New possibility of application of bacteriophages to prevent infectious complications in free skin grafting (bacteriophages in skin grafting)

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ABSTRACT

Aim. To prevent infectious processes in the area of a recipient wound in free skin grafting with a split-graft.

Materials and methods. A method was developed for immobilizing bacteriophages in the area of split-thickness skin grafts through transferring a solution containing bacteriophages into a gel form. Microbiological and clinical studies of the effectiveness of the proposed method were performed.

Results. The viability of bacteriophages in a gel dressing for up to 4 days was confirmed, as well as the reduced likelihood of local infectious complications in skin grafting.

Conclusion. The gel composition containing bacteriophages allows for a quick response to changes in current hospital microflora to effectively counteract the dangers of nosocomial infection.

Key words: bacteriophages, free skin grafting, split-thickness skin grafts, wound complications, microflora.

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INTRODUCTION

The number of patients who need plastic surgery to close soft tissue defects is steadily increasing. The relevance of the problem of closing soft tissue defects is explained both by an increase in frequency of military, domestic and man-made injuries, and by the growing level of technology in the field of vascular surgery, which ensures limb preservation in obliterating vascular diseases, including the presence of trophic ulcers of soft tissues [1].

Plastic closure of a skin defect with a split-thickness graft is one of the most common operations because of its relative safety and technical simplicity. However, one of the limiting factors is high sensitivity of the graft to the development of infection. This is determined by high prevalence of hospital flora with high virulence and antibiotic resistance, as well as by the technical peculiarities of these operations. The graft on recipient wounds is fixed by a multilayer bandage for a period of up to 4–7 days. Lack of light, nutrient availability, liquids, and favorable temperature regime create ideal conditions for the growth and proliferation of microorganisms under the bandage. During the first 4–5 days, the graft feeds only due to the diffusion of oxygen and nutrients from the vessels of the recipient wound and does not have its own vascular network, which makes it nearly defenseless against microorganisms [2].

The aim of the study was to prevent an infectious process in the area of a recipient wound in free skin grafting with a split graft, which is one of the fundamental conditions for an uncomplicated course of the early postoperative period.

To achieve this goal, it is necessary to solve two problems: to choose an agent capable of overcoming antibiotic resistance of the hospital flora and to maintain its concentration under the dressing, taking into account the need for minimal mechanical and chemical effects on the graft. Currently, to overcome the phenomenon of antibiotic resistance,
the possibility of using bacteriophages is being actively studied [3].

The basic principle of the treatment for infectious processes using bacteriophages was formulated by F. D’Hérelle, the discoverer of this type of viruses, as follows: “The bacteriophage should be introduced into the body in such a way as to realize the fastest and most intimate contact of it with bacteria to be destroyed” [4]. To create and maintain a high concentration of bacteriophages around the graft, we used modern wound dressings developed by the Russian company “Novye Perevyazochanye Materialy” LLC (Moscow).

MATERIALS AND METHODS

To protect the graft from hospital infection, a method of local application of bacteriophages has been developed [5]. Previously, the method of retrospective analysis identified the actual hospital microflora, which is the cause of local wound complications in skin grafting. Using commercially available specimens of bacteriophages, a set to which the identified hospital pathogens are sensitive was prepared. Then a dressing with the selected bacteriophages was created for the recipient wound. To immobilize the bacteriophages, the solution in which they are located was transferred into a gel state, which was then applied to the graft.

The blank for the dressing is a film made of polyvinyl alcohol, a hydrophilic biocompatible polymer, which is a suitable matrix to immobilize bioactive substances [6]. The polymer film contains a phosphate buffer to create an acid-base medium (pH 6.6 – 7.8) optimal for bacteriophages. The thickness of the film is 40 microns, but when adding a solution with bacteriophages, the film absorbs it, swells within 30–60 seconds and transforms into gel with formation of a gel plate.

The method was carried out in the following way. Split skin grafting was performed. After fixing the graft on the recipient wound, a bandage was prepared intraoperatively. To achieve this, 0.05–0.2 ml/cm² solution of bacteriophages, to which the identified hospital pathogens were sensitive, was added to the film of polyvinyl alcohol. As a result, the film and solution transformed into a gel plate. Then, the graft and recipient wound were covered with the resulting gel plate.

To control the viability and efficiency of bacteriophages immobilized in a gel dressing, bacteriological studies were performed in vitro and in vivo. In the in vitro study, a gel plate containing bacteriophages was obtained by the proposed method. The inability and bioavailability (release) of bacteriophages from the gel dressing were determined on the lawns of the Staphylococcus aureus test strain.

In the Petri dish with a lawn of the of Staphylococcus aureus test strain, as a matter of control, a drop of a solution containing bacteriophages was applied (control 1), as well as a sample of the wound coating produced by applying a physiological solution onto the film (control 2), and a prototype wound coverage of 1 cm², obtained by applying a bacteriophage solution onto the film. It was then incubated at 37 °C, the results were assessed visually after 24 hours and by the presence or absence of lysis zones. To determine the duration of the viability period of bacteriophages in the gel, its samples of 1 cm² were applied to the lawns of test cultures 48, 72, and 96 hours after the formation of the gel plate.

In clinical practice, the proposed method was used after performing split skin grafting in 25 patients with chronic soft tissue wounds. All patients participating in the clinical study signed an informed consent to do this, and the study was carried out in accordance with the requirements of the Declaration of Helsinki of the World Medical Association (as revised in 2013).

The criterion for the inclusion of patients in the study was the state of the recipient wound surface, estimated at 16–17 points on the scale of wound readiness for free split skin grafting [7]. The control group consisted of 108 patients with chronic soft tissue wounds who underwent split skin grafting in the period 2014–2017. The viability and bioavailability (release) of bacteriophages from the gel dressing in vivo was determined in 4 patients the experimental group. In these patients, bacteriophages with sensitive test strains of Staphylococcus aureus were used as an active antibacterial agent. At the first dressing, the gel covering the graft was collected with a sterile spatula and applied to a Petri dish with the lawn of the Staphylococcus aureus test strain. The criterion for the effective
prevention of infectious processes in the graft area was considered to be a decrease in the frequency of local inflammatory complications.

Statistical analysis of the obtained data was carried out by Statistica 10.0 software. Fisher’s exact method was used to assess the statistical significance of differences when comparing qualitative effects in pairs of distributions. The critical value of the significance level was equal to 5% (p ≤ 0.05).

RESULTS

In a retrospective analysis of the microbiological research data, the control group of 108 patients demonstrated local purulent-inflammatory complications in 24 cases (22%) after free split skin grafting. These complications were represented by lysis and purulent fusion of the graft and were associated with the presence of Streptococcus pyogenes in the wound (5 cases) and non-fermenting gram-negative bacteria, such as Pseudomonas aeruginosa (6 cases), Acinetobacter spp. (4 cases). According to the manufacturer (“Microgen” of the Ministry of Health of Russia), the ability to lyse strains of Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus, Escherichia coli, Proteus mirabilis, and Klebsiella pneumoniae is possessed by the commercial official drug “Polyvalent pyobacteriophage”, which was chosen as an anti-infective agent to protect the split skin graft. The treatment group had 1 (4%) case of purulent fusion in a part of the graft (p = 0.045).

When analyzing the results of the bacteriological studies in vitro, a “negative colony” appeared in the place of a phage drop (control 1), that is, a lysis zone (complete suppression of the visible growth of a microorganism) (Fig. 1). The same lysis zone was discovered in the gel containing the phage. In the gel region containing the saline solution, the lysis zones were not detected. The lytic properties of bacteriophages were retained 48, 72, and 96 hours after the formation of a gel plate from the bacteriophage solution (Fig. 2).

A clinical example of using the proposed method is described below.

Patient K., born in 1962, was admitted to the department of purulent surgery of the City Clinical Hospital No. 30 in Nizhny Novgorod on January 16, 2019 with the following diagnosis:

Decompensated type 2 diabetes mellitus (target HbA1 level < 7.5%). Diabetic polyneuropathy, sensorimotor form. Diabetic foot syndrome, neuroischemic form, Wagner II, condition after amputation of IV–V toes of the left foot dated 12.06.2018. Ischemic heart disease: atherosclerotic cardioarosclerosis. Chronic heart failure II A (FC II). Stage II arterial hypertension, 2nd degree. Risk 3. Dyslipidemia. Grade 2 obesity.

On the lateral surface of the left foot, a 3 × 4 cm wound was formed, about 0.4 cm deep. Microbiological investigations revealed the presence of wound exudates, therein an association of Pseudomonas aeruginosa and Proteus mirabilis 10^7 CFU/ml.
After the transition of the wound process to phase II and the elimination of microorganisms from the wound, it was decided to close the chronic wound of the lateral surface of the left foot with a free split skin graft. A bacteriophage solution was prepared (“Polyvalent pyobacteriophage”), which, according to the bacteriological analysis, hospital pathogens were sensitive to. Bacteriophages were kept in a liquid medium in 20 ml vials.

On 19.01.2019 free split skin grafting was performed with a 0.3 mm thick split flap, which was taken from the antero-lateral surface of the left thigh. After fixing the split skin graft, a bandage for the recipient wound was prepared intraoperatively. To achieve this, 10 ml of the “Polyvalent pyobacteriophage” solution was applied onto a 10 × 10 cm film made of polyvinyl alcohol and containing a phosphate buffer with a pH (6.6 ÷ 7.8) in the amount of (1 ÷ 3) × 10⁻⁵ mol/g. As a result, a gel plate was formed. Then, the graft and the recipient wound were covered with the obtained gel plate (Fig. 3). Aseptic dressings were applied onto the gel plate and then removed after four days (Fig. 4).

At a visual examination, the graft was viable, fixed to the recipient wound, and covered with a thin layer of gel. There were no signs of an infectious process. The gel was collected with a sterile spatula and applied to a Petri dish with a test culture. After a day of exposure, transparent lysis zones of the test culture were revealed in the area of gel application (Fig. 5), which indicates the presence of a bacteriophage with lytic activity ++++ (4 plus points) in the gel.

**DISCUSSION**

Currently, the following basic bacteriophage properties determining feasibility of their use in the prevention and treatment of surgical infection can be distinguished [3]:

– lack of influence on the physiological microflora;

– stimulation of specific and non-specific immunity factors;

– possibility of being used in patients with allergic reactions to antibiotics;

**Fig. 3.** Gel containing a bacteriophage is applied to the graft

**Fig. 4.** The 4th day after free split-thickness skin grafting. The graft is viable and coated with gel

**Fig. 5.** Petri dish with negative colonies (lysis zones) on the lawns of the Staphylococcus aureus test strain: 1 – in the places of applying a fresh solution containing bacteriophage (control); 2 – in the places where the gel obtained from a bacteriophage solution and located on the wound in the form of a bandage for 4 days was applied
full compatibility with any drugs;
lack of toxic and teratogenic effects.

Traditional topical application of bacteriophages is maintaining a moist environment around the wound by irrigating the wound and dressing with a solution containing bacteriophages [4]. Taking into account that as gauze dressings become dry, bacteriophage activity decreases sharply, it is necessary to periodically and abundantly moisten dressings with a bacteriophage solution. There is also a need to change dressings frequently, which leads to the misuse of resources and does not provide graft rest.

In order to simplify the application technology and lengthen the period of bacteriophage activity on the wound surface, foreign and domestic researchers are actively searching for technologies that allow bacteriophage immobilization in the structure of polymer carriers. Modern technologies [8] propose a method of covalent bacteriophage immobilization on a nanostructured support in the form of non-woven nanofibrous material of polycaprolactone. In this case, bacteriophages are located in a given position: the capsid is firmly bound to the carrier, and the tail remains free, which allows them to actively influence bacteria. In another study [9], with the aim of industrial production of wound dressings with bacteriophages, the effect of the type of polymer matrix on the activity of bacteriophages immobilized in the structure of coatings by introducing a polymer into a solution and subsequent drying by different methods was investigated.

The best results were obtained by the authors when staphylococcal and Pseudomonas phages in the structure of the polymeric biodegradable dressings of polyester bromide were immobilized using freeze-drying. However, a fundamental disadvantage inherent in all methods of industrial immobilization of bacteriophages on a bandage is that it is impossible for a surgeon in an operating room to select a bacteriophage for the pathogen that is relevant in a given medical organization, taking into account the sensitivity of a particular strain of a microorganism.

In addition, it is necessary to solve the technically difficult problem of preserving the bacteriophages viability during creation, transport, and storage of the dressing. In the following study [10], it is proposed to apply a solution containing bacteriophages onto a collagen hemostatic sponge and then cover the graft with it. However, it is known that a collagen sponge contains boric and acetic acid, and in an acidic medium, bacteriophages are inactivated, because their maximum activity is manifested at pH from 6.6 to 7.8 [4].

Thus, to date, the search for an effective and inexpensive way to counter the danger of nosocomial infection has not led to success. Modern microbiological laboratories provide the surgeon with accurate information about the actual hospital microflora and its sensitivity to antibacterial drugs and have methods for determining the sensitivity of microflora to bacteriophages and selecting an effective bacteriophage for the conditions of a particular medical organization. The possibility of prophylactic use of bacteriophages under these conditions to prevent an infectious process caused by nosocomial microorganisms becomes real.

CONCLUSION

Thus, the problem of bacteriophage immobilization with the preservation of their function in the skin grafting area for a period of up to 4–5 days has been solved. The gel composition created ex tempore and containing bacteriophages makes it possible to quickly respond to changes in the actual hospital microflora and effectively counteract the danger of nosocomial infection.

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**Authors contribution**

Beschastnov V.V. – conception and design of the study, justification of the manuscript and critical revision of the manuscript for important intellectual content. Ryabkov M.D., Pavlenko I.V., Leon’t ev A.E., Tulupov A.A., Kichin V.V. – analysis and interpretation of data. Yudanova T.N. – final approval of the manuscript for publication.

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Possibilities of radionuclide diagnostics of Her2-positive breast cancer using technetium-99m-labeled target molecules: the first experience of clinical use

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ABSTRACT

Background. The main purpose of the Her2/neu status determination in clinical practice is to determine the indications for the appointment of targeted therapy. The main methods for detecting the Her2/neu status are the immunohistochemical method (IHC) and the fluorescence in situ hybridization (FISH); however, despite their widespread use, they have a number of significant disadvantages. Over the past few years, radionuclide diagnostics using a new class of alternative scaffold proteins that meet all the requirements for optimal delivery of radionuclides to tumor cells has become widespread.

Aim. To study the possibility of clinical use of a radiopharmaceutical based on technetium-99m-labeled target molecules for the diagnosis of breast cancer with the Her2/neu overexpression in humans.

Materials and methods. The study included 11 patients with breast cancer (T 1–4N0–2M0) before systemic therapy: 5 patients with Her2/neu overexpression; expression of the marker was not detected in 6 patients. In all cases, morphological and immunohistochemical studies were performed. In case of Her2/neu 2+, FISH analysis was performed. The radiopharmaceutical was prepared immediately before administration, after which it was slowly injected intravenously into the patient. Scintigraphic studies in the “WholeBody” mode and SPECT of the chest organs were performed 2, 4, 6 and 24 hours after injection.

Results. Radiochemical yield, radiochemical purity and activity before administration were (80 ± 4)%, (98 ± 1)%, and (434 ± 19.5) MBq, respectively. The greatest uptake by normal organs was observed at a time interval of 6 hours in the kidneys and at a moderate activity in the liver and lungs at the same time interval. The organ with the highest absorbed dose was the kidneys; significant accumulation was also detected in the adrenal glands, gallbladder, liver, pancreas, and spleen. The smallest accumulation of the studied drug was observed in the brain (0.001 ± 0.000) mGy and skin (0.001 ± 0.000) mGy. The effective dose was (0.009 ± 0.002) mGy. The difference between tumors with positive and negative Her2-neu expression was found at all time points. In this case, the best indicator was determined after 2 hours of drug injection (p < 0.05).

Conclusion. Based on the results obtained, it can be indicated that the investigated radiopharmaceutical can be considered as a new additional method for the diagnosis of Her2-positive breast tumors.

Key words: alternative scaffold proteins, radionuclide diagnostics, Her2/neu, breast cancer, targeted therapy.

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