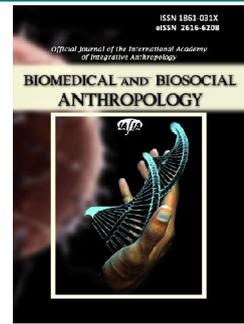




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The study of antimicrobial properties of film materials based on biopolymers and antiseptics

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The development of new biomaterials with improved properties is becoming increasingly important in a wide range of applications. However, some of the most sought-after properties are anti-microbial properties, which can help prevent unwanted wound infections, especially in the face of growing antibiotic resistance of bacteria. The aim of the study was to study the effect of antimicrobial biomaterials based on calcium alginate, as a polymer system of local prolonged delivery of quaternary ammonium compounds, on reference and clinical strains of microorganisms. Samples of antimicrobial biomaterials contained decamethoxin (0.03-0.07 wt%), and polymers (polyvinyl alcohol and calcium alginate). Reference and clinical strains of Staphylococcus aureus, Escherichia coli, Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa were used for the study. The sensitivity of strains of microorganisms was determined by the disk-diffusion method according to the generally accepted method. The result of antimicrobial activity was assessed after 24 hours. The mean (M), the mean error of the mean ($\pm m$), and the criterion for the significance of differences (p) were calculated. The presence of differences between the research data was assessed by the Student's t-criterion. The results were considered reliable at $p < 0.05$. High antimicrobial properties of the studied samples of antimicrobial biomaterials based on calcium alginate and decamethoxin have been established. It was revealed that the samples of polymeric biomaterials have a higher activity against gram-positive microorganisms compared to gram-negative strains. The composition is not inferior to the antimicrobial effect of a solution of decamethoxin and chlorhexidine in relation to all strains of microorganisms.

Key words: antimicrobial biomaterials, decamethoxin, calcium alginate, antibiotic resistance.

Introduction

Advances in medicine in the modern world allow to realize the human desire to improve the quality and duration of life [7, 8, 9]. A significant role in achieving these goals is played by the success of the development and use of new biomaterials used in medicine to support the vital functions and normal functioning of the body [12, 17, 20]. Biomaterials are materials that are in constant contact with body tissues, so they must meet a number of requirements. Despite the fact that among biomaterials an important place is occupied by metals, inorganic and carbon compounds, the priority belongs to polymers. Natural biomaterials have a therapeutic advantage over inert synthetic ones [2, 11, 14, 20, 22, 26].

The development of new biomaterials with improved properties significantly expands the possibilities of their

practical application. Antimicrobial properties are extremely important, which prevent the occurrence of infections and increase the effectiveness of control of their pathogens [13]. Infections associated with health care (IAHC) are an urgent problem in the field of health care, as they complicate the course of the disease, increase the risk of mortality and create a high financial burden on the health care system. In recent years, there has been an increase in the number of patients suffering from wounds and burns that are difficult to treat and heal [4].

There is an increased risk of severe infections caused by antimicrobial-resistant microorganisms [23]. The growing resistance of bacteria to antibiotics has caused great concern, so the World Health Organization has recently described the problem as threatening the

achievements of modern medicine [24, 25].

There is a need around the world for new strategies to achieve rapid wound healing and prevent the development of infectious complications. From these positions, researchers around the world are making every effort to develop bioactive materials that can play an active role in protecting and healing wounds and/or are able to isolate biomolecules and antimicrobials for the prevention and treatment of wound infections [6, 21].

With the spread of antibiotic resistance of microorganisms and against the background of reducing the effectiveness of antibiotics, the use of antiseptics as an antimicrobial component of bioactive materials becomes relevant. In domestic medical practice, a drug based on quaternary ammonium compounds - decamethoxine, which has been proven to be highly effective against a wide range of microorganisms belonging to 7 families and 16 genera [19], is used with great success.

The problem of local creation of long-term concentration of antiseptic drug is solved by choosing the matrix from which the antiseptic material should be released. Biocompatible natural polymers chitosan, collagen, elastin, alginate, hyaluronic acid and fibrinogen are used [1, 7, 13, 14, 18].

The main focus of drug research has long been the synthesis and detection of potent pharmacologically active agents for the treatment of diseases. However, it is now clear that the therapeutic benefit and efficacy of the drug are not directly correlated; this is closely related to the technology of manufacturing the drug and its delivery to the body. For effective healing of traumatic wounds and burns, there has always been a need to choose the optimal material that would cover the wound and prevent infection. It is important to develop biomaterials that can maintain volume and shape, maintain aseptic conditions, have prolonged antimicrobial properties, promote tissue repair, reduce wound healing time [1, 3, 5, 13].

The aim of the work is to study the effect of samples of antimicrobial biomaterials based on polyvinyl alcohol, calcium alginate and decamethoxine on reference and clinical strains of microorganisms.

Materials and methods

Reference strains of the Museum of Living Cultures of the Department of Microbiology, Virology and Immunology of National Pirogov Memorial Medical University, Vinnytsya Staphylococcus aureus ATCC 25923, Staphylococcus aureus ATCC 25927, Escherichia coli ATCC 25922, Escherichia coli ATCC 35213, Klebsiella pneumoniae ATCC 708603, Pseudomonas aeruginosa ATCC 27853, Acinetobacter baumannii ATCC BAA-747 and clinical isolates Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii were used for the study.

The test samples were an antimicrobial composition containing decamethoxine (0.03 wt. % - sample 1;

0.05 wt. % - sample 2; 0.07 wt. % - sample 3) and the ratio of film-forming biopolymers of 3.5 % calcium alginate (70.0 %) from 4.0 % polyvinyl alcohol (30.0 %). The technology of preparation of which consisted in the preparation of a solution of polymers in the specified ratio, followed by pouring into a special sterile round vessel made of polyethylene, 100 mm in diameter and drying at room temperature until completely dry. Disks with a diameter of 6 mm were prepared from microbiological research from ready dried film-like materials (thickness 1 mm) under sterile conditions.

Studies of susceptibility of strains of microorganisms were performed by disco-diffusion method according to the generally accepted method, in accordance with the order of the Ministry of Health of Ukraine on 3a 167 from 05.04.2007 "On approval of guidelines "Determination of sensitivity of microorganisms to antibacterial drugs " [16]. Meat-peptone agar (MPA) produced by PHARMACTIVE LLC, Ukraine was used for culturing microorganisms. A standard inoculum corresponding to 0.5 according to the McFarland standard (5×10^8 CFU/cm³) was prepared for the study. Disks of antimicrobial polymeric biomaterials were applied to the surface of MPA with lawn microorganisms sown with sterile tweezers, followed by incubation at a temperature of 35°C for 24 hours. The results were evaluated in reflected light by measuring the diameters of the growth retardation zones (DGRZ) of microorganisms around the disks with an accuracy of 1 mm. Standard filter discs moistened with 0.02 % decamethoxine and 0.05 % chlorhexidine solutions were used as controls.

The result of antimicrobial activity was evaluated after 24 hours. The study was performed at least 10 times for each of these strains of microorganisms.

Statistical analysis of the obtained results was performed using standard software packages "STATISTICA+" and "Microsoft Excel 2010". We calculated the arithmetic mean (M), the arithmetic mean error ($\pm m$), the criterion for the reliability of differences (p). The presence of differences between the studied indicators was assessed by Student's t-test. The results were considered reliable at values of $p < 0.05$.

Results

The results of the studies indicate high antimicrobial properties of samples of biomaterials based on decamethoxine and calcium alginate. It should be noted that the studied samples of antimicrobial biomaterials were not inferior to the antimicrobial action of control solutions of decamethoxine and chlorhexidine. It was found that these polymeric biomaterials with antimicrobial properties showed variable antimicrobial activity against gram-positive and gram-negative microorganisms. Thus, the effect of the studied samples on gram-positive strains significantly exceeded the activity on gram-negative strains of microorganisms (Table 1).

It was found that the studied samples of biomaterials based on decamethoxine showed significant antimicrobial

Table 1. Sensitivity of reference and clinical strains of microorganisms to antimicrobial biomaterials based on calcium alginate (M±m).

Strains of microorganisms	The diameter of the zone of growth retardation of microorganisms, mm, (n=10)				
	Chlorhexidine	Decamethoxine	1 sample *	2 sample **	3 sample ***
<i>Acinetobacter baumannii</i> ATCC BAA-747	9.100±0.90	9.300±0.960	11.40±0.68	12.60±0.68	13.00±1.20
p1	>0.05	-	<0.001	<0.001	<0.001
p2		>0.05	<0.001	<0.001	<0.001
<i>Acinetobacter baumannii</i> 28	11.70±0.90	12.70±1.30	12.20±1.04	12.30±0.76	11.40±0.76
p1	>0.05	-	>0.05	>0.05	>0.05
p2	-	>0.05	>0.05	>0.05	<0.05
<i>Acinetobacter baumannii</i> 56	7.800±0.960	8.100±0.740	9.500±1.000	9.500±1.000	10.80±0.64
p1	>0.05	-	<0.01	<0.01	<0.001
p2	-	>0.05	<0.05	<0.01	<0.001
<i>Acinetobacter baumannii</i> 50	7.700±0.980	7.400±0.560	9.700±1.160	11.40±0.92	11.70±0.96
p1	>0.05	-	<0.001	<0.001	<0.001
p2	-	>0.05	<0.001	<0.001	<0.001
<i>Acinetobacter baumannii</i> 5/12	9.100±0.920	9.200±1.040	11.30±0.76	12.40±0.92	12.90±1.32
p1	>0.05	-	<0.001	<0.001	<0.001
p2	-	>0.05	<0.001	<0.001	<0.001
<i>Staphylococcus aureus</i> ATCC 25927	12.60±0.56	13.30±1.36	11.50±0.90	13.20±0.48	14.00±1.40
p1	>0.05	-	<0.05	>0.05	<0.05
p2	-	>0.05	<0.05	>0.05	>0.05
<i>Staphylococcus aureus</i> ATCC 25923	14.30±0.56	14.50±0.70	11.50±0.80	17.40±0.68	14.50±0.70
p1	>0,05	-	<0.001	<0.001	>0,05
p2	-	>0.05	<0.001	<0.001	>0,05
<i>Staphylococcus aureus</i> 18	13.80±0.48	14.00±0.40	12.90±0.54	17.50±0.70	14.70±0.56
p1	>0.05		<0.01	<0.001	<0.01
p2		>0.05	<0.01	<0.001	<0.05
<i>Escherichia coli</i> ATCC 35213	11.20±0.64	8.400±0.480	8.600±0.720	9.700±0.420	8.900±0.720
p1	<0.001	-	<0.001	<0.001	<0.001
p2	-	<0.001	0>0.05	<0.001	>0.05
<i>Escherichia coli</i> ATCC 25922	10.20±0.64	7.600±0.600	8.200±0.320	9.400±0.600	8.400±0.480
p1	<0.001	-	<0.001	<0,05	<0.001
p2	-	<0.001	<0.05	<0.001	<0.01
<i>Escherichia coli</i> 3	11.20±0.64	8.900±0.360	8.800±0.640	9.700±0.420	9.300±0.420
p1	<0.001	-	<0.001	<0.001	<0.001
p2	-	<0.001	>0.05	<0,01	>0.05
<i>Klebsiella pneumoniae</i> ATCC 708603	7.200±0.320	7.200±0.320	7.200±0.320	7.200±0.320	7.300±0.420
p1	>0.05	-	>0.05	>0.05	>0.05
p2	-	>0.05	>0.05	>0.05	>0.05
<i>Klebsiella pneumoniae</i> Ø3030	7.200±0.320	7.200±0.320	7.400±0.480	8.000±0.400	7.900±0.360
p1	>0.05	-	>0.05	<0.01	<0.01
p2	-	>0.05	>0.05	<0.01	<0.01
<i>Pseudomonas aeruginosa</i> ATCC 27853	9.700±0.480	8.600±0.720	8.500±0.500	9.600±0.480	9.700±0.420
p1	<0.01	-	<0.001	>0.05	>0.05
p2	-	<0.01	>0.05	<0.01	<0.01

Continuation of table 1.

Strains of microorganisms	The diameter of the zone of growth retardation of microorganisms, mm, (n=10)				
	Chlorhexidine	Decamethoxine	1 sample *	2 sample **	3 sample ***
<i>Pseudomonas aeruginosa</i> 17	9.700±0.480	8.800±0.640	8.700±0.420	9.700±0.420	9.800±0.320
p1	<0.05	-	<0.01	>0.05	>0.05
p2	-	<0.05	>0.05	<0.01	<0.01

Notes: * - sample 1 contains 0.03 % decamethoxine; ** - sample 2 contains 0.05 % decamethoxine; *** - sample 3 contains 0.07 % decamethoxine; p1 - the significance of differences in relation to chlorhexidine; p2 - the significance of differences in relation to decamethoxine.

properties against reference and clinical strains of *A. baumannii*, which is a widespread microorganism that colonizes wounds and is classified by the WHO as pathogens of critical priority. Their antimicrobial activity against some strains of *A. baumannii* significantly exceeded that of control solutions of decamethoxine and chlorhexidine. Thus, the DGRZ of the reference strain *A. baumannii* ATCC BAA-747 under the influence of control solutions of chlorhexidine and decamethoxine was 9.100 and 9.300 mm, respectively. Whereas under the influence of samples of antimicrobial biomaterials № 1, № 2, № 3 were 11.40, 12.60, 13.00 mm, respectively. For clinical strains of *A. baumannii* 28, control solutions and samples of antimicrobial biomaterials showed their properties at almost the same level. The DGRZ of *A. baumannii* 28 was 11.70, 12.70, 12.20, 12.30, 11.40 mm, respectively. The effect of control solutions of antiseptics and test samples № 1, № 2, № 3 on most of the studied clinical strains of *A. baumannii* significantly exceeded that of control solutions. DGRZ *A. baumannii* 50 control samples of disks with chlorhexidine and decamethoxine were 7.700 and 7.400 mm, respectively; DGRZ *A. baumannii* 50 under the influence of the studied samples №1, №2, №3 - 9.700, 11.40, 11.70 mm, respectively.

The activity of the test samples was slightly lower against *E. coli* (DGRZ *Escherichia coli* ATCC 35213 around samples of biomaterials № 1, № 2, № 3 - 8.600; 9.700; 8.900 mm) than the effect of control discs with chlorhexidine (11.20 mm) and decamethoxine (8.400 mm). A similar trend was observed for clinical strains of *E. coli*.

The effect of antiseptics in control solutions and in the composition of polymeric biomaterials on the reference and clinical strains of *K. pneumoniae* and *P. aeruginosa* was uniform, but quite effective. Thus, the DGRZ of the reference strain *K. pneumoniae* ATCC 708603 under the influence of control solutions of chlorhexidine and decamethoxine was 7.200 mm, and under the influence of test samples № 1, № 2, № 3 reached 11.40, 12.60, 13.00 mm, respectively. Registered growth retardation of *P. aeruginosa* ATCC 27853 in controls around chlorhexidine and decamethoxine - 9.700; 8.600 mm, while when using samples № 1, № 2, № 3 - 8.500; 9.600; 9.700 mm.

The highest antimicrobial effect of polymer compositions based on decamethoxine, as well as control test disks with antiseptics, showed against reference and clinical strains of *S. aureus*. Under the control of chlorhexidine and

decamethoxine control solutions, the DGRZ of *S. aureus* ATCC 25927 was 12.60 and 13.30 mm, respectively, and in the case of test objects № 1, № 2 and № 3 were 11.50; 13.20 and 14.00 mm respectively. The antimicrobial activity of sample № 3 significantly exceeded the effect of control solutions on the reference and clinical strains of *S. aureus* (see Table 1).

Discussion

Prevalence of antibiotic-resistant bacteria classified by WHO as the first priority category (critical priority level), which include *A. baumannii* (resistant to carbapenems), *P. aeruginosa* (resistant to carbapenems), Enterobacteriaceae (resistant to carbapenems), and growth microorganisms classified in the second category with a high level of priority (*S. aureus*), is of great concern. Any injury is characterized by a high probability of developing an infectious process in the wound. In conditions of high risk of microbial colonization by these microorganisms, wounds of different genesis require effective local antimicrobial treatment. The effectiveness of repair is currently characterized not only by the term of wound healing. No less important is the aesthetic result. In addition, it is important to ensure the comfort of treatment to combine optimal therapy and quality of life of the patient [15].

In the conditions of wide distribution of resistant microorganisms and against the background of reduced efficiency of antibiotics, the use of antiseptics becomes relevant again. Given the ability of antiseptics to affect bacteria regardless of their metabolic status, to effectively destroy even antibiotic-resistant strains, they should be considered more promising for topical application to destroy biofilms and kill bacterial cells. The high sensitivity of pathogens of wound infections to antiseptics dictates the need for their wider use in the schemes of complex prevention and treatment [11, 25].

To achieve the expected impact on microorganisms and develop recommendations for effective use of antiseptics, it is necessary to develop optimal forms of local delivery of antiseptics to the affected area, which will have a detrimental effect on a wide range of wound pathogens in any form of life. the end result of the phase [10].

Studies have shown that antimicrobial biomaterials based on the natural polymer of calcium alginate and polyvinyl alcohol are depot forms capable of releasing

concentrations of the antiseptic decamethoxine effective against a wide range of priority gram-positive, gram-negative wound infections. Polymer matrix based on calcium alginate and polyvinyl alcohol has its own positive therapeutic properties. At the same time, the natural origin of this polymer makes it a suitable substitute for the extracellular matrix as a natural environment for skin cells. The emollient, soothing, astringent, anti-inflammatory and antioxidant properties of natural products can be useful for the wound healing process [1, 13, 14].

Thus, the development and comprehensive preclinical study of the properties of antimicrobial biomaterials based on decamethoxine and natural polymers as a carrier matrix, the study of the effectiveness of new biomaterials for the prevention and treatment of local infectious and inflammatory processes are relevant and promising.

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