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In vitro study of the antimicrobial activity of hydrogel-based matrices impregnated with antibiotics against the leading microorganisms of bone infection

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Abstract

Purpose To evaluate the results of a comparative analysis of the *in vitro* antimicrobial activity of polymer hydrogels and PMMA impregnated with antibiotics against test cultures of the leading pathogens of bone infection. **Materials and methods** A comparative analysis of the antimicrobial activity of the polymer hydrogel and PMMA impregnated with antibiotics was carried out. The bactericidal efficacy and prolongation of action of the following microbe-antibiotic pairs were assessed: *MSSA* – gentamicin, *MSSE* – cefazolin, *MRSA* and *MRSE* – vancomycin, *A. baumannii* – tobramycin. The duration of the study is 7 days. Statistical comparison of groups was carried out using the Mann-Whitney and Wilcoxon tests; the median and 95 % CI were used to describe the data. **Results** The obtained indicators of the zone of inhibition of the growth of microorganisms around the polymer hydrogel were several times higher than the results of PMMA ($p < 0.05$ for all periods). The antimicrobial activity of hydrogels in all cases lasted more than 7 days. Hydrogels impregnated with vancomycin showed the lowest rate of contraction of the inhibition zone. The best antimicrobial effect was demonstrated by the compared samples with cefazolin in relation to MSSE, the maximum index of the zone of suppression of which, on average, for the hydrogel was 29.3 mm, PMMA – 22.3 mm. **Discussion** Samples from polymer hydrogel impregnated with antibiotics demonstrated prolonged pronounced effective antimicrobial activity against the leading pathogens of orthopedic infections in comparison with PMMA. The bactericidal efficacy of the polymer hydrogel significantly exceeded the activity of PMMA already from the first day of the study ($p = 0.002$). The action of the hydrogel throughout the study was uniform without abrupt changes or dips ($p > 0.05$), in contrast to bone cement, whose activity against all test cultures significantly decreased ($p = 0.042$) on the 2nd day of the study. **Conclusion** Polymeric biodegradable hydrogels impregnated with antibiotics have a greater antimicrobial potential compared to PMMA. Uniform, slow and controlled release of the drug and effective inhibition of the growth of microorganisms, as well as prolonged action show that these properties may turn hydrogels into an effective depot system.

Keywords: antimicrobial activity, polymeric biodegradable hydrogel, bone cement, orthopedic infection, *in vitro* study, polymethyl methacrylate (PMMA)

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INTRODUCTION

Due to a growth of severe mechanical injuries and increased surgical activity, the number of infectious complications also increases, among which the most common is implant-associated infection (IAI). The incidence of the latter after osteosynthesis of long bone fractures ranges between 1.8 and 27 %, after primary arthroplasty between 0.3 and 2.4 % [1]. In the absence of proper treatment or a long course of IAI, it can turn into a chronic osteomyelitis with damage to all bone tissue structures. Despite the constant improvement of bone infection treatment methods, poor outcomes still remain at a high level (57 %) [2], which ultimately leads to deterioration in the patients' quality of life.

The IAI etiology is based on microorganisms capable of forming three-dimensional biofilms that are difficult to treat [3-5]. Their eradication is the primary task of practicing orthopedic surgeons in the treatment of bone infection. Surgical treatment, accompanied by mechanical destruction of microbial biofilms on the surface of the implant and bone tissue, followed by etiotropic systemic antibiotic therapy, frequently does not provide the desired effect. The poor outcome of this treatment method is explained by the ability of the surviving fragments of the bacterial biofilm to restore its integrity within 24 hours [6]. Systemic therapy is not able to create high concentrations of antibiotics in the infection nidus, necessary for the eradication of biofilms [7, 8], due to insufficient blood supply to the affected area of the bone [9, 10]. Systemic treatment, acting on planktonic forms of pathogens [7, 11], only prevents the spread of microbial biofilms in the patient's body.

The solution to the problem, along with the removal of the infected implant followed by a thorough debridement of the focus, was the creation of local depot systems saturated with antibacterial drugs and allowing high doses in the focus for several weeks, 1000 times or more exceeding the minimum inhibitory concentrations (MIC) for planktonic types [12, 13].

The most studied and widely used carrier depot system in clinical practice is bone cement based on

polymethyl methacrylate (PMMA). The creation of local concentrations in the focus of infection, the temporary filling of formed defects and large spaces after surgical debridement [8, 9, 14, 15], as well as mechanical strength, make bone cement a very attractive material for treating bone infections. However, there are numerous shortcomings (non-biodegradability [16], limited range of antibiotics that can be impregnated into the PMMA structure; hydrophobic surface [17]; release of only 10 % of the impregnated antibiotic [16]; uneven distribution of the drug agent in the matrix and its uneven elution; change in characteristics structure of cement and warranty properties by manual mixing the constituent PMMA components with an antibiotic; high polymerization temperature up to 120 ºC, which can cause necrosis of adjacent tissues [10]; traumatic re-operation to remove bone cement [7, 15]; high toxicity of volatile vapors of methyl methacrylate [18, 19]) do not allow us to recommend it as an ideal carrier depot system.

Based on the above, biodegradable transport systems impregnated with antibiotics have been currently attracting more and more attention. They have been shown to be effective in *in vitro* and *in vivo* studies [20].

Unfortunately, at present there are no materials and medical devices approved for clinical use that have the full range of characteristics required for an ideal transport system. In particular, the depot system, in addition to being capable of prolonged controlled release of the antibiotic at effective concentrations, must have high hydrophilicity, which reduces the risk of bacterial biofilms to form on the implant surface and in the bone; the ability to biodegrade, eliminating the need for repeated surgical intervention; mechanical properties close to the properties of biological tissues, to exclude damage to soft tissues and pain in the affected area; as well as simple manufacturing and application techniques. Biodegradable hydrogels based on synthetic polymers have the closest to the required set of properties.

Purpose To evaluate the results of a comparative analysis of the in vitro antimicrobial activity of polymer hydrogels and bone cement impregnated with antibacterial drugs in relation to test cultures of the most common pathogens of orthopedic infections.

MATERIALS AND METHODS

Purified sterile solutions of unsaturated derivatives of polyvinyl alcohol with a concentration of 8 g/100 ml were used for the preparation of samples based on polymer hydrogel that had been previously prepared at the Department of Biopolymers at the Russian Mendeleev Chemical Technical University. Synthesis of polymers was carried out according to previously developed procedures [21].

The samples of carrier matrices saturated with antibiotics were created in an operating room on the basis of the department for the consequences of injuries of the musculoskeletal system and osteoarticular infection of the Federal State Budgetary Institution *Priorov NMRC for TO* of the Ministry of Health of the Russian Federation in compliance with the conditions of asepsis and antisepsis.

Since the hydrogel is formed within a few seconds, the mixing of its constituent components was carried out directly in a three-component 5-ml syringe *Plastipack BD*. The required amount of the antibiotic, pre-measured on analytical scales with a sensitivity of up to 0.1 mg, was added to a vial containing 5 ml of sterile polyvinyl alcohol and stirred until the drug was completely dissolved. The resulting solution was poured into a three-component syringe with a pre-cut cone-shaped tip. Next, Mohr's salt and ammonium peroxydisulfate were added successively, mixing thoroughly each time. Upon cross-linking of the material, cylinders sized 13 × 8 mm were shaped.

Similarly, samples were prepared from bone cement based on PMMA (Synicem 1, France) without antibiotics. The mixing of the constituent components (5 g of copolymer and 2.5 ml of liquid monomer) with the necessary antimicrobial agents was performed intra-operatively manually in accordance with the manufacturer's instructions. Once the mass reached a plastic state, a dense filling of the volume of a three-component syringe was performed using a sterile spatula. Next, cylinders were shaped, equal to the volume of the hydrogel samples.

The prepared for comparison matrices were transported in sterile test tubes to the laboratory of microbiology of the Priorov NMRC for TO for their study on the same day.

The following drugs were impregnated into the compared materials: cefazolin, vancomycin, gentamicin, and tobramycin. The choice of antimicrobial agents was based on their wide availability, prevalence of use in clinical practice and biochemical profiles with different molecular weight and solubility.

The investigation of the antimicrobial activity of the samples was conducted against the most common microbes detected in bone infection: *Staphylococcus aureus*, sensitive to methicillin (*MSSA*), and *Staphylococcus aureus*, resistant to methicillin (*MRSA*), sensitive to methicillin *Staphylococcus epidermidis* (*MSSE*) and resistant to methicillin *Staphylococcus epidermidis* (*MRSE*), and agents of gram-negative microflora – *Acinetobacter baumannii* (*A. baumannii*) [22]. The activity of the following pairs of "microbe – antibiotic" was studied: *MSSA* – gentamicin, *MSSE* – cefazolin, *MRSA* and *MRSE* – vancomycin, *A. baumannii* – tobramycin. All test-cultures were sensetive to the chosen medical drugs.

The initial concentration of vancomycin in one test sample with a volume of 1.13 cm^3 was approximately 67.8 mg, cefazolin – 45.2 mg, gentamicin and tobramycin – 18 mg, respectively. The weight of

antibiotics to saturate the carrier matrices was determined taking into account the MICs of the preparations for each test culture of microorganisms.

As test strains, cultures of microorganisms isolated from the material of patients with chronic osteomyelitis of long bones and periprosthetic infection of large joints (intra-operative biopsies of affected areas of bone and soft tissues) were used, who were hospitalized at the department of consequences of injuries of the musculoskeletal system and joint infection of the Federal State Budgetary Institution Priorov NMRC for TO of the Ministry of Health of the Russian Federation. Conventional methods were used for culturing material samples. Identification of isolated microorganisms and determination of their antibiotic resistance was carried out using a bacteriological automated analyzer Vitec 2 compact (BioMerieux, France).

The course of the study was identical to the determination of antibiotic resistance of microorganisms to antibiotics using the disk diffusion method.

A microbial suspension of the test culture was prepared in saline at a concentration corresponding to the turbidity standard of 0.5 McFarland. It was applied to the surface of Mueller-Hinton agar in a Petri dish, evenly distributed and dried. The studied paired samples (polymer hydrogel and bone cement) impregnated with the same antibiotic were placed on the surface of the infected agar in compliance with the rules of asepsis and incubated in a thermostat at 37 ºC for 24 hours.

The result was evaluated by the presence of a growth inhibition zone (GIZ) of the test culture around the test sample. The results were taken into account on the basis of measuring the size of the zone (if any) from the edge of the cement or hydrogel sample to the edge of the growth inhibition zone using a ruler-template designed for this purpose (HiMedia PW297, India).

In order to identify the prolonged antimicrobial activity of the test samples, the antibiotic-containing material was transferred daily to pre-prepared Petri dishes with infected Mueller-Hinton agar. After 24 hours, the zone of inhibition of the test culture, if any, was measured. This manipulation was repeated for 7 days: on days 1, 2, 3, 6 (72 hours of incubation) and 7 days.

To increase the reliability of the results of a comparative analysis of the antimicrobial efficacy of the studied matrices, the study was performed in three parallel samples in relation to all test cultures. Thus, we studied 3 blocks of samples from polymer hydrogel and bone cement impregnated with the same type of antibiotic.

Statistical data processing was carried out using the IBM SPSS Statistics 22. Data are presented as Me and 95 % CI. The results of three parallel studies for each microbe-antibiotic group are given as arithmetic mean values. The Mann-Whitney test was used to compare differences between groups. Values were considered significant at $p < 0.05$. The Wilcoxon test was used to compare the antimicrobial activity of the same matrix at different times. Values were considered significant at $p < 0.05$.

RESULTS

Antimicrobial activity of the compared samples impregnated with gentamicin against MSSA

On days 1 and 2, the diameter of the MSSA growth inhibition zone around the hydrogel was 12 mm (Fig. 1). On the 3rd day, the growth inhibition zone (GIZ) of bacteria slightly decreased to 10 mm. Seventy-two hours (on the 6th day), a smooth insignificant decrease in the activity of the hydrogel was observed and the GIZ was 8 mm. On the 7th day of the study, the GIZ of microorganisms extended for a distance of 6 mm from the edge of the sample. The release of gentamicin from the hydrogel matrix during the entire period of the study proceeded evenly without abrupt changes.

In the PMMA series, on day 1 of the study, the GIZ against MSSA was 5 mm, which is twice lower than the antimicrobial efficiency of the polymer hydrogel (Fig. 1). Bone cement also showed uneven elution of the impregnated gentamicin on the first day of the study. On day 2, there was a picture of a decrease in antimicrobial activity, which was uneven and one-sided. The GIZ was 3 mm, which is 4 times less than the efficiency of the polymer hydrogel on the same day. On the 3rd day, the effect of bone cement was still uneven, one-sided with a tendency of a reduced effect to 2 mm. On the 6th day of incubation of the sample, the diameter of the GIZ was 5 mm, the activity was one-sided. On the 7th day of the study, the elution of gentamicin from the cement matrix completely stopped; there was no GIZ against *MSSA*.

Antimicrobial activity of the compared samples impregnated with vancomycin against MRSA

During 6 days of the study, the GIZ against MRSA around the polymer hydrogel was the same and measured 7 mm (Fig. 2). Antimicrobial activity proceeded evenly. On the 7th day, the effectiveness of inhibiting the growth of microorganisms decreased insignificantly to 6 mm. The uniformity of vancomycin release from the hydrogel did not change by the 7th day.

The GIZ around the bone cement on the 1st day practically did not differ from the activity of the polymer hydrogel and amounted to 6 mm. However, on the 2nd day, the bactericidal effect of the PMMA sample sharply decreased to 1 mm (Fig. 2). On the 3rd day, the measurement of GIZ showed 2 mm, which is slightly higher compared to the previous indicator. On the 6th day (after 72 hours), the activity of bone cement again decreased to 1 mm. On the 7th day of incubation, the antimicrobial effect of bone cement was not recorded as the release of vancomycin had completely stopped by that time. Moreover, the suppression area was uneven throughout the entire study period.

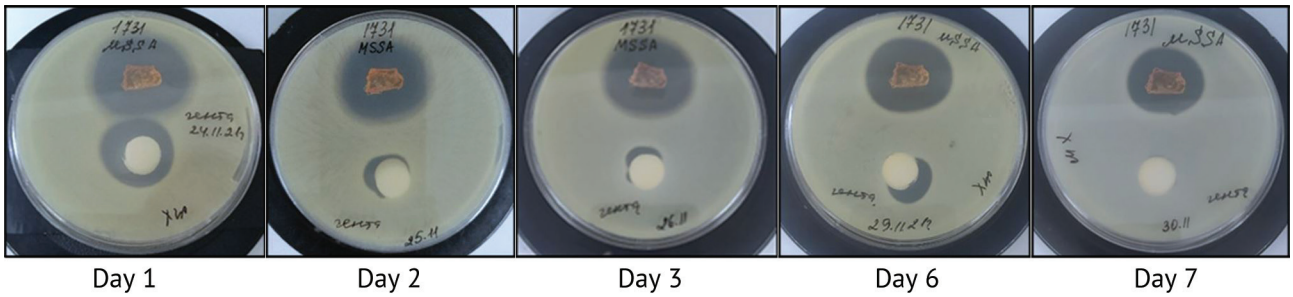

Fig. 1 Antimicrobial activity of compared samples saturated with gentamicin against MSSA in 1 parallel study. In the upper half of the Petri dish there is a polymer hydrogel, in the lower half there is a sample of bone cement

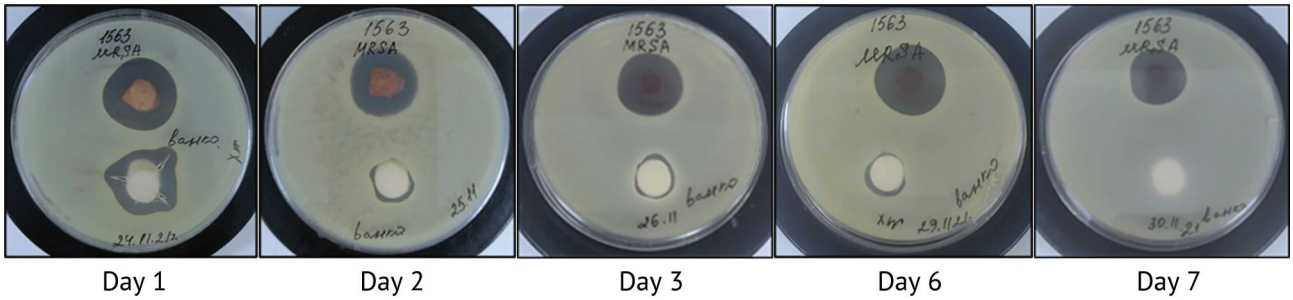

Fig. 2 Antimicrobial activity of compared vancomycin-impregnated samples against *MRSA* in 1 parallel study; in the upper half of the Petri dish there is a polymer hydrogel, in the lower half there is a sample of bone cement

Antimicrobial activity of the compared samples impregnated with cefazolin against MSSE

In relation to MSSE, a pronounced antimicrobial activity of both studied samples was observed; the zones of inhibition of the growth of microorganisms by cefazolin overlapped each other in both cases (Fig. 3).

On the 1st day of MSSE incubation, the diameter of the GIR for the hydrogel was 16 mm and 13 mm for the bone cement. On the second day, the antimicrobial activity of the polymer hydrogel increased to 20 mm, while that of the bone cement, on the contrary, decreased to 10 mm. On the 3rd day, the zone of growth suppression in both samples was slightly higher in comparison with the previous result: for polymer hydrogel it was 29 mm, for bone cement – 19 mm. After 72 hours of incubation (on the 6th day) from the moment of the last transfer of the samples, the antimicrobial activity of the hydrogel sample decreased to 22 mm, PMMA did not change. On the 7th day, the following indicators were recorded: polymer hydrogel – 18 mm, bone cement – 13 mm. The release of the antibiotic in both cases occurred evenly throughout the study period.

MRSE Antimicrobial activity of the compared samples impregnated with vancomycin against MRSE

On the 1st and 2nd day of incubation, the growth inhibition zone of the test strains around the hydrogel was 9 mm (Fig. 4). On the 3rd day, the activity decreased to 6 mm. After 72 hours (on the 6th day) of the study, suppression of the MRSE growth was 11 mm. On the 7th day, the antimicrobial activity of the hydrogel was 5 mm. The release of the antibiotic proceeded evenly.

The bactericidal effect of bone cement based on PMMA against MRSE was observed on the first 2 days of the study and amounted to 5 mm and 1 mm, respectively (Fig. 4). From day 3 to day 7, the elution of the drug completely stopped, and, therefore, there was no antimicrobial activity of the cement sample.

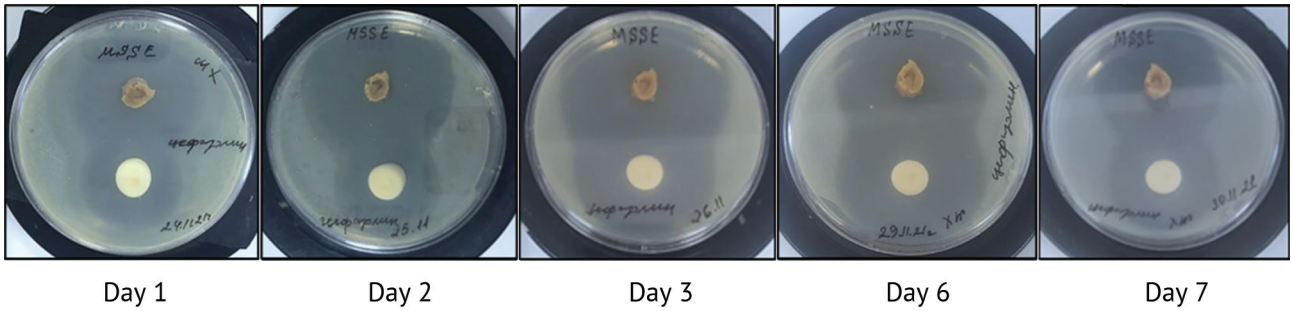

Fig. 3 Antimicrobial activity of the compared samples saturated with cefazolin against MSSE in 1 parallel study; in the upper half of the Petri dish there is a polymer hydrogel, in the lower half there is a sample of bone cement

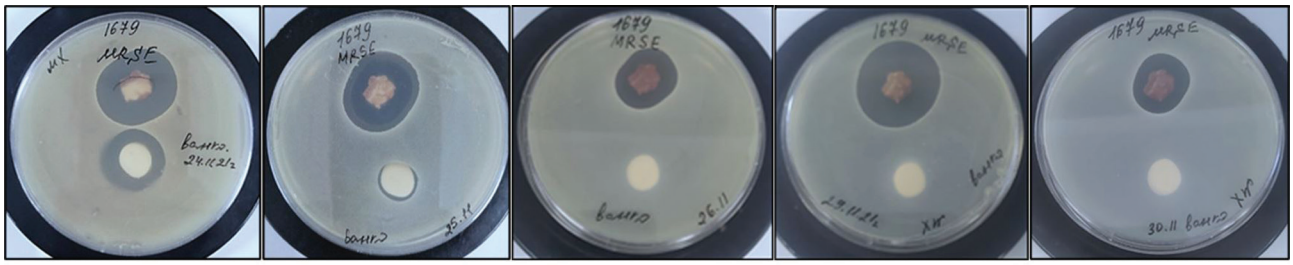

Fig. 4 Antimicrobial activity of the compared vancomycin-impregnated samples against MRSE in 1 parallel study; in the upper half of the Petri dish there is a polymer hydrogel, in the lower half there is a sample of bone cement

Antimicrobial activity of the compared samples impregnated with tobramycin against A. baumannii

On the 1st and 2nd days, the diameter of the GIZ around the hydrogel sample was 10 mm; on the 3rd day of incubation, it was 8 mm. On the 6th day, an increase in the growth inhibition zone of the test culture up to 11 mm was observed as a result of 72 hours of incubation. On the 7th day of the study, bactericidal activity decreased to 7 mm (Fig. 5). The elution of the drug was uniform on all days of the study.

The antimicrobial activity within a diameter of 8 mm in the sample based on PMMA was recorded only on the 1st day of the study, while the area of the GIZ was somewhat uneven. No activity was observed from days 2 to 7 (Fig. 5).

The arithmetic mean values of the microbe-antibiotic pairs of all parallel studies are presented in Table 1. The statistical data of the comparative analysis are shown in Table 2.

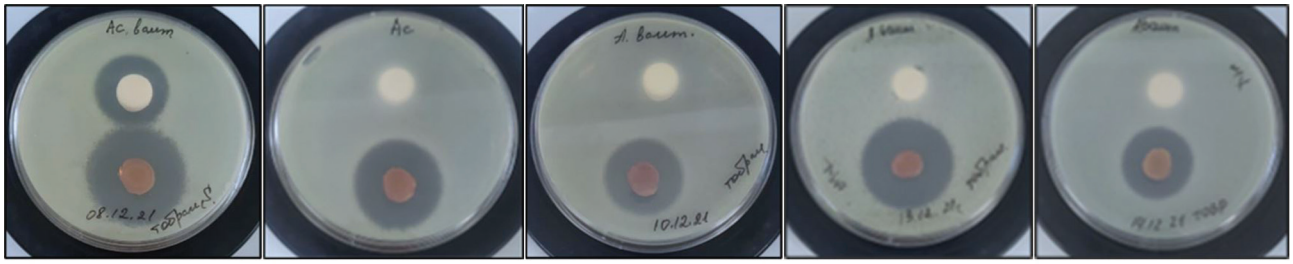

Fig. 5 Antimicrobial activity of the compared samples saturated with tobramycin against A. baumannii in 1 parallel study. In the upper half of the Petri dish there is a sample based on bone cement, in the lower half – based on polymer hydrogel

Table 1

Arithmetic mean values of the diameters of growth inhibition zones for each microbe-antibiotic pair

Pathogen / antibiotic	Diameter of growth inhibition zones in mm by days*									
	Polymer hydrogel					Bone cement (PMMA)				
	1	2	3	6	7	1	2	3	6	7
MSSA / gentamicin	13.6	12	10	10.3	8.7	8	4.3	4.3	4.7	2.7
MRSA / vancomycin	10	10.3	9	8.7	7.7	5.3	3	1.7	1.7	0.3
MSSE / cephazolin	27.3	27.7	29.3	25.7	23.7	22.3	12	16	15	13
MRSE / vancomycin	12.7	12.3	11	12.3	9.3	6.3	3.7	1.7	1.7	0.3
A. baumannii / tobramycin	13.3	11.7	11	12.3	10	8	0.3	0	0	0

Table 2

Statistical Comparison of the Antimicrobial Activity of Polymer Hydrogel and Bone Cement from the 1st to the 7th day of the study

	Day1	Day 2	Day 3	Day 6	Day 7
Significance of differences (p)* (Mann-Whitney test)	0.002	0.000	0.001	0.001	0.001
Significance of differences (p)** of zone diameter at the time-points of the experiment for bone cement (Wilcoxon test)	–	0.042	0.043	0.655	0.063
Significance of differences (p)** of zone diameter at the time-points of the experiment for hydrogel (Wilcoxon test)	–	0.276	0.343	0.785	0.053
Diameter of the hydrogel zone, Me (95 % CI)	14 (11; 19)	12 (10; 18)	11 (9; 18)	12 (10; 17)	10 (8; 15)
Diameter of the cement zone, Me (95 % CI)	8 (5; 14)	3 (2; 7)	2 (1; 8)	1 (1; 7)	0 (0; 5)

*Statistical significance ($p < 0.05$) indicates the confirmation of differences in the diameters of the zones of inhibition of the growth of microorganisms: * – for polymer hydrogel and bone cement at the same time; ** – separately for polymer hydrogel and bone cement*

DISCUSSION

As our analysis showed, all samples of polymer biodegradable hydrogel impregnated with gentamicin, vancomycin, cefazolin and tobramycin demonstrated prolonged (more than 7 days) pronounced effective antimicrobial activity against the leading bone infection pathogens in comparison with the samples made of bone cement based on PMMA. As can be seen from Table 1, the difference in the diameters of the zones of inhibition of the tested cultures every 24 hours of incubation in Petri dishes was mainly 1-2 mm, which indirectly indicates a slow gradual decrease in the activity of the polymer hydrogel as a result of controlled diffusion of antibiotics from the matrix volume.

The largest zone of inhibition was observed for cefazolin released from both the hydrogel and PMMA (Fig. 3). Antimicrobial activity in the case of both compared samples was equally uniform and continued more than 168 hours. The bactericidal profile in two parallel samples increased gradually with a maximum peak on the 3rd day of the study. Subsequently, the activity of the hydrogel decreased gradually in comparison with bone cement. The inhibitory effect of the latter dropped sharply within 48 hours of incubation. Moreover, the efficacy of the hydrogel matrix saturated with cefazolin was superior to that of PMMA throughout the study.

The activity of hydrogel impregnated with vancomycin against resistant strains of staphylococcus decreased the slowest. The inhibition of the growth of microorganisms remained practically unchanged during the first 3-4 days of the study (Fig. 2 and 4).

Bone cement samples impregnated with gentamicin, vancomycin and tobramycin showed the worst result. The antimicrobial activity of cement matrices containing gentamicin and vancomycin was uneven from the first days of the study. Elution of antibiotics on the 2nd day of incubation proceeded mainly from one half of the cement surface; and from 3rd to 6th days from 1/3 of its area (Figs. 1 and 2). The antimicrobial effect of tobramycin was generally short and continued only 24 hours (Fig. 5).

It should be noted that the activity of bone cement in all microbe-antibiotic pairs in our study significantly decreased ($p = 0.042$) within 48 hours of the study (Table 2).

In general, our data are comparable with those of other authors. Thus, Chang Y reported a two-day antimicrobial effect of vancomycin loaded into bone cement at a concentration of 1 g per 40 g of PMMA [24]. Cunha MT et al. revealed a sharp decrease in the antimicrobial activity of vancomycin and gentamicin impregnated in PMMA after 24 hours from the start of the study [26]. Schiefer UR in his work observed a decrease in the elution of gentamicin from the cement matrix within 24 hours of the study [27]. Dziuba GG, studying the elution of vancomycin from articulating spacers based on PMMA, revealed a significant decrease in the release of the drug on the 2nd day after implantation [28]. However, some authors obtained results that differ from ours. Thus, prolonged antimicrobial activity of PMMA samples saturated with gentamicin for more than 14 days [23] and vancomycin for more than 1 month was demonstrated [24]. In the latter case, the activity of not the sample itself, but its daily eluate, was studied by diffusion into agar.

As is known, the effectiveness and duration of the antimicrobial activity of depot systems depend on the rate of drug elution from the matrix volume. The key reason for different rates of antibiotics release is their different physicochemical properties (molecular weight, crystallinity, charges, solubility) [10]. Most likely that the high elution of cefazolin from bone cement due to its low molecular weight (M = 454 Da) and good water solubility [29] explains the high activity of PMMA against MSSE obtained in our study. Therefore, gentamicin and tobramycin, which have a low particle mass (477 Da and 467 Da, respectively) and high water solubility compared to vancomycin (1449 Da) [17, 30, 31], should also have high level of release from PMMA and, as a result, antimicrobial activity. However, the bactericidal efficacy and duration of release of all three antibiotics from the cement material in our study was almost equally low. One can refer to the low content of aminoglycosides in bone cement samples compared to the concentration of vancomycin (18 mg versus 67.8 mg, respectively), but with the same dose of antibiotics in the polymer hydrogel, the activity of these drugs did not differ significantly and was equally prolonged.

Thus, it can be concluded that in the case of loading an antibiotic into a hydrogel, there is a certain correlation between the structure and physicochemical properties of preparations with the dynamics of their desorption and antimicrobial activity. No such clear correlation was observed for bone cement in our study. It is likely that the release from PMMA and its antimicrobial activity are determined by the granulometric composition of the drug substance.

Thus, Paz E et al. explain the short-term inhibitory effect of vancomycin by its low elution from PMMA, since drug particles have a high molecular weight and poor water solubility [29]. The meta-analysis also showed that large fractions of vancomycin (> 99 %) impregnated in PMMA remain isolated in the pores of the material and lose bioavailability after its solidification [30]. The mass transfer of particles for vancomycin, according to the literature, ranges from 0.0001 to 0.008, regardless of the degree of its saturation in bone cement. Thus, when loading 6 g of vancomycin in 60 g of bone cement, only tens of milligrams of the antimicrobial agent were available [30]. In another study, a 4-fold increase in the content of vancomycin in PMMA slightly prolonged its antimicrobial activity from 2 to 5 days [25].

The short-term bactericidal profile of PMMA is associated with the release of mainly surface-bound antibiotic particles [8], since diffusion from the bulk of the cement is difficult and less involved. An increase in the concentration of the drug in the cement is more conducive to the appearance of near-surface areas from which the drug can be eluted, but only a small amount remains bioavailable [30]. Dusane DH et al. determined that the release of gentamicin from bone cement was carried out mainly from its outer layers with a thickness of 100 μm [8]. As a result, an 8-fold increase in the content of gentamicin in PMMA enhances the release by only 11 % [23]. According to Liawrungrueang W, the inclusion of a lyophilized form of liquid gentamicin in the PMMA matrix can improve the elution properties of the drug and antimicrobial activity, in contrast to its powder form [23, 32].

Uneven and uncontrolled suppression of microorganisms is a consequence of the inhomogeneous distribution of the medicinal agent in the cement matrix during manual mixing of the components. The elution of excessive concentrations of the drug by some areas of the cement may affect the state of osteoblasts and osteoregenerative properties of bone tissue or become a source of recurrence of infection and the emergence of resistant strains of microorganisms when insufficient concentrations are released by other areas of the sample [10, 26, 33-35]. Moreover, due to hydrophobic properties of PMMA, a sharp premature cessation of antibiotic release from bone cement and its antimicrobial activity is associated with the adhesion of microorganisms to the implant surface [14-16, 26]. Thus, van Vugt TAG et al. found bacterial colonization on the surface of explanted spacers in patients with arrested PJI and a triple negative bacteriological culture [36]. Some studies showed the facts that the surface of spacer beads is contaminated with microbes during the continued elution of the antibiotic [37]. Schiefer UR et al. detected bacterial growth in cylindrical cement samples impregnated with gentamicin after 72 hours of inoculation with cultures of *S. aureus* and *S. epidermidis* [27]. G. Balato et al. obtained a similar result of S. epidermidis biofilm formation on tobramycin and gentamicin cement discs after 72 hours of cultivation and MRSA biofilms on PMMA vancomycin discs after 96 hours of observation [38]. The detection of separate colonies of *P. aeruginosa* along the edge of the GIZ by sudying the antimicrobial activity of bone cement saturated simultaneously with tobramycin and vancomycin indicates the tendency of PMMA to generate resistant or slowly growing strains of microorganisms (persisters) [14]. In addition, the study of a smear from the inner inhibition zone of *S. aureus* after 3 days of its inoculation with an antibiotic-containing cement bead showed the presence of viable strains [8].

Our study demonstrated that the antimicrobial efficacy of the biodegradable polymer hydrogel was significantly higher than the activity of PMMA ($p = 0.002$) already from the first day of incubation (Table 2). The bactericidal action of the hydrogel throughout the observation period was uniform without abrupt changes or dips. As can be seen from Table 2, the diameter of the growth inhibition zone on the 2nd day of the study in hydrogel samples compared to bone cement had no significant differences ($p = 0.276$).

The correct rounded shape of the zone of inhibition of test cultures around the polymer hydrogel during the entire observation period, the initial concentrations loaded into the sample volume, the gradual decrease in antimicrobial activity and the values obtained on the 7th day of observation (95 % CI: – 8;15) may speak about the prolonged action of hydrogels and the uniform distribution of antibiotic particles in the structure of the material, regardless of their molecular weight and water solubility. Soft biodegradable hydrogels can provide a diffusion-based mechanism and release high effective antibiotic concentrations over a long period of time, which differs them from PMMA that is limited by surface depletion of the antimicrobial agent and has a minimal potential for elution from the matrix volume [30]. Moreover, the hydrophilic surface that prevents the adhesion of microorganisms, elasticity, simple manufacturing technique, minimum material hardening time (up to 5 sec.), biocompatibility, biodestructibility and lack of toxicity [21] make the hydrogel matrix a more suitable and effective local transport system for bone infection treatment.

To confirm our results, further studies are underway *in vivo*.

CONCLUSION

Polymer biodegradable hydrogels impregnated with antibiotics have a greater antimicrobial potential compared to bone cement that is commonly used in clinical practice. A uniform slow and controlled release of the drug and effective inhibition of the growth of microorganisms along with prolonged action are the properties that make the biodegradable hydrogel matrix an effective local transport system.

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