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Experimental Substantiation of Antimicrobial Efficiency of a New Composite Polymeric Material Based on Poly(2-Hydroxyethyl Methacrylate) under the Action of Low-Intensity Current without External Power Supplies

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Abstract. Active use of polymeric materials has become an integral part of all areas of modern medicine. Wound dressings capable of prolonged release of drugs directly into the lesion occupy a special place among them. The possibility of using such materials in the presence of low-intensity currents without external power supplies in a comprehensive treatment program for patients with burn injuries remains promising. The aim of the work is to study experimentally the antimicrobial efficacy of a new composite polymeric material based on poly(2-hydroxyethyl methacrylate), saturated with the antiseptic decamethoxine, under conditions of low-intensity current without external power supplies. The method of free radical thermal polymerization of a mixture of liquid monomer 2-hydroxyethyl methacrylate, crosslinking agent triethylene glycol dimethacrylate, polymerization initiator azobisisobutyronitrile was used for the synthesis of composite polymeric material. In addition, fourfold volume of distilled water as a pore-forming agent and decamethoxine as an antimicrobial component were administered. Known dressings of synthetic and biological origin were selected for comparison, some of which were pre-soaked in a 0.02% solution of decamethoxine. The study of conductivity of the materials without external power supplies was performed on the surface of a dense nutrient medium in a Petri dish using VITA-01M measuring device. Determination of antibacterial properties was performed by diffusion into agar. The obtained results allowed to establish the ability of the suggested polymeric material to conduct low-intensity currents without external power supplies, exceeding the duration of other traditional dressings. Comparison of antimicrobial activity of the studied samples confirmed the synergism of the action of physical factors and a new polymer-based composite material with the addition of antimicrobial substance to inhibit the growth of the test museum and clinical strains of *Staphylococcus aureus*. The ability of low-intensity currents without external power supplies to potentiate the antimicrobial properties of a new composite polymeric material based on poly(2-hydroxyethyl methacrylate), modified with a pore-forming agent, with the addition of decamethoxine was experimentally established.

Introduction

Active use of polymeric materials has become an integral part of all areas of modern medicine [1]. For therapeutic purposes they are mainly used in the form of composites, where macromolecular compounds play a role of the base carrier or have their own physiological activity [2]. Wound

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dressings for temporary closure of wound defects of various genesis, which have numerous advantages over traditional means of wound healing (ointments, sprays, powders, etc.) occupy a special place among them [3]. Further rapid development of the chemical industry has expanded the properties of polymeric substances, which nowadays are not limited to biocompatibility, atraumaticity, lack of local irritant, allergic or toxic effects [4]. This relates to the development of unique materials capable to provide long uniform release of medicinal substances directly into the center of injury providing their high therapeutic concentration [5]. It is these materials that are necessary to provide qualitative care to patients with burn injuries at different stages of its provision [6]. Immediately after the burn, in addition to terminating the thermal agent by neutralizing hyperthermic exogenous, endogenous damage factors, it is important to close the wound surface, which becomes extremely vulnerable to the development of various complications [7]. One of them is active development of microflora in necrotic tissues, which leads to the development of sepsis, multiple organ failure and lethal consequences [8].

Scientists all over the world are working on the technology of maximum inhibition of the development of microflora in the eschar in order to use it as biological coating of burn wounds. One such technology may be the use of a new composite polymeric material based on poly(2-hydroxyethyl methacrylate) (PHEMA) (Fig. 1) saturated with antiseptic drugs under conditions of low-intensity current without external power supplies.

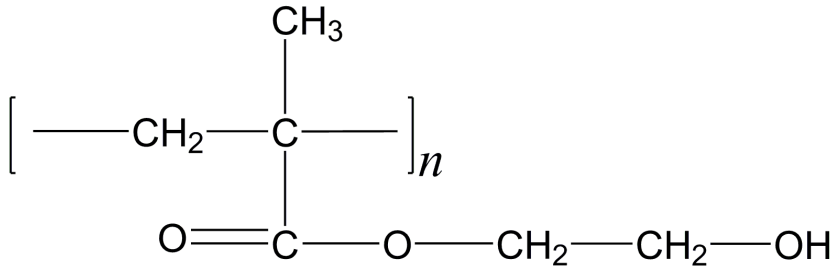


Fig. 1. General formula of poly(2-hydroxyethyl methacrylate).

Modification of this material with water as a pore-forming agent in situ significantly expanded the scope of its use (Fig. 2).

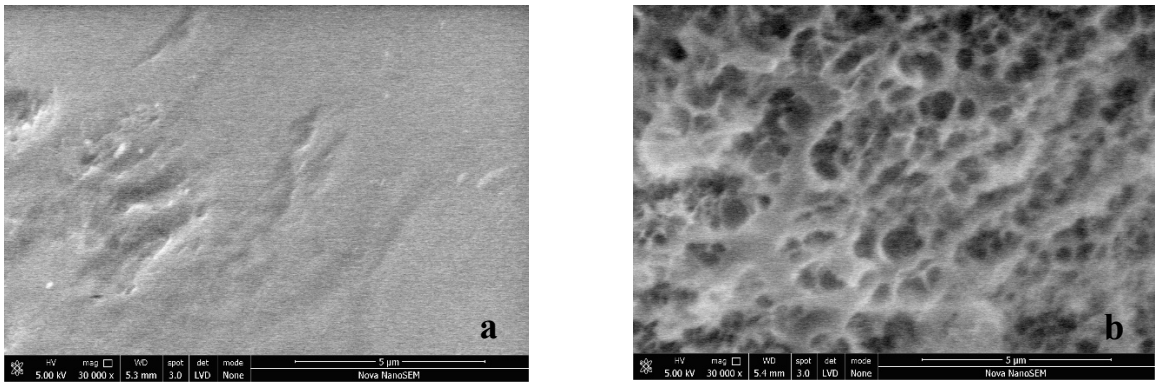


Fig. 2. Image of samples after freeze-drying: initial poly(2-hydroxyethyl methacrylate) (a); poly(2-hydroxyethyl methacrylate) synthesized by addition of water (b). Images were obtained by low-vacuum scanning electron microscopy (LVSEM) [10].

Aim of Paper

The aim of the work is to study experimentally the antimicrobial efficacy of a new composite polymeric material based on poly(2-hydroxyethyl methacrylate) saturated with the antiseptic decamethoxine under the action of low-intensity current without external power supplies.

Materials and Methods

Samples of study material were synthesized by free radical thermal polymerization in polypropylene-polyethylene cylindrical container at the temperature range of 70–90ºC. The polymerization mixture consisted of liquid monomer 2-hydroxyethyl methacrylate (Sigma-Aldrich, Germany), a crosslinking agent triethylene glycol dimethacrylate (1% of monomer weight), polymerization initiator azobisisobutyronitrile. In addition to distilled water used as pore-forming agent (75.4-78.7 wt% (1:4)) and 3,0 % decamethoxine (Pharmhim, Ukraine) as antimicrobial substances were added.

For comparison, wound dressings commonly used in clinical practice today were chosen. They included both synthetic wound dressings (activated carbon material (ACM), "Dnipro", Ukraine; hydrogel dressings, including those filled with dimexide ("Arma-Gel", Ukraine)) and biological ones (lyophilized porcine xenograft, "Institute of Biomedical Technologies", Ukraine). The use of ACM and xenomaterials provided for their preliminary immersion into 0.02% decamethoxine solution.

The study of conductivity of the materials without external power supplies was performed on the surface of a dense nutrient medium in a Petri dish using a measuring device VITA-01M, which contained an electrode-donor made of copper with a diameter of 0.5 mm and an electrode-acceptor made of aluminum-magnesium-zinc composite of the same diameter, interconnected by a conductor of the first kind. The test materials were placed alternately under each of the electrodes.

Determination of antibacterial properties of the studied materials in the form of disks 6 mm in diameter and 2 mm thick was performed by diffusion into agar. To do this, a layer consisting of a thoroughly mixed nutrient agar medium with a daily test culture of the microorganism (*Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* 17) was poured into the cup. The microbial load amounted to 10^7-10^8 microbes per 1 ml of medium, which was determined by an instrumental investigative method according to the McFarland standard. Mueller-Hinton agar was used for the study. Evaluation of antimicrobial activity was performed 24 hours later by the degree of growth retardation in the area of application of the studied sample [11].

The obtained results were processed statistically using data processing software Microsoft Excel 2016 and "Statistica 5.5" (licensed № AXXR910A374605FA) [12].

Research Results

Electrodes located on the surface of the nutrient medium generated a constant microcurrent through the measuring device without external power supplies with a force of 13.8 ± 3.4 μA. When the test specimens were placed under the electrode-donor, the current did not differ significantly and was 12.6 ± 3.5 μA for composite polymeric material, 13.1 ± 4.4 μA for ACM, 11, 9 ± 3,1 and 12, 3 ± 3.4 μA for hydrogels, 12.3 ± 4.5 μA for xenograft (Fig. 3). Subsequently, dynamic decrease in these parameters was determined for all materials with the disappearance of conductivity properties up to 8 days, except for the composite which retained the ability to conduct current even 14 days later.

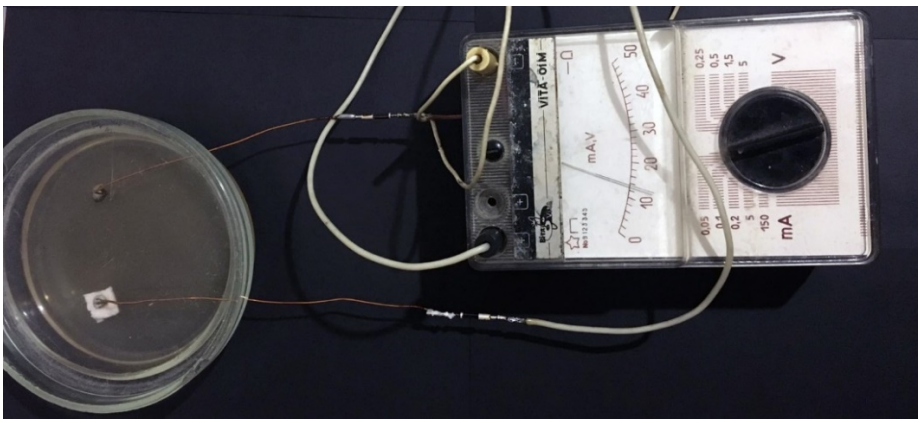


Fig. 3. VITA-01M device for studying the conductivity of a composite polymeric material placed on the surface of a dense nutrient medium.

Analysis of the results of antimicrobial properties allowed to establish the growth retardation zone of more than 10 mm of only the samples of xenograft and the studied polymeric composite based on PHEMA, which constituted 11.7 ± 0.4 mm and 23.4 ± 0.3 mm, respectively, for *S. aureus* ATCC 25923, 10.09 ± 0.5 mm and 21.2 ± 0.4 mm, respectively, for *S. aureus* 17 (Fig. 4b).

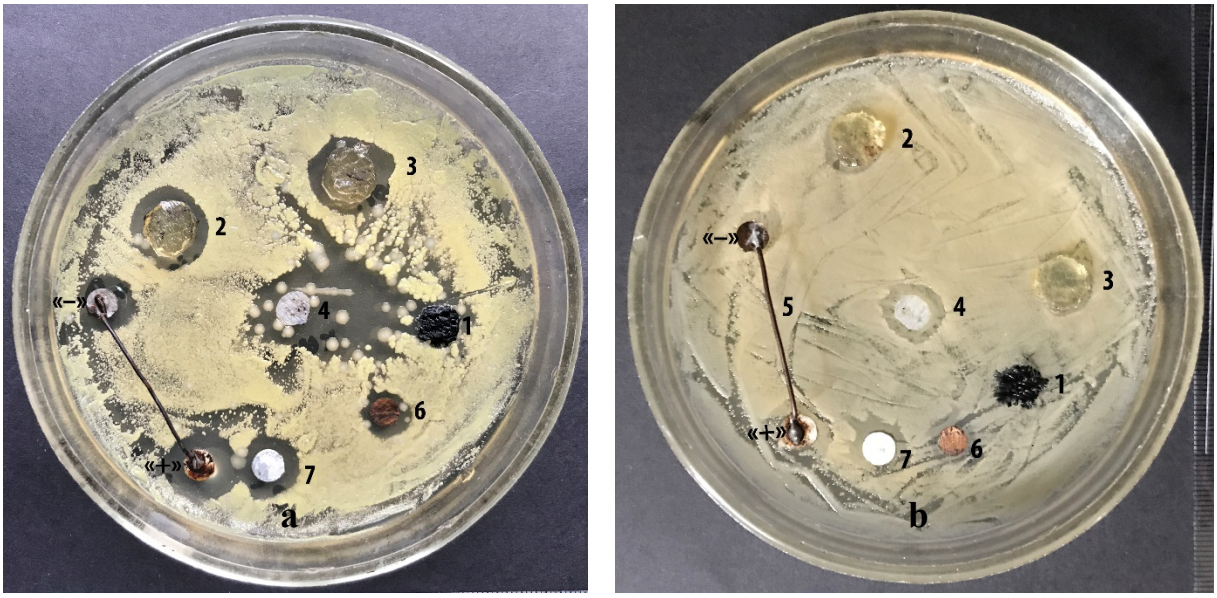


Fig. 4. Study results of antimicrobial activity of intact test samples against *S. aureus* ATCC 25923 (a) and *S. aureus* 17 (b).
Notes: 1 - activated carbon material; 2 - hydrogel material without filler; 3 - hydrogel material with dimexide; 4 – xenograft; 5 - biogalvanic device; 6 - electrode-donor; 7 - electrode-acceptor.

Under the action of biogalvanic currents in a closed circuit on *S. aureus* ATCC 25923 changes in the antimicrobial properties of some test materials were determined, which differed depending on the location of the electrodes. In particular, under the receptor electrode around the ACM material, growth retardation zone of microorganisms of 11.4 ± 0.2 mm was observed (Fig. 5a), which was larger compared to intact samples, but inferior to samples located under electrode-donor of electrons, where the growth retardation zone accounted for 24.4 ± 0.5 mm (Fig. 5b). Under the action of low-intensity current xenomaterials, as well as other samples, also showed an improvement in antimicrobial properties, but the growth retardation zone was clearly defined by secondary growth of the culture. Quite unexpectedly, the growth retardation of microorganisms due to the action of purely physical factors was also determined around the electrodes, which were located directly on the agar nutrient medium (Fig. 5).

The studied composite polymeric materials based on poly(2-hydroxyethyl methacrylate) demonstrated significantly stronger antimicrobial effect on *S. aureus* ATCC 25923, forming the growth retardation zone under the electrode-acceptor measuring 21.3 ± 0.5 mm and 28.5 ± 0, 4 mm under the electrode-donor, which differed significantly from the initial values in intact samples ($p \leq 0.05$).

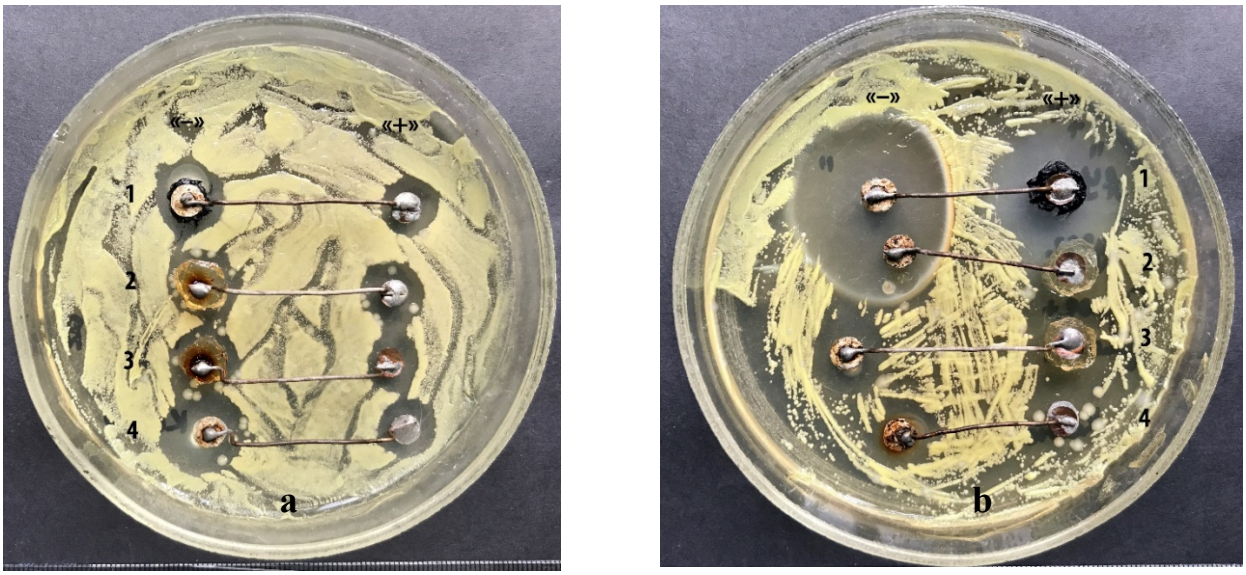


Fig. 5. Study results of antimicrobial activity of the test samples located under the electrode-acceptor (a) and the electrode-donor (b) against *S. aureus* ATCC 25923.
Notes: 1 - activated carbon material; 2 - hydrogel material without filler; 3 - hydrogel material with dimexide; 4 – xenograft.

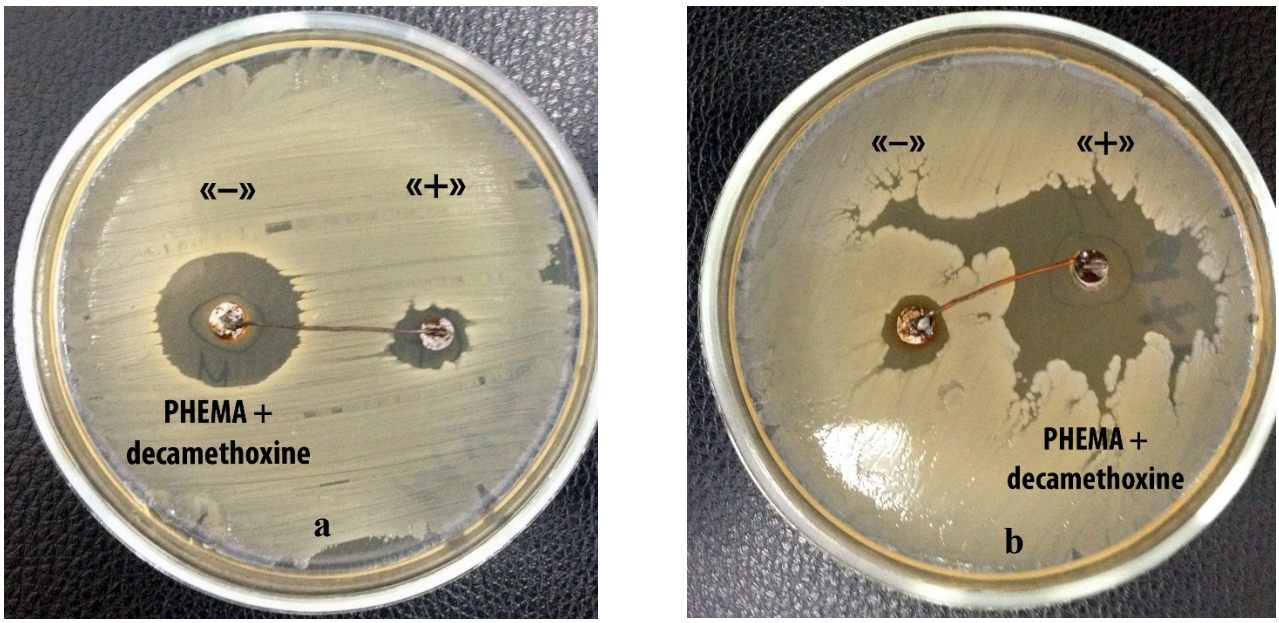


Fig. 6. Study results of antimicrobial activity of samples based on poly(2-hydroxyethyl methacrylate) located under the electrode-acceptor (a) and electrode-donor (b) against *S. aureus* ATCC 25923.
Notes: PHEMA - poly(2-hydroxyethyl methacrylate).

Naturally, the results of the evaluation of the antimicrobial activity of the studied samples against the resistant clinical culture of *S. aureus* 17 were lower, which showed a stable growth around all materials under the conditions of the electrode-acceptor (Fig. 7a). Under the action of electrode-donor of electrons, the formation of a clear growth retardation zone around ACM materials with the size of 14.7 mm ± 0.3 mm and xenograft pre-soaked in 0.02% decamethoxine solution measuring 13.5 ± 0.4 mm was observed (Fig. 7b).

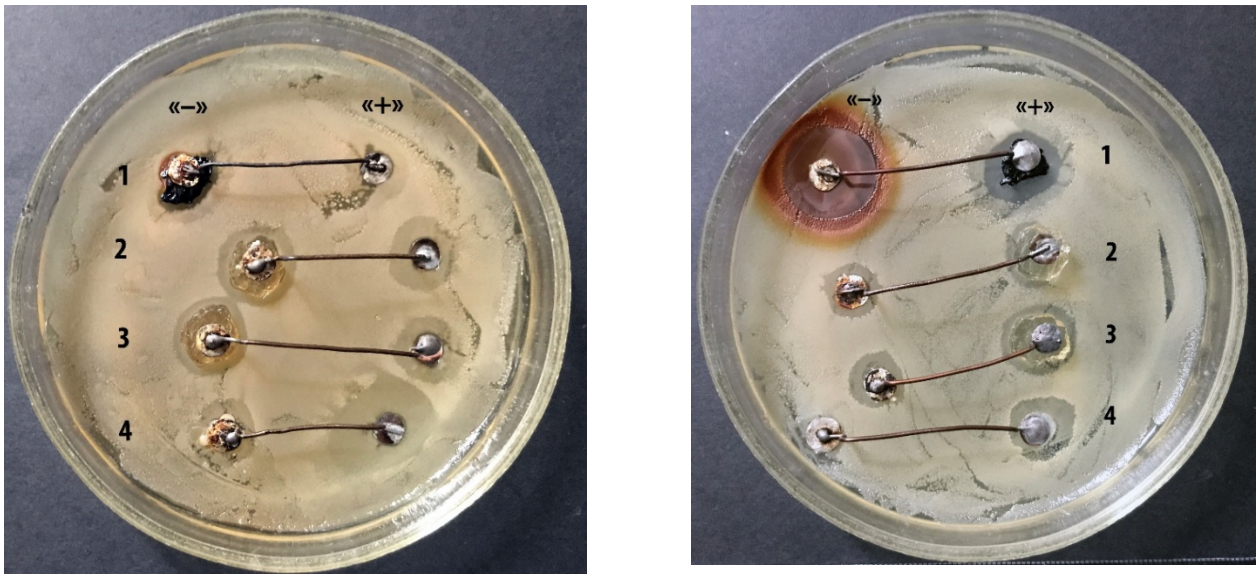


Fig. 7. Study results of antimicrobial activity of the test samples located under the electrode-acceptor (a) and the electrode-donor (b) against *S. aureus* 17.
Notes: 1 - activated carbon material; 2 - hydrogel material without filler; 3 - hydrogel material with dimexide; 4 - xenograft.

Developed composite polymeric materials showed a consistent sufficiently high antimicrobial effect inhibiting the growth of *S. aureus* 17 in diameter of 20.1 ± 0.4 mm under the electrode-acceptor (Fig. 8a) and 26.4 ± 0.3 mm under the electrode-donor (Fig. 8b).

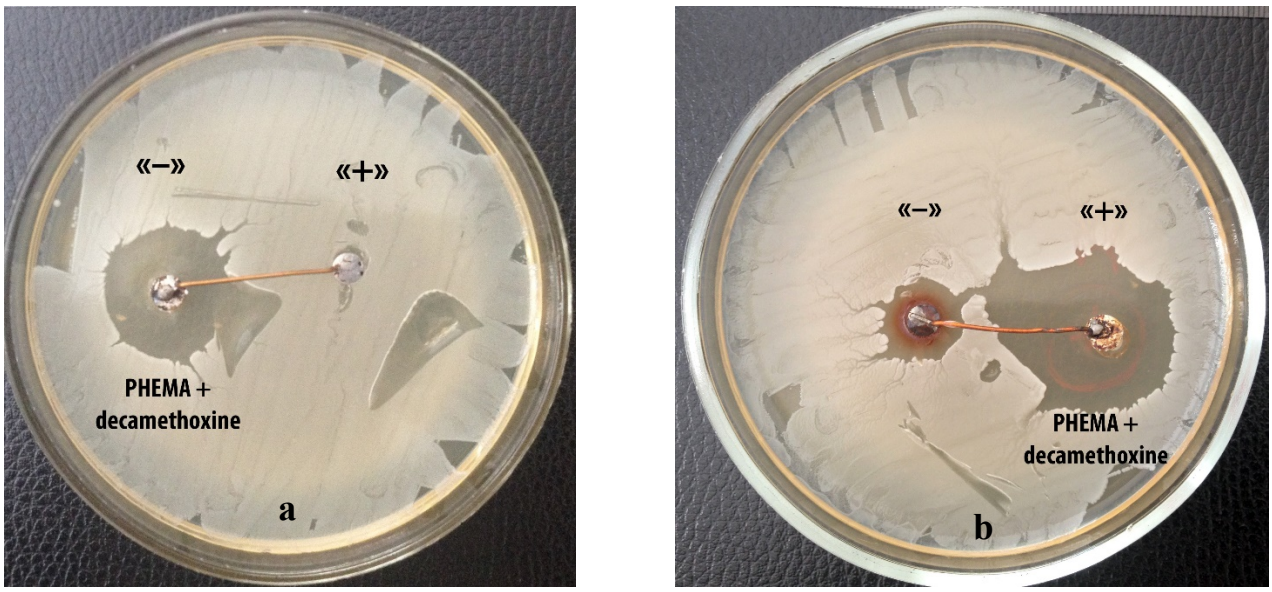


Fig. 8. Study results of the antimicrobial activity of samples based on poly (2-hydroxyethyl methacrylate), located under the electrode-acceptor (a) and the electrode-donor against *S. aureus* 17.
Notes: PHEMA - poly(2-hydroxyethyl methacrylate).

Thus, the quality of care provided is essential in the further course of the disease and the key to successful treatment. The development of effective ways to combat microbial contamination of the wound from the first minutes remains one of the priorities of medicine [13]. Among the broad range of antimicrobial agents and materials wound dressings, capable of gradual prolonged release of active substances into the wound area are of great interest [14]. Composite polymeric material on the basis of poly(2-hydroxyethyl methacrylate), modified by a pore-forming agent with various antimicrobial fillers, have such properties. The results of previous studies have established the optimal composition and proportions of the components of such a composite, which would best respond to the basic physical, mechanical and clinical requirements [15]. In the study we were able to establish another

property of the suggested material, which is not typical of most traditional medical materials - the conduction of electric currents of varying intensity, including low-intensity current without external power supplies. After all, there are reliable data that confirm the ability of such currents to have a detrimental effect not only on museum strains of microorganisms, but also on their resistant clinical forms [16]. It is clear that the action of currents only has a number of limitations and does not provide a reliable antimicrobial effect, especially with regard to gram-negative bacteria, which currently occupy a leading position among patients of inpatient surgical departments [17]. Not to mention stable forms of coexistence of microorganisms that have developed the ability to form biofilms [18]. Therefore, the established in the study synergism of antimicrobial action of physical factors and a new polymer-based composite material with the addition of decamethoxine allows to effectively inhibit the development of *S. aureus* microorganisms, surpassing the properties of other materials.

Conclusions

1. The obtained results allowed to establish the ability of the suggested polymeric material to conduct low-intensity currents from external power supplies.
2. Microbiological study results conclusively confirm the ability of such currents to improve the antimicrobial properties of the polymeric material based on poly(2-hydroxyethyl methacrylate) modified with microporosity and the addition of decamethoxine.

We consider it expedient to continue the study to investigate the effect of new polymeric material on other strains of microorganisms with the addition of antiseptics under conditions of low-intensity current without external power supplies and further study of efficacy on experimental animals, possible introduction into clinical practice of burn treatment.

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