials (Mahmudin et al., 2016). The intense absorption is due to the surface plasmon resonance which describes the collective excitation of electrons (Umadevi et al., 2012). The change in the color of reaction mixture might be due to the resonance of frequency from electromagnetic field with electron motion which results in strong absorption (Saranya et al., 2017). In the present investigation, broad absorption was observed which might be due to the size distribution of the particles (Mafune et al., 2000). The synthesis was rapid under the influence of parameters like temperature and pH. It was observed that synthesis was maximum at 90^{o}C and at pH 9. The reaction rate increased with increase in temperature from 30 to 90 ^{o}C and it was observed that at 90 ^{o}C, there was gradual shift of absorbance peak towards UV range which was maximum in comparison with other temperatures tested (Suppl.1). The influence of temperature on particle size has been previously studied which indicates formation of nuclei from silver ions at elevated temperature and prevents the secondary reduction process of pre-formation of nuclei (Phanjom and Ahmed, 2017). Similarly, when pH was increased from 5 to 9, maximum nanobactericides synthesis was observed at pH 9 (Suppl.2). The synthesis was rapid and completed within 25 minutes of incubation time. At pH 9 the increased absorbance and shifting of peak towards UV range indicated the effect of pH for reduction of metal salts. The influence of intrinsic factors to mediate the synthesis is well demonstrated in previous studies which was justified in the present study (Nagarajan and Kuppusamy, 2013; Mendis et al., 2016). The possible role of phyto-components as reductive agents was predicted with FTIR analysis of synthesized nanobactericides (Fig.2a,b). The absorption peaks obtained in FTIR analysis corresponded to various functional groups (Table - 1). The FTIR analysis of nanomaterials predicts the bio-molecular interaction which envisions the functional groups associated with the complex interaction (Pawlikowska-Pawlęga et al., 2012). It has been reported that IR absorption give rise to stretching , bending vibrations at varied frequency range which confines to the characteristic functional groups (Parida et al., 2008; Chaitanya et al., 2011; Lin et al., 2014). The obtained results coincides with majority of scientific reports which confers the role of phyto-components in mediating the synthesis and stabilization of nanomaterials which is one of the prime factor for attenuating the applicative property (Kuppusamy et al., 2016). In the present investigation, the crystalline nature of nanobactericides was confirmed with XRD analysis which displayed intense absorbance peat at 2θ angle which denoted the (111), (200),(220) and (311) planes which clearly suggested the face-centered cubic of silver which justifies the previous findings (Agnihotri et al., 2014). Similarly, for zinc nanobactericides, the most intense peak at 2 theta is obtained at 40.89 along the (102) orientation. The diffraction peaks are in accordance with the typical zincite structure of ZnO diffraction and all the peaks were well indexed to hexagonal ZnO wurtzite structure (Fig. 3). The results coincides with earlier findings (Khalil et al., 2014). The morphological characteristics of nanoparticles were studied using TEM analysis which depicted the polydispersity of synthesized nanobactericides with different size and shape. The average size of the synthesized nanobactericides was found to be 20±5 nm (Fig.4). The characterization results obtained in the present study are in accordance with the earlier findings

(Syed et al., 2016b; Al-Shabib et al., 2016). In the present investigation, more emphasis was attributed towards evaluating the synthesized nanobactericides against range of human pathogenic microorganisms. The antimicrobial activity of synthesized nanobactericides was measured as zone of inhibition via well diffusion assay (Fig.5). In comparison of activity, the synthesized silver nanobactericides expressed highest zone of inhibition against Gram -ve *Escherichia coli* strain 55 with 24 mm zone of inhibition and least activity was conferred against *Klebsiella pneumoniae* strain 104 with 12 mm zone of inhibition. Similarly, the zinc oxide nanobactericides displayed highest activity against *Methicillin-resistant Staphylococcus aureus* with 20 mm inhibition zone and least activity was compared obtained against *Acinetobacter baumannii* resistant strain 210 with 13 mm zone of inhibition. In order to confirm the minimal inhibitory concentration of nanobactericides, minimal inhibitory concentration assay was carried out in triplicates and rezazurin was used as growth indicator of the test pathogens. The optical density of the test organisms seeded with nanobactericides was measured at 600 nm, interestingly, the MIC results (Table 2) were in accordance well diffusion assay. In the present investigation, the synthesized nanobactericides were further bio-functionalized onto bacterial cellulose films. The nanobactericides were externally embedding by pipeting on the pre-synthesized bacterial cellulose films. The film was immersed into nanobactericides solution and incubated at 37 ^{o}C for one hours During which, the nanobactericides were able to bind with bacterial cellulose thus forming complex. The developed film was completely dried under strict aseptic condition to remove the moisture content. The dried film was subjected to biophysical characterization to study the mechanical properties of developed BCF. The binding of nanobactericides with film was confirmed with scanning electron microscopy which displayed the nesting of nanobactericides between the cellulose fibers. The results of biophysical characterization of BCF can be cited in table 3.Interestingly, the inclusion of silver nanobactericides significantly changed the mechanical properties of bacterial cellulose which resulted in increase in the young's modulus which was found to be 5809,79±899,91. The tensile strength was increased by two orders in comparison with net bacterial cellulose as mentioned in the table 3. Similar observation was also seen with inclusion of zinc nanobactericides which displayed slightly increase in the young's modulus and doubled elongation rate. The tensile strength also increased which was found to be 105,17±26,26. The nesting of both silver and zinc oxide nanobactericides showed similar observation on the physical and mechanical properties of bacterial cellulose. The obtained results are in accordance with earlier findings which also states the influence of nanomaterials on the physicochemical properties of bacterial cellulose (Pal et al., 2017; Moniri et al., 2017). The mode of embedding /immobilization of nanobactericides directly facilitates the interaction with bacterial cellulose (Vivekanandhan et al., 2012). Further, it offers advantage of size-tunable properties of nanobactericides, different size of nanobactericides can be immobilize which can influence the antibacterial properties. It has been well studied and demonstrated that lower the size of nanomaterials higher will be the antimicrobial activity. Also with such pipetting of nanobactericides results in freely available onto the surface of bacterial cellulose thus increasing the rigidity and prevents from oxidation by providing chemical stability and it can offer better bactericidal properties (Luan et al., 2012; Liyaskina et al., 2017). The obtained results are in accordance with study conducted Benavente et al., 2017 which highlighted the inclusion of silver nanoparticles onto the cellulose membrane and its antibacterial potential. According to the SEM analysis, the size of the silver and zinc oxide nanobactericides ranged between 20 to 100 nm (Fig.6). The SEM analysis also displayed the agglomeration of particles thus forming monolayer onto the surface of BCF. The interaction of nanobactericides with bacterial cellulose is yet to be completely elucidated, studies predict the electrostatic interaction between nanobactericides with electron rich oxygen atoms present in bacterial cellulose (Maria et al., 2010). It is interesting to elucidate the exact mechanism of forming layer which will be the subject of future studies. The bactericidal activity of developed BCF was tested against the selected drug resistant pathogens and activity was measured as zone of inhibition across the BCF blocks (Fig.7). Interestingly, the binding of nanobactericides with cellulose had no impact on the bactericidal properties. The efficacy of nanobactericides as antimicrobial agents have been studied earlier but to best of our knowledge, scanty reports are available on the evaluation of nanobactericides against wide range of drug resistant pathogens including both Gram +ve and Gram -ve (Ansari et al., 2011; Sadanand et al., 2016;). The test pathogens are reported to cause severe microbial infections and are one of the major contributors of nosocomial infections. The tested pathogens belong to ESKAPE group which are reported to leading contributors of multi-drug resistant across the globe in comparison to other microbial pathogens (Santajit and Indrawattana, 2016). The range of antimicrobial resistance mechanisms among ESKAPE pathogens includes enzymatic inactivation, modification of drug targets, alteration of efflux pumps and biofilm production (Syed et al., 2016a,b; Santajit and Indrawattana, 2016). The antimicrobial results obtained in present investigation highlights the importance of nanobactericides as emerging tool in combating drug resistant pathogens. In the present investigation, silver nanobactericides showed significant activity against all the test pathogens in comparison with ZnO nanobactericides. The complete schematic representation of present investigation has been summarized in figure 8. The efficacy of silver nanomaterials have extensively demonstrated and the results are in accordance with previous scientific reports (Syed et al.,2016a). The attempt in the present study is carried out based on the interest of developing dressing materials for better wound healing process with respect to drug resistant pathogens. It is reported that combined microbial infections are causing severe threats among the immuno-compromised patients and at hospital with poor sanitary conditions. These pathogens can easily be transmitted to healthy individuals and can also enter the ecosystem and affect all forms of lives. Hence developing new potent antimicrobial agents with minimal side effects is highly essential in current scenario. The combinatorial antibiotic therapy is often not feasible which can lead to organ failures owing to high dose of antibiotics (Ventola, 2015). The results obtained in present investigation are promising enough to report the activity of nanobactericides against multi-drug resistant pathogens which can open new avenue towards developing novel bio-functionalized antimicrobial agents.

4. Conclusion

The present study attributes towards expanding scientific knowledge on development of novel bio-functionalized nanobactericides against drug resistant microbial pathogens. The development of nanobactericides via phytogenic source expressed facile process towards producing silver and zinc oxide nanobactericides which were functionalized onto bacterial cellulose films. The efficacy of nanobactericides and bio-functionalized bacterial cellulose films displayed significant bactericidal activity against tested multi-drug resistant pathogens. The obtained results envisage the potential of nanobactericides during the post-antibiotic era.

Acknowledgements

Authors are thankful for facilities provided by Siberian Federal University and Krasnoyarsk State Medical University to carry out the present study.

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