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Study of the Antibacterial Activity of Levofloxacin Biocomplexes Based on Natural and Synthetic Polymers on Medical Meshes

Abstract

In the presented article, a model system was created and tested on cultures to determine the antibacterial activity of composites without metal nanoparticles, as well as those containing silver (Ag^o) and magnetite (Fe_3O_4) nanoparticles. During surgical operations for abdominal hernias, special antisterile synthetic meshes made of polyvinylidene fluoride were impregnated with polymer matrices, their homopolymer mixtures, and solutions with levofloxacin. The polymer- and polymer/drug-impregnated meshes were placed in bacterial media in Petri dishes, and their antibacterial activity was compared based on the diameter of the resulting lysis zones. For control purposes, levofloxacin alone was also impregnated into synthetic meshes with a size of 1 cm^2 and a mass of 30-40 mg, and experiments were conducted. Each synthetic mesh was impregnated with polymer samples comprising about 15-20 % of the mass, a metal oxide-metal nanoparticle coated with a polymer chain, and approximately 5 micrograms of levofloxacin. It was found that the homogeneous systems containing metal nanoparticles both levofloxacin free and the levofloxacin-loaded samples had a stronger microbial effect. This led to an increase in the area of the lysis zone. This effect is naturally related to the antibacterial properties of the metal nanoparticles themselves, which make the composite more effective in killing microbes. Additionally, compared to samples without nanoparticles, the lysis zone remains stable for a longer period.

Keywords: *levofloxacin, lysis zone, magnetite, polymer, composite, nanoparticle*

Introduction

The convergence of nanotechnology and medicine has led to the active development of a new field of research – nanobiotechnology – which creates interesting opportunities for the discovery of new materials.

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It is known that the synthesis of Ag and Fe_3O_4 nanoparticles in biological media without using chemical reagents is widely applied. The simplicity, non-toxicity, and environmental compatibility of this method are noteworthy. The synthesis of Ag^o nanoparticles in the presence of biomolecules such as starch, dextran, gum arabic, chitosan, enzymes, amino acids, and fungi allows for stable long-term maintenance, a primary principle for biocompatibility. Ag^o nanoparticles have also been synthesized using bacterial proteins and plant extracts as reducing agents, with positive results in drug delivery applications in medicine (Park et al., 2012; Xiliang et al., 2014). The bactericidal and antimicrobial activity of Ag^o nanoparticles depends on the chemical and biological coating surrounding their surface. For instance, the positive charge on the surface of Ag^o nanoparticles enables them to remain in the bloodstream for a long time, a crucial requirement for drugs and reagents used against cancer (Hiramatsu et al., 2004).

Research

Recently, the bactericidal property of Ag^o nanoparticles has been linked to their slow oxidation upon contact with the environment, resulting in an excited state. For example, the impact of 3-25 nm Ag^o nanoparticles on the survival times of Gram-negative microorganisms like E. coli, V. cholerae, and P. aeruginosa has been studied (More et al., 2023). The physical and chemical properties of Ag^o nanoparticles make them suitable for use as carriers and protective materials in medicine, either alone or in compositions with various natural polymers (Humbatova et al., 2017). The field of application of Ag^o nanoparticles depends on their synthesis method and the nature of the compound used as a stabilizer (Helmlinger et al., 2015; Huang et al., 2008; Schlinkert et al, 2015; Hengbo et al., 2004). When natural polymers are used both as reducing and stabilizing agents, the physical stability of the Ag^o nanoparticles obtained is high, and the toxicity of the final product is lower, facilitating its application in green synthesis in medicine. The physicochemical properties of the obtained nanoparticles affect their biological indicators, so factors like size, distribution, surface area and energy, aggregation tendency, solubility, toxicity, and biocompatibility must be evaluated after the synthesis of Ag^o nanoparticles (Ping-Chang et al., 2014; Richard et al., 2008).

Ag^+ ions and Ag^o-based compounds are highly effective against microorganisms, demonstrating strong biocidal activity against 12 types of bacteria, including E.coli (Shankar & Rhim, 2015; Xi-Feng Zhang et al., 2016). Modern dressing materials can gradually release silver, helping to prevent toxicity and ensure a therapeutic dose of silver for wounds (Sangiliyandi et al., 2016; Cabrini et al., 2011). Research results show that these dressings can destroy common wound pathogens, including Staphylococcus aureus and Pseudomonas aeruginosa (Thakkar et al., 2010). The antibacterial activity of chitosan tripolyphosphate nanoparticles loaded with various metal ions has been investigated by Du and colleagues, showing increased antibacterial activity due to the loaded metal ions (Wen-Li et al., 2009).

In this regard, Fe_3O_4-based composites among metal oxide nanoparticles also form the foundation of highly effective antibacterial preparations (Prabhu et al., 2015). Although these composites have wide applications in optics, mechanics, biotechnology, engineering, microbiology, electronics, and other fields, research on green synthesis and their antimicrobial activity has rapidly increased in the past decade (Khwaja et al., 2016; Pelgrift & Friedman, 2013).

Fe_3O_4 nanoparticles possess unique properties, including small size, high conductivity, surface modification, and others. These nanoparticles are significant in delivering antibacterial drugs to infected sites, increasing effective drug concentrations, thereby enhancing antibacterial effects, reducing drug doses, and overcoming bacterial resistance (Bharathi et al., 2019). Studies have shown that iron oxide nanoparticles coated with chitosan have antioxidant activity and exhibit potential antibacterial activity against both Gram-positive and Gram-negative pathogens (Vallabani et al., 2020; Bhuiyan et al., 2020).

Experimental Section

In this research, poly-N-vinylpyrrolidone (PVPr) with an average molecular weight of 360,000 was used, obtained from Fluka. Gum arabic (GA), with a chemical purity sufficient for biological

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research (98 %), was used in synthesis processes without further purification (CAS number 9000-01-5, obtained from Sigma Aldrich).

Arabinogalactan (AG), with sufficient chemical purity for biological research, containig less than 15 % moisture, was used in synthesis processes without further purification (CAS 9036-66-2).

The preparation of magnetite (Fe_3O_4) nanoparticles was performed according to the methodology in reference (Mahdavi et al., 2013), using $FeCl_2$ and $FeCl_3$ salts as the iron oxide source.

Levofloxacin (Lfx) with the chemical formula $C_{18}H_{20}FN_3O_4$ and ATC code J01MA12, has 99 % bioavailability, with 83 % excreted unchanged by the kidneys (CAS number 100986-85-4). It is a white-yellowish solid crystal with a molar mass of $M(C_{18}H_{20}FN_3O_4)$ = 361.368 g/mol, and a density of 1.5 g/cm³. Commercially known as Levaquin, it is used in drug form as a hemihydrate $C_{18}H_{20}FN_3O_4 \times ½\ H_2O$ and was obtained as a hemihydrate from Sigma Aldrich for use as a reagent.

Chitosan (CS) is a white, yellowish solid called deacetylated chitin or poly-D-glucosamine, CAS number 9012-76-4, average molecular weight 230 kDA, insoluble in water, well soluble in 1 % acetic acid, polyaminosaccharide with a linear structure with 85 % degree of deacetylation.

Meshes containing polyvinylidene fluoride, used for abdominal hernia treatment, were impregnated with Lfx alongside synthesized hydrogels to test their antibacterial activity in vitro. The method for impregnating polymer gel-antibiotic onto the mesh and evaluating its antimicrobial activity was improved according to reference (Kuleshova, 2015).

The impregnation of the polymer gel-antibiotic into the meshes was performed according to the methodology proposed by us, and the assessment of its antimicrobial activity was performed using the traditional method. So, meshes of 1 cm^2 size and 0.02 g mass were cut in sterile conditions. First, suspensions of synthesized gels with a concentration of 50 mg in 15 ml of distilled water are prepared. On the other hand, 10 ml of solution containing 5 μg of Lfx is prepared. Meshes are soaked with the appropriate hydrogel sample and released from the solvent at 35-40ºC. 20-25 % of the mass of nets has gel. At the end, 0.2 ml of Lfx was absorbed into the mesh-gel composite over the entire surface and dried again at low temperature. Then, the mesh-gel-drug composite is stored under sterile conditions and directed to the next antibacterial tests.

Results and Discussion

The antibacterial properties of homopolymers and graft copolymers used in this study were examined by the disk diffusion method. To evaluate these properties, Staphylococcus aureus (Gram-positive bacteria) and Escherichia coli (Gram-negative bacteria), Pseudomonas aeruginosa, and Candida were used as test cultures, which are the main causative agents of purulent-inflammatory processes. In the disc diffusion method, a suspension with 0.2 billion microbial cells per 1 ml is prepared from the daily culture of the microorganism, i.e., a small amount is taken from the daily microbial culture on the skew agar surface with a bacteriological loop on a sterile physiological solution, and a suspension with 1 billion microbial cells per 1 ml is adjusted to the standard and brought to the limit. This was then poured into Petri dishes containing nutrient agar (Meat-Peptone Agar, MPA) and spread evenly. The dishes are gently moved so that the suspension is spread equally in all directions. After that, the remaining suspension is sucked through a pipette and placed in the disinfectant solution. The dishes are kept at 37°C for 10 min to allow the solution to dry slightly. After that, the dishes are removed from the thermostat, divided into 2 or 4 parts with a pencil, 1 cm^2 meshes impregnated with polymer gel-levofloxacin are placed on the surface (sectors) of the nutrient medium where the microbe is planted, with a distance from each other, and gently pressed with tweezers so that the squares are well let it get wet. Then the MPAs are placed in a thermostat with a temperature of 37°C. As the meshes absorbed moisture, the impregnated substances diffused into the agar, killing the microbes. After 24-48 hours, the plates were removed, and results were recorded. The results were compared with those of meshes impregnated without levofloxacin. Antimicrobial indicators were determined based on the area of the lysis zone. Larger lysis zones indicate higher antimicrobial properties.

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Homopolymers and graft copolymers samples are CS, AG, PVPr, and their mixtures. Visual images of the lysis zones around the meshes observed over a one-week period are shown in Figures 1 and 2.

Figure 1

Effect of natural and synthetic homopolymers, graft copolymers, and their mixtures with Levofloxacin, absorbed onto synthetic meshes, on Staphylococcus, Pseudomonas aeruginosa, and Escherichia coli microbes

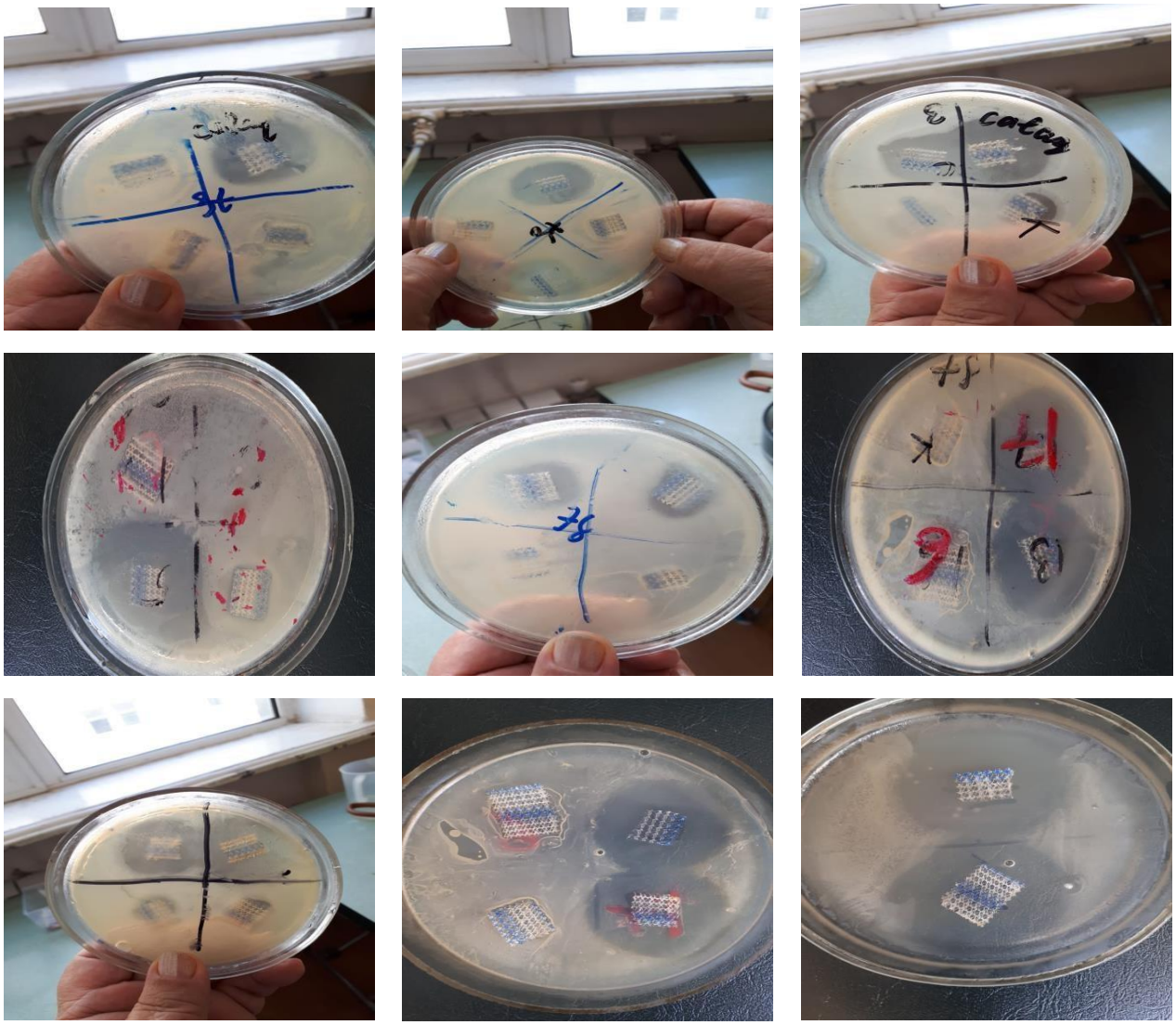

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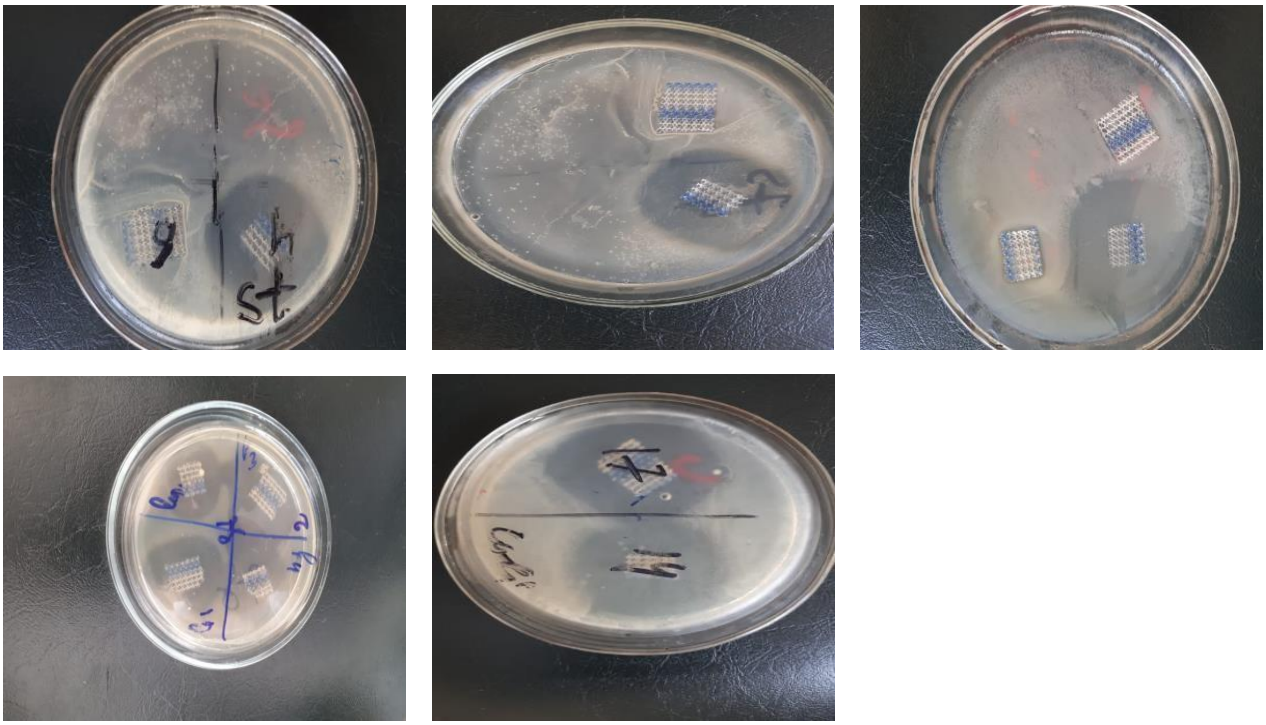
Figure 2

The effect of biocomplexes of colloidal solutions containing Ag^o and Fe_3O_4 nanoparticles coated with natural and synthetic polymers, infused with levofloxacin, on Staphylococcus, Pseudomonas aeruginosa, and Escherichia coli bacteria



Various polymer and graft copolymer blends impregnated into mesh were initially cut into square shapes, and their antibacterial properties were studied using the disk-diffusion method. To assess these properties, standard test cultures were selected, representing common causative agents of purulent-inflammatory processes: Staphylococcus aureus (as a Gram-positive bacterium), Escherichia coli (a Gram-negative intestinal bacterium), and Pseudomonas aeruginosa (a Gram-negative, pigment-producing bacterium). Additionally, homopolymer samples combined with silver nanoparticles were impregnated into mesh and tested for antibacterial activity. The antibacterial efficacy observed over a 3-week period was evaluated by measuring the lysis zone area (Table 1).

Table 1

Determination of antibacterial properties of natural and synthetic-based polymers, their graft copolymers, Ag^o and Fe_3O_4 nanoparticle complexes, and Lfx -immobilized samples

Substances		Test cultures		
		Staphylococcus aureus(mm)	Esherichia coli (mm)	Pseudomonas aeruginosa, (mm)
1	AG	20	18	20
2	AG-Lfx	24	20	24
	AG-Ag^o	21	19	21
	AG-Ag^o-Lfx	28	27	25
	AG-Fe_3O_4- Lfx	29	28	23
3	CS	+ (5-7)	+ (4-6)	+ (2-3)

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	CS-Ag^o	11	9	8-9
4	CS-Lfx	24	28	28
5	CS-Ag^o-Lfx	37	34	39
6	CS-Fe_3O_4-Lfx	35	38	40
7	PVPr	+ (7-9)	+ (5-6)	+ (4-6)
	PVPr-Ag^o	24	39	40
8	PVPr-Lfx	35	25	35
9	PVPr-Ag^o-Lfx	38	41	39
10	PVPr-Fe_3O_4- Lfx	36	43	46
	CS-PVPr-Lfx-Ag^o	41	48	52
Physiological solution (control)		+	+	+

Note: *The numbers indicate the diameter of microbe-free zones in millimeters, while "+" indicates full inhibition.*

As shown in the table, most of the newly developed formulations (meshes) demonstrated varying degrees of antimicrobial effects against bacteria. The most active bactericidal agents were the samples containing Ag^o and Fe_3O_4 nanoparticles. These samples exhibited strong lethal effects on both Gram-negative (Ps. aeruginosa and E. coli) and Gram-positive (St. aureus) bacteria, with the sterile zones around the mesh measuring between 35–40 mm.

In all trials, complete inhibition was observed in the control samples. Due to their biological activity, these samples demonstrated antibacterial activity even without nanoparticles, making them valuable components in materials with bactericidal properties. All experiments were repeated three times for accuracy.

Conclusion

Based on previous and recent test cultures with the above samples, it can be concluded that free arabinogalactan, poly-N-vinylpyrrolidone, CS, and its graft copolymers with poly-N-vinylpyrrolidone exhibit bactericidal properties even without antibiotic immobilization. This feature enhances the antibacterial effect of the final product, requiring a smaller amount of drug for immobilization. It was found that antibiotic-free AG formed a lysis zone of 20 mm against Staphylococcus, while Ag^o- and nanoparticle-containing polymer composites created a 30 mm lysis zone. Free PVPr formed lysis zones of 32–34 mm against all three microbes. Additionally, the graft copolymer of PVPr and CS exhibited bactericidal effects, forming lysis zones of 30–40 mm. After Lfx immobilization on these polymer samples, bactericidal activity increased, surpassing the activity of free Lfx. These findings suggest that drug formulations structured with these polymers, or their graft copolymers with nano metal oxides and metal nanoparticles, can maintain their biological activity for at least three weeks. This stability makes them suitable for long-term drug delivery application.

Research also determined that when homogeneous systems containing Ag^o and Fe_3O_4 nanoparticles were loaded with and without Lfx, antimicrobial efficacy increased. The lysis zone expanded, indicating enhanced bactericidal properties attributed to the inherent antibacterial effects of silver and magnetite nanoparticles. This improvement makes the composite more effective against microbes, with the lysis zone remaining stable for longer periods compared to samples without nanoparticles.

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