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Research Article

Antibacterial wound coating based on chitosan and povidone, obtained by 3D printing

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ABSTRACT

The objective of this study was to develop a method for forming an antimicrobial wound coating based on chitosan and polyvinylpyrrolidone using 3D printing technology.

The properties of the coating were then studied *in vitro* and *in vivo* to improve the treatment outcomes of deep burns. The resulting coating was a 4% hydrogel of medium molecular weight chitosan with the addition of 1% povidone iodine and dermal fibroblasts. After transplantation, the coating was covered with "Foliderm" film. The coating was formed using an extrusion 3D bioprinter, with printing parameters determined experimentally. The samples were first studied *in vitro*. Scanning electron microscopy was used to evaluate the coating's microarchitecture and its interaction with dermal fibroblasts. A colorimetric test was conducted to assess cell metabolic activity and cytotoxicity, and antimicrobial activity against reference strains of *Staphylococcus aureus* was analyzed. An experiment was conducted to evaluate the *in vivo* properties of the coating. Nineteen male Wistar rats were used in the study. An injury was inflicted that resulted in a deep thermal contact burn, affecting all layers of skin and subcutaneous fatty tissue, with an area of approximately 20 cm^2. The animals were divided into three groups: experimental (with the application of the developed coating), comparative (using the traditional and widespread method of treatment with Levomekol ointment) and control (without treatment).

The study lasted for 38 days and found that the developed coating is highly biocompatible, atraumatic, elastic, and adheres well to wounds. Chitosan was used to create a porous structure with channels running parallel to each other. The coating cells are evenly distributed on the surface of the matrix, specifically on the walls of the pores. The inclusion of 1% povidone iodine in the polymer resulted in high antimicrobial activity without significantly affecting the activity of the cells in the composition. The experiment on applying a coating for treating deep thermal burns demonstrated that the developed coating had a positive effect on the wound healing process. This effect was characterized by a higher rate of epithelization and a significantly lower incidence of infectious complications compared to other experimental groups. In the histological study, the experimental group outperformed the control and comparison groups in the quality of the formed granulation tissue, the number of newly formed capillaries, and the severity of the local inflammatory process.

Keywords: 3D bioprinting; chitosan; fibroblasts; povidone iodine; thermal burns; wound coating.

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Научная статья

Антибактериальное раневое покрытие на основе хитозана и повидона, полученное методом 3D-печати

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АННОТАЦИЯ

Целью настоящего исследования являлись разработка антимикробного раневого покрытия, изготовленного методом 3D-печати, на основе хитозана и поливинилпирролидона и последующее исследование его свойств *in vitro* и *in vivo* с целью улучшения исходов лечения глубоких ожогов.

Материалы и методы. Полученное покрытие состояло из 4 % гидрогеля среднемолекулярного хитозана с добавлением 1 % повидон-йода и дермальных фибробластов. После трансплантации область раны с покрытием защищалась наложением пленки «Фолидерм». Для формирования покрытия использовался экструзионный 3D-биопринтер, параметры печати которого были определены экспериментально. Полученные образцы первоначально детально изучены *in vitro*. Были выполнены сканирующая электронная микроскопия для оценки микроархитектуры покрытия и ее взаимодействия с дермальными фибробластами, колориметрический тест для оценки метаболической активности клеток и анализа цитотоксичности, анализ антимикробной активности по отношению к эталонным штамам *Staphylococcus aureus*. Далее выполнен эксперимент по оценке свойств покрытия *in vivo*. Исследование проведено на 19 самцах крыс линии Wistar. В качестве наносимой травмы выбран глубокий термический контактный ожог (омертвление всех слоев кожи и подкожно-жировой ткани) площадью около 20 см^2. Животных разделили на три группы: опытную (с применением разработанного покрытия), сравнительную (с использованием традиционного и широко распространенного метода лечения мазью «Левомеколь») и контрольную (без лечения). Период наблюдения составил 38 сут.

Результаты исследования показали, что разработанное покрытие имеет высокую биосовместимость, атравматичность, эластичность и адгезию к ране. Использование хитозана позволило получить пористую структуру, причем поры образуют каналы, расположенные параллельно друг другу. Клетки в составе покрытия распластаны и хорошо распределены по поверхности матрицы (по стенкам пор). Добавление в состав полимера повидон-йода в концентрации 1 % позволило добиться высокой противомикробной активности без значимого влияния на активность включенных в состав клеток. Эксперимент с применением покрытия для лечения глубокого термического ожога показал, что разработанное покрытие оказывало положительное влияние на ход раневого процесса, заключающееся в более высокой скорости эпителизации и значительно меньшей частоте возникновения инфекционных осложнений на фоне других экспериментальных групп. При гистологическом исследовании опытная группа также превосходила контрольную и группу сравнения по качеству формируемой грануляционной ткани, числу новообразованных капилляров и выраженности местного воспалительного процесса.

Ключевые слова: повидон-йод; раневое покрытие; термический ожог; трехмерная биопечать; фибробласты; хитозан.

Как цитировать

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研究文章

基于壳聚糖和聚维酮的3D打印抗菌创面涂层

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简评

本研究旨在开发一种基于壳聚糖和聚乙烯吡咯烷酮的3D打印抗菌创面涂层，并并对其体外和体内特性进行后续研究，以改善深度烧伤的治疗效果。

材料与方法。所制备涂层由4%中分子量壳聚糖水凝胶、1%聚维酮碘和真皮成纤维细胞组成。移植后，使用Foliderm薄膜对涂层创面进行保护。涂层是用3D生物打印机制成，其打印参数通过实验确定。首先对获得的样品进行了详细的体外研究。通过扫描电子显微镜评估了涂层的微观结构及其与真皮成纤维细胞的相互作用，通过比色试验评估了细胞的代谢活性并对细胞毒性进行分析，对金黄色葡萄球菌参考株进行抗菌活性分析。接下来，我们进行了一项实验，以评估涂层在体内的特性。研究对象是19只雄性Wistar大鼠。选择面积约为20平方厘米的深度热接触烧伤（所有皮肤层和皮下脂肪组织坏死）作为致伤部位。动物被分为三组：实验组（使用开发的涂层）、对比组（使用传统且广泛使用的Levomecol软膏进行治疗）和对照组（未进行治疗）。观察期为38天。

研究结果表明，所研制的涂层具有很高的生物相容性、无创伤性、弹性和对伤口的粘附性。壳聚糖的使用使得多孔结构成为可能，孔隙形成相互平行的通道。涂层中的细胞分布在基质表面（沿孔壁）。在聚合物组合物中添加浓度为1%的聚维酮碘，可以获得较高的抗菌活性，而不会对组合物中包含的细胞的活性产生明显影响。应用该涂层治疗深度热烧伤的实验表明，所研制的涂层对伤口的愈合过程有积极的影响，与其他实验组相比，上皮化率更高，感染性并发症的发生率明显降低。在组织学检查中，实验组在肉芽组织形成的质量、新形成毛细血管的数量和局部炎症的严重程度方面也优于对照组和对比组。

关键词： 聚维酮碘；伤口敷料；热烧伤；三维生物打印；成纤维细胞；壳聚糖。

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BACKGROUND

The skin is a vital organ that shields all internal organs from external factors [1] that can damage the skin and impair its protective and barrier functions. Wound coverings are used to restore damaged skin areas and promote rapid skin regeneration. An ideal wound dressing possesses mechanical strength for easy application by surgeons to the injury site, flexibility, elasticity, good adhesion to the wound surface, and antibacterial and regenerative properties to prevent infection and promote rapid epithelialization [2]. Currently, several wound dressings in the form of films, fibers, hydrogels, and sponges are available in clinical practice [3]. Hydrogels have been used effectively in numerous biomedical applications as a substitute for extracellular matrix to promote wound healing [4, 5]. When used as a wound coating, hydrogels should be nonallergenic and nontoxic, maintain a moist environment, promote efficient oxygen exchange, protect the wound from microbial organisms, and absorb wound exudate. Chitosan is a semicrystalline polysaccharide obtained by alkaline chitin deacetylation. It is widely used as a biomedical material because of its biocompatibility, biodegradability, nontoxicity, and antimicrobial and antifungal effects [6]. Additionally, chitosan promotes wound healing by mimicking hemostasis and intensifying the formation of new tissues [7, 8].

Povidone, which contains iodine as its main active component, is used to treat wounds of various origins in medical practice. Povidone iodine is an antiseptic solution that consists of a complex of povidone (polyvinylpyrrolidone, PVP), hydrogen iodide, and elemental iodine and inhibits the growth and reproduction of microorganisms [9]. The PVP iodine complex releases free iodine in solution, which reacts with amino acids' -SH and -OH groups to eliminate viruses, fungi, and bacteria by iodizing lipids and oxidizing cytoplasmic and membrane compounds [10]. PVP, a hydrophilic polymer, is used as a carrier in the pharmaceutical and biomedical fields. Numerous PVP-based systems for delivering various active components of natural and synthetic origin have been developed [11–13].

Wound coatings are formed using classical and modern methods, including solution irrigation, leaching, freeze-drying, electrospinning, and 3D printing [14, 15]. Classical methods are simpler and more accessible than electrospinning and 3D printing. However, controlling the size and distribution of pores and overall architecture of the wound coating is challenging using classical matrix formation methods. In recent years, 3D printing wound coatings with antibacterial properties using chitosan has gained popularity among researchers. For example, researchers have demonstrated that by adding pectin and lidocaine to chitosan, it is possible to 3D print biodegradable heat-sensitive wound coatings that have good elasticity and the ability to swell and absorb exudate [16]. A wound coating has been produced using 3D printing with chitosan and alginate [17]. Morphological studies have demonstrated the suitability and accuracy of 3D printing for creating well-controlled hydrogel structures with mechanical stability, making it easy to handle such matrices.

Several studies have reported PVP use in 3D printing. Collamaran et al. combined PVP with ramipril to create matrices with prolonged drug release [18]. Okwuoasa et al. developed tablets based on PVP that released the drugs dipyramidol and theophylline simultaneously [19]. Furthermore, Dores et al. used PVP to print tablets containing thiophylline [20]. Literature review showed the potential use of chitosan and PVP for a wound coating with versatile properties. These polymers are already used in medical practice. Combining them to form a complex wound coating could result in a product with the combined properties of each component.

This study aimed to develop a method for forming an antimicrobial wound coating with chitosan and PVP using 3D printing. The properties of the coating were then studied *in vitro* and *in vivo*.

MATERIALS AND METHODS

Chitosan solution preparation with povidone iodine

First, to prepare a 4% solution, chitosan (Heppe, Germany) is dispersed in water. The mixture is stirred on a laboratory top drive stirrer at a 1000 rpm speed for 30 minutes at room temperature. Povidone iodine solution up to 1% is then added and stirred for 5 minutes. Then, acetic acid (Reachim, Russia) up to 2% is added and stirred under the same conditions for another 10 minutes.

Printing

The 3D bioprinter Dr. Invivo 4D (ROKIT, South Korea) was used to create volumetric matrices through the extrusion method. Ink was supplied to the movable printing table with glass through a pneumatic dispenser attached to a syringe nozzle. The printer was controlled using the preinstalled software, Android OS, and a touch screen on the outer side of the device. The application used was SREATORK version 1.68. The initial 3D modeling was performed using Autodesk Fusion 360 software version 2.0.1772 and model slicing using NewCreatorK software version 1.57.80.

During printing, ink was dispensed from a 10 mL syringe onto a movable printing stage with a glass surface, resulting in the creation of 3D matrices, which were converted into an insoluble form by treating them with a 10% sodium bicarbonate solution for 10 minutes, followed by washing with distilled water. The samples were then placed in a lyophilizer chamber (Labconco Free

Zone Triad 2.5L, USA) and frozen for 4 hours at –30°C. The solvent was removed under reduced pressure and at a temperature of +5°C for 24 hours.

Cytotoxicity test

The DF1 human skin fibroblast cell line (Institute of Cytology, St. Petersburg, Russia) was used for the cytotoxicity study (MTT test). The cells were cultured in a CO_2 incubator at 37°C in a humidified atmosphere containing air and 5% CO_2 in DMEM/F12 nutrient medium (Dulbecco's Modified Eagle Medium; Gibco) containing 1% essential amino acids, 10% (vol/vol) thermally inactivated fetal bovine serum (FBS; HyClone, USA), 1% L-glutamine, 50 U/mL penicillin, and 50 μg/mL streptomycin.

For the experiment, 5 × 103 cells/100 μl/well were seeded in 96-well plates and cultured for 24 hours to allow attachment. Finally, the plates were incubated for an additional 4 hours to allow MTT conversion. After 24 hours, the medium was replaced with complete nutrient medium, and the wells were incubated with printed samples for 3 days. At the end of the incubation period, the medium was removed, and 50 μl/well of DMEM/F12 medium with tetrazolium dye (MTT) (0.1 mg/mL) was added. The cells were incubated in a CO_2 incubator at 37°C for 2 hours. Following supernatant removal, the formazan crystals formed by metabolically viable cells were dissolved in 50 μl/well of dimethyl sulfoxide and transferred to clean wells. The optical density at 570 nm was measured on a flatbed spectrophotometer using polynomial regression analysis in Microsoft Excel.

Scanning electron microscopy

The ultrastructure of the samples was studied using a Carl Zeiss Supra 55VP scanning electron microscope (Carl Zeiss, Germany). Images were obtained with the Microcapture 2.2 hardware and software system.

Antimicrobial activity test

The preliminary determination of the minimum inhibitory concentrations of povidone iodine against the tested *Staphylococcus aureus* strains, including MSSA (ATCC292123) and MRSA (ATCC43300), revealed its antimicrobial activity. The MIC was <1% for both cultures. Each sample was placed in a separate sterile tube containing 3 ml of Mueller–Hinton broth (MHB) and was repeated three times. Each sample was immersed in MHB. Furthermore, 50 μl of the bacterial suspension was immersed in MHB as a positive control. The negative control consisted of sterile MHB. The tubes were incubated for 24 hours at 37°C. After a day of incubation in the presence of samples, the optical density of MHB with bacteria was measured to obtain the antibacterial activity of the samples. Additionally, 200 μl from each tube was added to a 96-well flat-bottom plate, in four repeats. The optical density was measured at 600 nm using a Spectrostar Nano spectrophotometer.

The antibacterial activity of the samples was determined using the cup method. A bacterial suspension with an optical density of 0.5 McF (1 × 10 × 8 CFU/mL) was spread on the surface of Mueller–Hinton agar (MHA) in petri dishes using a cotton swab. Each test sample measuring 1.5 × 1.5 cm was introduced with sterile forceps onto the prepared bacterial lawn. The dishes were incubated at 37°C, and the antimicrobial effect of the samples was evaluated after 18 h. A positive result was indicated by a zone of growth retardation around the sample.

In vivo evaluation of coating properties

The experimental stage of the study was conducted at St. Petersburg State Pediatric Medical University with the participation of staff from the Department of Medical and Biological Research of the Research Center and Department of Thermal Injuries of the Kirov Military Medical Academy. Bioengineered structures in the form of a rounded mesh with a diameter of 50 mm, thickness of 3, and mesh size of 1 mm were used for the samples. The construct was 3D bioprinted using extrusion technology with biocornyls consisting of 4% chitosan hydrogel and 1% povidone iodine, along with 1 × 106 rat dermal fibroblasts per 1 mL. Following transplantation onto the wound, the construct was covered with Folliderm film, a 12-μm-thick lavsan tracking membrane with high-density, submicron-sized (≈0.4 μm) through-pores. This membrane facilitates water and gas exchange while protecting the wound surface from external infections.

A thermal contact burn of IIIB severity with an area of approximately 20 cm^2 was chosen as the inflicted injury. The severity of this injury, with the marked area and depth of the lesion, indicates the difference between the effects of different types of treatment on the wound process.

The study involved 19 male Wistar rats weighing 250 ± 8 g. The animals were randomly divided into three groups (Table 1).

Group 1 (experimental group) received treatment with the developed coating for the injury. Group 2 (comparative group) was treated with the traditional method for such injuries, which involves dressings with Levomekol ointment. Group 3 (control group) received no treatment after the burn injury.

Manipulations were performed on rats under injection anesthesia (intraperitoneal injection) using a mixture of Zoletil®100 (25 mg/kg) and Rometar (10 mg/kg) [21]. After anesthesia induction, the rats were fixed on the operating table. The site for application of the burn was previously dislodged and depilated. All biobjects were given a contact burn of IIIB degree with an area of 20 cm^2 using the equipment and model of burn injury described in the corresponding patent [22].

The burn application parameters were consistent across all groups. Group 3 rats served as the control

Table 1. Experimental animal groups
Таблица 1. Экспериментальные группы животных

Group No.	Group name	Number of rats
1	Necrectomy, fibroblast construct, Folliderm film	6
2	Necrectomy, treatment with bandages and Levomekol ointment	6
3	Control group without treatment	7

group and received no treatment. In groups 1 and 2, necrectomy was performed on the injured tissues up to the fascia, and the wound edges were sutured to the underlying muscle tissue 2 days after the injury. In group 1, a bioengineered construct was transplanted and subsequently treated with a Folliderm film after necrectomy. In group 2, an aseptic dressing was applied using Levosin ointment. In group 3, no necrectomy was performed. Dressings in the first two groups were performed every other day, with assessment of the wound area and weight of the animals [23]. The experiment lasted for 38 days, during which a punch biopsy (8 mm in diameter) was taken from the wound area for histomorphologic study every 7 days. The sample was taken to the entire depth of the skin up to the muscle layer.

Histomorphologic study

The histological sections, 5 μm in thickness, were evaluated using an automatic rotary microtome with the STS HM 355S slice transfer system (Thermo Fisher Scientific, USA). The sections were stained with hematoxylin and eosin using the standard technique. The evaluation focused on the number of newly formed capillaries and signs of inflammation.

Statistical analysis

The data were analyzed using GraphPad Prism 9.0 software (USA), and the results were evaluated through one-way ANOVA analysis of variance. $P < 0.05$ was considered statistically significant.

RESULTS

Wound coating structure

During the development of wound coatings, it was discovered that the optimal solution for our tasks is the use of bioengineered mesh structures that are 3 mm thick and have a mesh size of 2–3 mm. The structure was printed using the extrusion 3D bioprinting method from biocornyls, which consist of 4% chitosan hydrogel with the addition of 1% povidone iodine and 1 × 106 rat dermal fibroblasts per 1 ml. Moreover, samples without the addition of povidone iodine were prepared for comparison of antibacterial properties. Figure 1 presents the samples obtained.

In vitro research

Figure 2 displays the appearance of human skin fibroblasts cultured in the presence of nutrient medium after incubation with chitosan and chitosan with povidone iodine matrices.

The morphology of cells in control and experimental samples was similar. They had a characteristic elongated spindle shape, indicating no cytotoxic effect of the samples on the cells. However, a smaller number of cells was determined in the samples with medium after incubation with chitosan and povidone iodine compared to the control sample.

Cell viability was assessed using the MTT method, and the results are presented in Figure 3.

Data reveals that the addition of povidone to the coating composition has a minor effect on cell viability.

The fibroblast–matrix interaction was evaluated by fixing cells cultured on lyophilized matrix for 2 days with glutaric aldehyde and analyzing them using scanning electron microscopy. Figure 4 shows the ultrastructural picture of the investigated construct.

The lyophilized material composed of chitosan and povidone exhibits a porous structure with channels running parallel to each other. The pore diameter ranges from 20 to 90 microns. This ordered pore structure is a result of the self-organization of the chitosan solution during the freezing stage, followed by phase separation of the homogeneous polymer solution [24, 25]. Additionally, the cells are evenly distributed on the matrix surface, specifically on the pore walls. The ultrastructural analysis results suggest that there is good cell adhesion on the pore surface of chitosan-based matrices.

When evaluating antibacterial activity, the optical density values of incubation solutions with samples were significantly lower than the positive control without sample introduction (Fig. 5). The antibacterial properties were visible to the naked eye (Fig. 6).

Furthermore, samples with povidone iodine added were found to be statistically significantly more active than samples with chitosan alone (Fig. 7).

In a further comparison of antibacterial activity using the cup method, it was observed that samples impregnated with povidone iodine exhibited antibacterial properties, whereas samples consisting of chitosan alone did not show sufficient activity to inhibit the growth of Staphylococcus aureus on nutrient dense medium (Fig. 8).

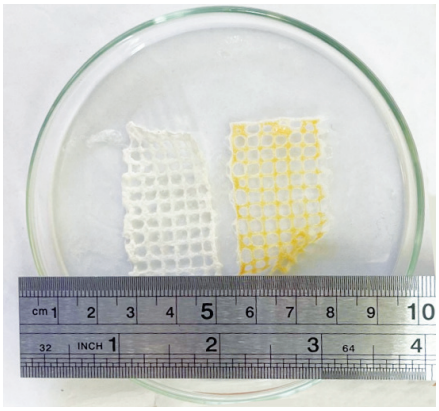


Fig. 1. Appearance of matrices based on chitosan (left) and chitosan with povidone-iodine (right)
Рис. 1. Внешний вид матриц на основе хитозана (слева) и хитозана с повидон-йодом (справа)

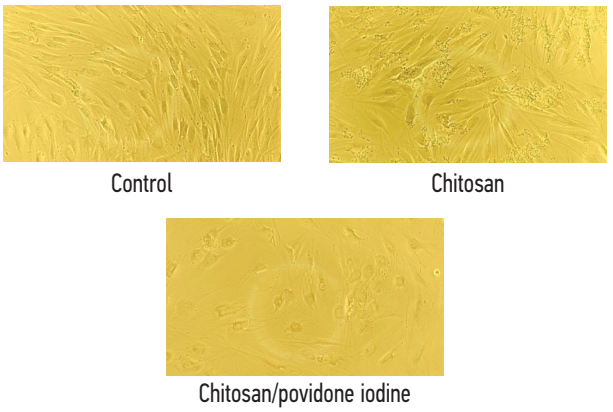


Fig. 2. Optical microscopy of human skin fibroblasts
Рис. 2. Оптическая микроскопия фибробластов кожи человека

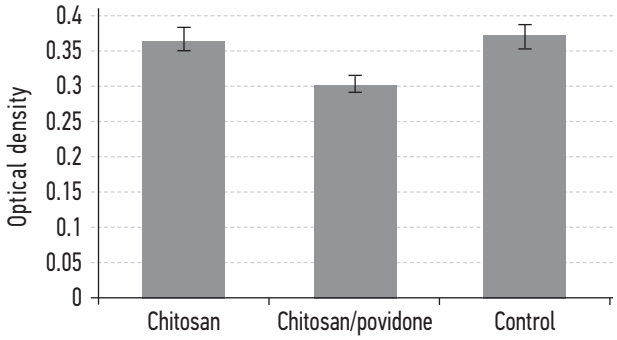
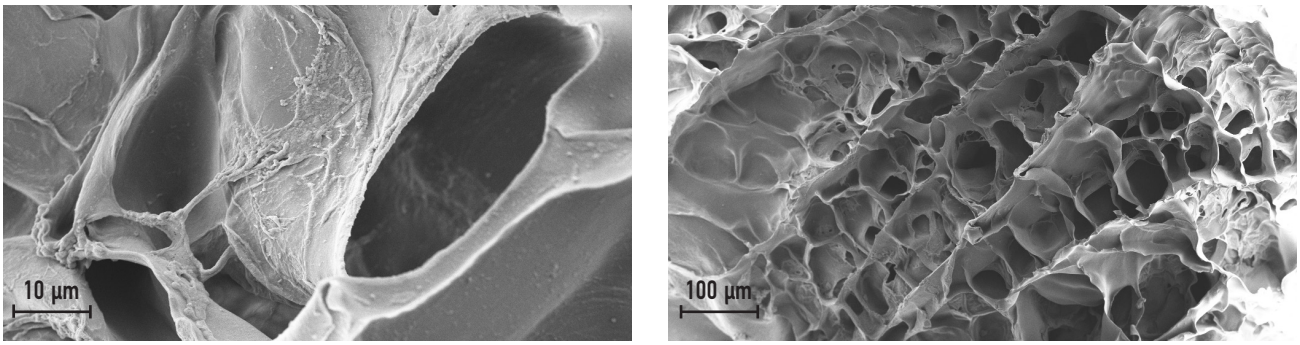


Fig. 3. MTT assay was performed on human skin fibroblasts
Рис. 3. МТТ тест фибробластов кожи человека



а *б*

Fig. 4. Scanning electron microscopy of human skin fibroblasts after 2 days of cultivation on a matrix of chitosan with povidone. Increased ×5000 (*а*); ×500 (*б*)
Рис. 4. Сканирующая электронная микроскопия фибробластов кожи человека после 2 сут культивирования на матрице из хитозана с повидоном. Ув. ×5000 (*а*); ×500 (*б*)

The results indicate that chitosan-based samples impregnated with povidone iodine exhibit antibacterial action against reference strains of *Staphylococcus aureus*. Significant differences in antibacterial action were observed between chitosan-based and povidone-iodine-impregnated samples. The introduction of povidone iodine as an additional component enhanced the antibacterial effect of chitosan-based samples. Methicillin-resistant strain susceptibility to the samples was a crucial finding. It appears that susceptibility to the tested samples is not dependent on the antibiotic profile of *S. aureus*. However, further studies are required on clinical isolates of this species and representatives of other taxonomic groups, including gram-negative pathogens.

In vivo research

In vivo tests of the bioengineered construct demonstrated a positive effect on the wound healing

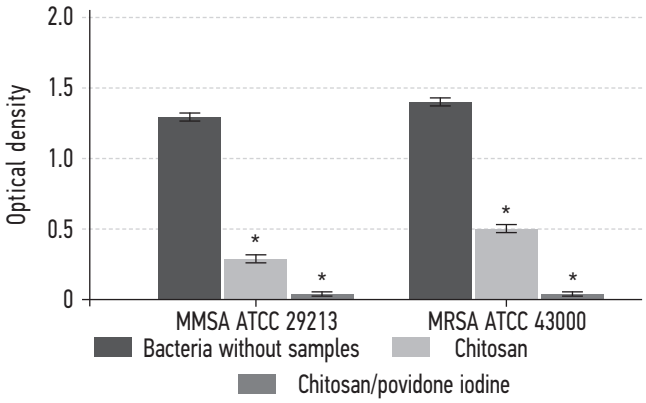


Fig. 5. Comparison of the antibacterial activity of samples in relation to control reference strains of *S. aureus*. Measurement at wavelength 600 nm. * — $p < 0.05$
Рис. 5. Сравнение антибактериальной активности образцов в отношении контроля эталонных штаммов *S. aureus*. Измерение при длине волны 600 нм. * — $p < 0,05$

Fig. 6. Test tubes after 24 hours of incubation of MRSA in a nutrient medium. QC—bacterial culture control (left); 8 — nutrient medium with MRSA and a sample of chitosan with vidon-iodine (right)
Рис. 6. Пробирки через сутки инкубации MRSA в питательной среде. КК — контроль культуры бактерий (слева); 8 — питательная среда с MRSA и образцом из хитозана с повидон-йодом (справа)

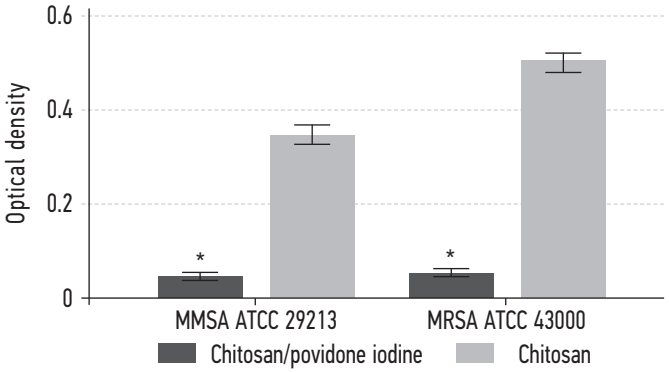


Fig. 7. Comparison of the antibacterial activity of samples from chitosan and with the addition of povidone-iodine against reference strains of *S. aureus*. Measurement at a wavelength of 600 nm. * — $p < 0.05$
Рис. 7. Сравнение антибактериальной активности образцов из хитозана и с добавлением повидон-йода в отношении эталонных штаммов *S. aureus*.Измерение при длине волны 600 нм. * — $p < 0,05$

process (Fig. 9). This effect was characterized by a higher rate of epithelization and a significantly lower incidence of infectious complications compared to other experimental groups.

In the histologic examination, the experimental group showed superior results compared to the control and comparison groups regarding the quality of granulation tissue formed, number of newly formed capillaries, and severity of local inflammation (Fig. 10).

Detailed characteristics of each group

In Group 1, a thin elastic scab was formed 3 days after necrectomy and transplantation of the bioengineered construct with Folliderm film. The scab was removed 17 days after the injury, and no suppuration was observed in this group of animals. After the scab was rejected, it was found that it did not impede epithelization, and the cleared wound reduced in size at an average rate of 2.7% ± 0.2% per day. The wound bed consisted of fresh granulation tissue, with the edges fully closed by experiment day 38, resulting in a thin, normotrophic scar formation. The biopsy of granulation tissue from the wound bed on experiment day 21 showed an average of 29 ± 4 capillaries per visual field.

In group 2, a scab formed 3 days after necrectomy. However, it was rougher than the scab formed in group 1. Scab rejection occurred on day 24 from the moment of injury, and suppuration of the scab was observed in 80% of cases. The wound bed was covered with granulation tissue with fibrin plaque. The latter was much coarser and thicker in comparison with the granulation tissue in animals in group 1. The wound contracted at an average rate of 1.8% ± 0.3% per day. On day 38, the wound area was 3.6 ± 0.8 cm^2. The biopsy of granulation tissue from the wound bed on experiment day 21 showed an average of 16 ± 2 capillaries per visual field.

In group 3, scab rejection occurred 33 days after the injury. The scab was rough, and suppuration was observed in 100% of cases. After scab detachment, the wound was represented by granulation tissue with signs

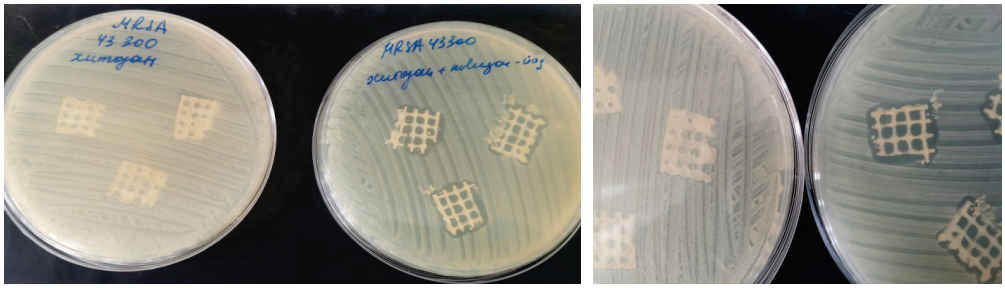


Fig. 8. Zone of MRSA growth inhibition around povidone-iodine specimens
Рис. 8. Зона подавления роста MRSA вокруг образцов с повидон-йодом

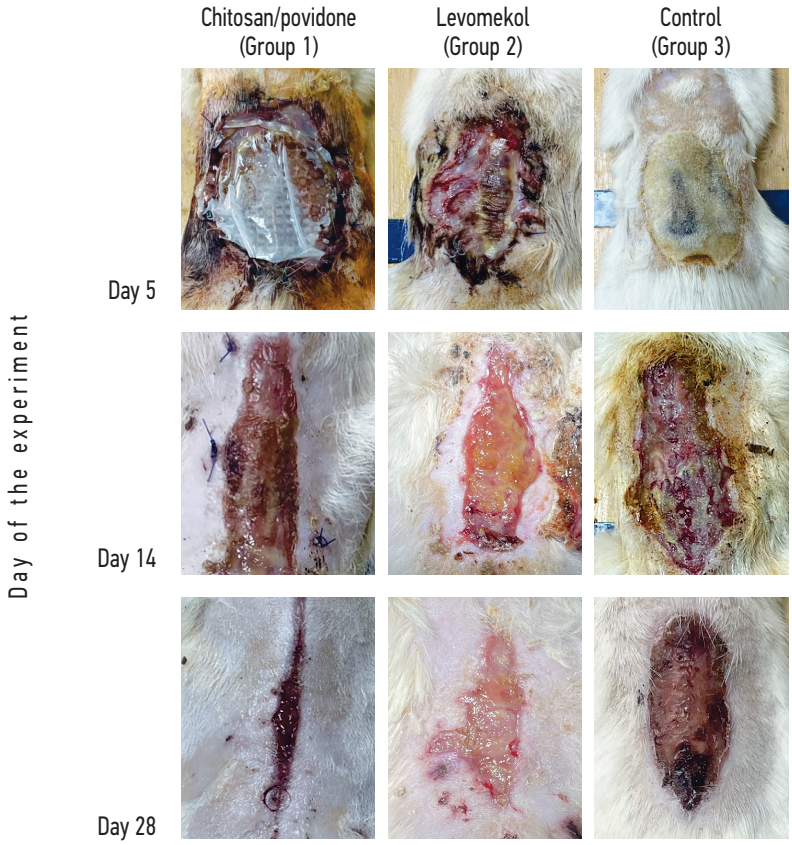


Fig. 9. Dynamics of the wound process in the study groups
Рис. 9. Динамика раневого процесса в исследуемых группах

of fibrosis up to 3 mm thick. The average rate of wound contraction was 0.3% ± 0.04%/day. The biopsy of granulation tissue from the wound bed on experiment day 21 revealed an average of 11 ± 2 capillaries per visual field.

CONCLUSIONS

The use of chitosan in the form of a 4% hydrogel to create a mesh structure resulted in a final product with high biocompatibility, atraumaticity, elasticity, and adhesion to the wound.

Adding povidone iodine to the polymer composition at a concentration of 1% enabled the bioengineered construct to exhibit high antimicrobial activity while minimally impacting the viability of the included cells. Moreover, the inclusion of povidone iodine in the composition resulted in a reduction of the neutrophil–macrophage cellular response in the wound area. This was evidenced by a less severe inflammatory reaction during scab rejection and granulation tissue maturation.

The use of 3D printing technology in the manufacturing of bioengineered constructs ensures the maintenance of aseptic conditions and required humidity, pH, and temperature. This technology guarantees the maintenance of aseptic conditions during the creation of the construct and of the required humidity, pH, and temperature.

The approach used to print the bioengineered construct ensured the preservation of the included cells and their continued high viability.

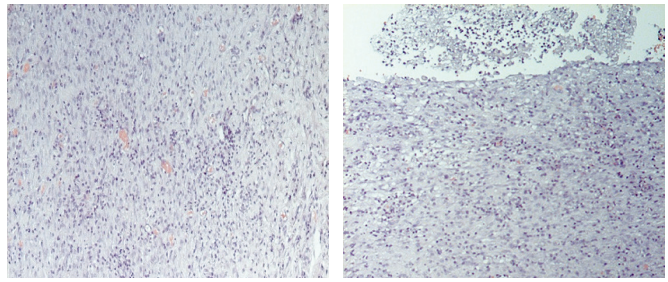


Fig. 10. Morphological preparations from the wound area, taken on the 21st day of the experiment (hematoxylin-eosin staining, ×100 magnification)
Рис. 10. Морфологические препараты из области ран, отобранные на 21-е сут эксперимента (окраска гематоксилин-эозин, ув. ×100)

ADDITIONAL INFORMATION

Authors' contributions. All authors contributed to the research and preparation of the article and read and approved the final version before publication. K.P. Golovko, concept and design development; V.E. Yudin, organization of work on the bioengineered construct; D.V. Ovchinnikov, verification of critical intellectual content and final approval of the manuscript for publication; I.A. Barsuk, execution of the experimental part of the work with animals; E.M. Ivankova, performing scanning electron microscopy; V.N. Alexandrov, literature review; Y.A. Naschekina, culture work and conducting cytotoxicity study; E.M. Gordina and S.A. Bozhkova, developing methodology and conducting study on antimicrobial activity of the coating.

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Conflict of interest. The authors declare that there are no obvious and potential conflicts of interest related to the publication of this article.

Ethical review. The study was approved by the local ethical committee of the Kirov Military Medical Academy of the Ministry of Defense of the Russian Federation (extract from Protocol No. 259 dated January 25, 2022).

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