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Original Article

Antibacterial Activity of New Compounds Based on Nanocomposites

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Abstract

Background: Antimicrobial resistance is a major problem in treatment and public health, and it has been increasing over the last few decades. Hence, serious measurements are needed to overcome this challenge. In this study, we evaluated antibacterial and antifungal activity of some nanocomposites including titanium dioxide (**5a**), polyimide nanocomposites containing cerium oxide (**5b**), silver-titanium dioxide nanoparticles prepared under desired conditions (**5c**), polyaniline/wheat husk ash PANI /WHA (**5d**), Ag-TiO_2 prepared by sol–gel route (**5e**), and cellulose-graphene (**5f**) against some bacterial and fungal strains, which are the most common agents in many infectious diseases.

Methods: The nanoparticles were prepared in desired condition. The agar dilution and well agar diffusion methods were used for determination of inhibition zoon and minimum inhibitory concentration (MIC) during preliminary evaluation of antimicrobial activity against *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans*.

Results: The results showed that the nanocomposites had good activity against gram-negative bacteria.

Conclusions: Our results showed that the inhibitory activity of these nanocomposites on gram-negative bacteria was better than gram-positive bacteria.

Keywords: Antimicrobial activity, Nanocomposite, Silver-titanium dioxide, Polyimide, Cellulose-graphene

Background

The high level of resistance in different microorganisms to a majority of antimicrobial agents is one of the global threats, and researchers have always been struggling to cope with infectious diseases causing mortality and morbidity worldwide. To overcome this threat, the synthesis and discovery of new compounds to replace existing antimicrobial agents is an effective strategy. Metal-based nanoparticles (NPs) and investigation of their antimicrobial activity could be one of such alternatives. The size of NPs provides high interactions in small molecule antibiotics (1,2) and high surface of adhesion in ligands; therefore, the use of NPs can be a good strategy to enhance interactions in target bacteria (3). Recent research on such NPs as TiO_2, cellulose-graphene, and polyaniline indicated encouraging results for antibacterial nature (4). TiO_2 has received a special attention because of its variety of scientific and technological applications, such as self-cleaning surface applications (5), photocatalyst (6), antibacterial agents (7,8), building materials (9,10), anti-fogging (11), and photodynamic therapy (12). In our previous study, we synthesized some NPs including TiO_2 (13), polyimide nanocomposites containing cerium oxide (14), silver-titanium dioxide nanoparticles (Ag-TiO_2) prepared under desired conditions (15) and sol–gel route (16), polyaniline/wheat husk ash PANI /WHA (17), and cellulose-graphene (18). In this study, we evaluated antibacterial and antifungal activity of these NPs against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans.*

Material and Methods

Preparation of Nanoparticles

TiO_2 NP was prepared by adding 12 mL of Ti(OC3H7)4 solution in 1.35 mL of MeOH for 3 min. The mixture was sonicated and agitated for 210 min at 70 0C, then it was added to hot solution. The smoothed sediment was washed with hot water and organic solvents to remove the adsorbed impurities, then it was calcined for 3 hours at different temperatures (13). Cerium oxide (1 g) was added into a solvent containing ethanol (95%) and 3-aminopropyltriethoxysilane (APTS) to prepare polyimide nanocomposites containing cerium oxide. The mixture was sonicated for 10 minutes and heated at 80°C for 1 hour. After adding *N*, *N*-dimethyl acetamide (DMAC), the mixture was placed in an ultrasonic device for 4 hours and a gray suspension of cerium oxide was prepared. To obtain a viscose polyamic acid, DMAC 4,4-oxydianiline (ODA, 0.0767 g) and pyromellitic

dianhydride (PMDA, 0.1524 g) were stirred for 2 hours at 80°C and 400-500 rpm. After 2 hours of preparing both the suspension and polyamic acid, the suspension was added to polyamic acid, then stirred for 24-48 h to get the condensed material at the same rate and temperature. In this stage, the magnet could not move within the material. The material was discharged into a crucible and the four-stage cooking was performed as follows: 1 h at 100°C, 2 hours at 200°C, 1 hours at 250°C, and 1 hour at 300°C (14). To obtain Ag-TiO_2 nanosol, photodeposition of silver on TiO_2 NPs was executed by adding a desired volume of aqueous $AgNO_3$ (10^{-3} M) followed by irradiation for 120 minutes with visible light source (200 W halogen lamp). Finally, the product was centrifuged and washed with distilled hot water and organic solvents to remove the adsorbed impurities, and then calcined for 3 hours at different temperatures (15,16). Polyaniline/wheat husk ash nanocomposite was prepared by adding 1 g of KIO_3 to 100 mL of sulfuric acid (1 M) and stirred by magnetic mixer until a uniform solution was obtained. Then wheat husk ash (1 g) and fresh distilled aniline monomer (1 mL) were added to stirred solution one after the other at room temperature for 5 hours.

Consequently, to separate the oligomers and impurities, the filtered product was washed several times with deionized water and then dried in oven for 24 h at 60°C (17). Inside the beaker, 4.5 g of cellulose and 0.5 g of graphene and acetone as a solvent were poured, and the solution was placed on heater while stirring until dry and then cellulose-graphene nanocomposites were obtained (18). Table 1 shows the size of NPs.

Antimicrobial Screening

Microbial Cultures and Growth Condition

Standards collection of *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes*, and *Candida albicans* were obtained from Urmia University of Medical Sciences, Iran. All the test strains were retained on nutrient agar slants (Sigma Aldrich, the US) at 37°C and subcultured on nutrient broth (Sigma Aldrich, the US) for 24 hours prior to antibiogram test. These bacteria and fungi were used as important pathogens in estimating the antimicrobial nature of the synthesized NPs (19).

Agar Diffusion Method-Individual Compounds Solution

The antibacterial activity of nanocomposites was determined according to Clinical & Laboratory Standards Institute (CLSI) (20). The prepared NPs were dissolved in 100% dimethyl sulfoxide (DMSO) to final concentrations of 5, 2.5, and 1 mg/mL. Bacterial suspension with the turbidity of 0.5 McFarland was spread on Mueller-Hinton agar (MHA) plates with a sterile swab (19). Afterward, wells of 8 mm diameter were punched into the agar medium using a sterile borer and were loaded and filled with 70 µL of nanocomposites and allowed to diffuse at 37°C for 24 hours and at 25°C for 48 hours for bacterial and fungal loads, respectively. After incubation, the diameters of the growth inhibition zones were measured in mm. All experiments were performed with three replications for all mentioned microorganisms. Data were expressed as mean ± standard deviation (21,22).

Agar Dilution Method

Our plates containing defined concentrations of nanocomposites were prepared by using a single stock of each nanocomposite and pouring the molten agar into petri dishes. Experiments were performed with six different concentrations of each NP (1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, and 31.25 µg/mL) to prepare doubling serial dilutions of nanocomposites in medium up to the sixth concentration. Negative control plates were prepared at the same way (23). Suspension of each microorganism with turbidity of 0.5 McFarland was transferred on MHA containing different concentrations of the nanocomposite to see their antimicrobial power. The growth of microorganism on MHA indicated that specific concentrations of NPs were unable to prevent the growth of the desired bacteria or fungi. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of an antimicrobial that inhibited the visible growth of a microorganism after overnight incubation. Also, minimum bactericidal concentration (MBC) was identified by determining the lowest concentration of antimicrobial agent that reduces the visibility of the initial bacteria or fungal inoculum by a preset reduction such as ≥99.9% (24). To get MBC and minimum fungicidal concentration (MFC), a loopful was taken from the MIC wells and streaked on plates. The growth was observed after incubation at 37°C for 24 hours and at 25°C for 48 h for bacterial and fungal loads, respectively (25).

Results

Measurement of Antimicrobial Activity

The antimicrobial natures of nanocomposites including TiO_2 (**5a**), polyimide nanocomposites containing cerium oxide (**5b**), silver-titanium dioxide nanoparticles prepared under desired conditions (**5c**), polyaniline/wheat husk ash PANI /WHA (**5d**), Ag-TiO_2 prepared by sol–gel route (**5e**), and cellulose-graphene (**5f**) were measured according to their zone of inhibition against desired microorganisms. The MICs for the nanocomposites are shown in Tables 2 and 3.

In present study the increasing of concentration

Table 1. The Size of Nanoparticles

Nanoparticle	TiO_2	Polyimide nanocomposites containing cerium oxide	Ag-TiO_2 Calcination temperature (350°C)	Ag-TiO_2 Calcination temperature (700°C)	PAN/WHA	Cellulose-graphene
Size (nm)	8	41	8.2	14.48	18	45

Table 2. Antimicrobial Results of Synthesized Nanocomposites at Three Different Concentrations (5, 2.5, 1 mg/mL)

Nanocomposites	*Pseudomonas aeruginosa*	*Escherichia coli*	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Candida albicans*
TiO_2 (**5a**)	14,12,12	0, 13, 12	0, 0, 0	14,12,14	0, 0, 0
Polyimide cerium oxide (**5b**)	10, 14, 12	12,12,12	0, 0, 0	10,12,12	0, 0, 0
Silver titanium dioxide (**5c**)	10, 12, 12	0, 14, 11	0, 0, 0	10,14,12	0, 0, 0
PANI /WHA (**5d**)	12, 12, 14	11, 14, 11	0, 0, 0	12,13,12	0, 0, 0
Ag-TiO_2 (**5e**)	12,16,13	10, 14, 11	0, 0, 0	12,15,12	0, 0, 0
Cellulose-graphene (**5f**)	10, 16, 12	10, 14, 11	0, 0, 0	10,14,12	0, 0, 0

Table 3. Minimum Inhibitory Concentrations of Synthesized Compounds (µg/mL) for Tested Bacteria

	Staphylococcus aureus	*Streptococcus pyogenes*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Candida albicans*
5a. TiO2	NA	800±0.28	NA	400±0.23	NA
5b. Polyimide	NA	800±0.82	800±0.8	400±0.81	NA
5c. Ag-TiO_2	NA	800±0.23	800±0.8	400±0.18	NA
5d. Polyaniline/WHA	NA	800±0.18	NA	400±0.23	NA
5e. Ag-TiO_2	400±.03	800±0.81	800±0.8	200±0.3	800±0.3
5f. Cellulose/graphene	800±0.6	800±0.8	400±0.6	200±0.3	NA

Data were expressed as mean ± standard deviation.
NA, No Activity.

of nanocomposites from 1 to 5 mg/ml lead to higher antibacterial activity against *Escherichia coli* , Pseudomonas aeruginosa an Streptococcus pyogenes from 12-15 mm.

For bacteria, the MICs of the compounds **5a**, **5b**, **5c**, and **5d** ranged from 400 to 800 µg/mL, while the MIC of the compounds **5e** and **5f** containing Ag-TiO_2 and cellulose/grapheme were determined in the range of 200 to 800 µg/mL. The MICs and MBCs values were similar for bacteria growth but about Candida. albicans, it was inhibited only by Ag-TiO_2 and minimal fungicidal concentration and MIC values were the same.

Discussion

In this study, the antimicrobial activity of some nanocomposites including TiO_2 (**5a**), polyimide nanocomposites containing cerium oxide (**5b**), silver-titanium dioxide NPs prepared under desired conditions (**5c**), polyaniline/wheat husk ash PANI /WHA (**5d**), Ag-TiO2 prepared by sol–gel route (**5e**), and cellulose-graphene (**5f**) were evaluated against important human pathogens. According to the results, gram-negative bacteria appeared to be more tolerant to silver than gram-positive bacteria (Table 1), except for *S. pyogenes*. Among six synthesized nanocomposites in this research, only Ag-TiO_2 prepared by sol–gel route (5e) had an inhibitory effect on *C. albicans*. According to the results shown in Table 1 and 2 , the order of inhibitory power was found to be 5e>5f>5c>5a>5d. Ag-TiO_2 nanocomposite prepared by sol–gel **5e** route exhibited stronger bactericidal effects than the silver-titanium dioxide prepared under desired conditions **5c**. Overall, recent findings show that the most important reason is the difference in particle size (26). The size of NPs is so critical for antimicrobial efficacy, so that as the size of NP becomes smaller, its efficiency in inhibiting the growth of microorganisms increases (27). The results of our report showed that antibacterial efficiency of Ag-TiO_2 was better than TiO_2 and these differences have also been found in recent studies (28). The Ag-TiO_2 NPs prepared by sol–gel route (**5e**) had higher efficiency in iinhibition of the growth of various microorganisms. Interestingly, the antibacterial activity of cellulose-graphene (**5f**) against most bacteria was equal to Ag-TiO_2 (**5e**). Although a comparative study of MIC values indicated that the inhibitory activity of these nanocomposites on gram-negative bacteria was better than gram-positive bacteria, the study conducted by Xie et al showed that the cellulose/graphene coated oxide-CuO nanocomposite films displayed significant antimicrobial activities against gram-positive bacteria (29). Haghi et al demonstrated the inhibitory influence of TiO_2 nanoparticles on the growth of pathogenic strain of E. coli and and proposed the manufacture use of titanium dioxide on various surfaces in hospitals to prevent infections (30). The results of an interesting comparative study between the antibacterial nature of TiO_2 and Ag- TiO_2, CuO-TiO_2 showed that Ag and Cu alone have maximum inhibitory activities, but combinations of Ag or Cu with TiO_2 were more active than TiO_2 (31). The difference in the structure of the cell membrane was one of the most important factors in this regard. In fact, the thick layer of peptidoglycan in gram-positive bacteria acts as a strong protector against external factors and protects the cytoplasmic membrane, but gram-negative bacteria do not have this layer (28,32). The NP diffusion rates was another important factor affecting antimicrobial activity (33).

Conclusions

Due to the antibacterial activity of **5e** and **5f** nanocomposites against *Escherichia* and *Pseudomonas*, they can be used as important antimicrobial agents in textiles and biomedical devices for continuously releasing NPs to provide

protection against infectious agents in hospital equipment.

Conflict of Interests

None.

Acknowledgments

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