Iranian Journal of Medical Sciences

The effect of organic scaffolds on the healing of polymicrobial wounds

The Use of Organic Health-Promoting Scaffolds "CM" in the Treatment of Infected Polymicrobial Skin Wounds

Affiliation: Operative surgery and topographic anatomy department. North-Western I. I. Mechnikov State Medical Academy, St. Petersburg, 195067, Russia.

****Running Title** (short form of the main title presented on the top of pages): The Use of Organic Health-Promoting Scaffolds "CM" in the Treatment of Infected Polymicrobial Skin Wounds

****Name of the institution where the work was done (If it is a Research study):**
North-Western I. I. Mechnikov State Medical Academy, St. Petersburg, 195067, Russia.

**** Conflict of interests:** We certify that we have participated sufficiently in the intellectual content, conception and design of this work or the analysis and interpretation of the data (when applicable), as well as the writing of the manuscript, to take public responsibility for it and have agreed to have our name listed as a contributor. We believe the manuscript represents valid work after revision. Neither this manuscript nor one with substantially similar content under our authorship has been published or is being considered for publication elsewhere, except as described in the covering letter. We certify that all the data collected during the study is presented in this manuscript and no data from the study has been or will be published separately. We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.
We hereby transfer, assigns, or otherwise conveys all copyright ownership, including any and all rights incidental thereto, exclusively to the Journal, in the event that such work is published by the Journal.
We give the rights to the corresponding author to make necessary changes as per the request of the journal, do the rest of the correspondence on our behalf and he will act as the guarantor for the manuscript on our behalf.

**** Funding:** I am pleased of behalf on esteemed co-authours to inform that we received no financial support for the research, authorship, and/or publication of article “The Use of Organic Health- Promoting Scaffolds in the Treatment of Infected Polymicrobial Skin Wounds”

**** Acknowledgement:** This manuscript has not been published and is not under consideration for publication elsewhere. We have no conflicts of interest to disclose.

** **Ethics committee approval code:** Study was approved by Local Ethical Committee of Mechnikov University (No 04-19 from 06.03.2019)

** **Authors' contribution:** We certify that we have participated sufficiently in the intellectual content, conception and design of this work or the analysis and interpretation of the data (when applicable), as well as the writing of the manuscript, to take public responsibility for it and have agreed to have our name listed as a contributor.

Introduction: The search for new methods to prevent and treat complicated skin wounds is one of the current issues of public health care. **Objective:** To evaluate the effects of an organic biodegradable scaffold on the healing rate of a polymicrobial skin wound in an experiment. **Materials and Methods:** The effect of an organic biodegradable scaffold was evaluated in 30 white male Wistar rats. The animals were divided into 3 groups such as the Control (spontaneous healing), Group 1 (scaffold 1), and Group 2 (scaffold 2). A model of fecal contamination was used to reproduce a purulent skin wound. A wound exudate was sampled to assess a microbial content in 0, 48, 96 and 168 hours from the beginning of observation. Data were analyzed using the Microsoft Office Excel 2019, Jamovi 1.0.1.9 and IBM SPSS Statistics 26. **Results.** By Day 19, a wound defect area was reduced by 98.4±1.34% ($p < 0.05$) from baseline in Group 2 animals, that made it possible to consider this wound healed. The process of complete wound healing in the Control and Group 1 lasted for 21-22 days. At the same time, by Day 11 the decrease of the wound defect area was 47.95 ± 1.78% ($p < 0.05$) and 25.2 ± 3.67 ($p < 0.05$) in Group 1 and the Control, respectively. Based on microscopy, wounds in all cases healed by secondary intention with an evident exudative inflammation. The morphometry demonstrated leukocyte infiltration of the exudate to be significantly lower in Group 2 than in Group 1 and the controls. A bacterial culture test of the wound exudate showed a polymicrobial pattern. In 168 hours post-injury P. aeruginosa and K. pneumoniae continued to grow in the control animals. **Conclusion:** The use of health-promoting scaffolds in the treatment of infected skin wounds in an experiment promotes wound healing.

Key words: infected wound, herbal medicines, modern dressing, treatment

Introduction

The search for new methods to prevent and treat complicated skin wounds is one of the current issues of public health care. A growth of the elderly population, antibiotic resistance, a rising incidence of diabetes and obesity worldwide have resulted in an increased number of patients with persistent (chronic) skin wounds [1, 2, 3, 4].

In Russia, more than 2.5 million people suffer from chronic, persistent wounds of the lower extremities [5]. There is a similar trend in many countries [6, 7]. In the USA, about 6.5 million people [8] receive treatment for chronic skin wounds, at an annual cost of $25 billion [9, 10, 11]. At present, the management of these patients is a heavy economic burden and accounts for 3-5.5% of the total health care budget costs in different countries [1,8].

Chronic skin wounds are heterogeneous pathologies, including complications of vascular diseases (venous and arterial ulcers) [12], diabetes mellitus (trophic foot ulcers), and bedsores [13, 14]. Chronic wounds of the lower extremities are a challenge for both

patients and their families; they are accompanied by infection, loss of limb function [15, 16], financial costs, and can cause sepsis and amputation. For example, the disability rate of patients with venous leg ulcers ranges from 10 to 30%, and, according to some authors, reaches 50%. In particular, a chronic ulcer develops in almost 10% of people during their lifetime, which is the cause of death [5]. Annually, 5% of diabetic patients will develop a diabetic foot, 1% of whom will require limb amputation. The five-year survival rate after one major amputation of the lower extremity is about 50% [11].

It is clinically important to prevent most of the complications by taking preventive measures that reduce the risk of chronic ulcer, as well as by proper management of an ulcer that has already developed. Given the high social and economic significance of the issue, the development of new methods of treatment and care of skin wounds is a priority task of modern public healthcare.

The use of wound dressings is an integral part of management of both acute and chronic wounds of the skin and soft tissues. To protect a wound against damage wounds dressings such as gauze, pile, cotton wool, tulle, etc. are traditionally used. However, the use of these materials is associated with a lot of adverse events such as wound drying, which worsens the healing process, secondary damage to the granulation tissue and epithelium when changing the dressing, and a high risk of wound infection [17]. Recently, attempts have been made to create wound dressings promoting wound healing [18]. According to the current knowledge of wound healing, these dressings should have specific qualities. When in contact with a wound surface, a dressing should provide a moist environment in a wound, promote elimination of excessive exudate, and maintain optimal temperature [19]. It should be biocompatible, water and oxygen semi-permeable, hypoallergenic, but cause no immune reactions, and promote tissue renewal processes. In addition, a dressing should not cause injury when being removed, and should also be cost effective [20]. Modern dressings have been developed Based on various synthetic and natural materials, in the form of semi-impermeable foams, films, hydrogels, fibers, colloids, alginates, and so on, which accelerate the process of wound healing [21, 22]. However, the problem of wound infection treatment is still not resolved.

To date, the presence of an infected wound, accompanied by a systemic inflammatory response syndrome, is an indication for systemic antibiotic therapy. At the same time, local infection or a so-called “critical colonization”, aggravating healing of a chronic wound, requires local antimicrobial therapy. The use of antiseptics is limited by their cytotoxic effect on body tissues, while local antibiotics associated with a high risk of microbial resistance [23].

The use of natural components with nonspecific antibacterial properties that do not cause microbial resistance [24], or have a local toxic effect on their own tissues [25], is promising in the treatment of skin wounds. Organic health-promoting scaffolds “CM”, composed of herbal extracts (St. John's wort, sage, yarrow), minerals, vitamins, and a solution of gentamicin are an example of such wound dressings. The scaffold is based on a natural biopolymer - gelatin (Marketing authorization No. FSR 2010/07797 dated on May 21, 2010, issued by the Federal Service for Supervision in Healthcare). Their use after dental implantation has demonstrated good results in terms of the duration of postoperative wound healing, as well as the recovery of oral cavity microflora [26, 27, 28]. Based on the data obtained, the scaffold was modified to be used in surgical treatment of infected skin wounds.

The research was aimed to evaluate effects of the organic biodegradable scaffold "CM" on the healing rate of an infected polymicrobial skin wound in the experiment.

Materials and Methods

30 white male Wistar rats aged 6 months and weighing 250±17.5 g were used in the experiment. The rats were kept in individual vivarium cages with water and food ad libitum. Animals were handled in accordance with the European Convention for Protection of Verterbrate Animals used in Experimental and other Studies, 1986.
All manipulations with animals were performed under adequate anesthesia. A mixture of Zoletil (230 µl, 100 mg/kg, Virbac) and Rometar (20 µl, 20 mg/ml, Bioveta) was injected intraperitoneally. A surgical field was prepared as follows: fur in the interscapular region was cut and shaved, the skin was treated three times with an antiseptic solution for 5 minutes and the field was delimited with sterile wipes. Additionally, infiltrative anesthesia was performed with 1.0 ml of a 0.5% lidocaine hydrochloride solution. Then the skin, subcutaneous adipose tissue and superficial fascia of the skin were excised with a scalpel in two semi-oval incisions. A wound bed was formed by the superficial muscle. A total wound area was 1.76 cm^2. A 5% solution of autofeces was injected into the formed wound at a volume of 0.5 ml per 100 g. of body weight. To prevent additional injury and contamination by surrounding microorganisms, the wound was covered with a dense multilayer fabric. In 48 hours post inoculation, the animals were divided into three groups such as the control group (n=10, untreated wound healing), Group 1 (n=10, wound healing with the use an organic biodegradable scaffold “CM” 1) and Group 2 (n=10, an organic biodegradable scaffold “CM” 2 used). A scaffold size was comparable to the skin wound area. Experiment results were recorded in all groups since grouping the animals (post infection day 2). Scaffolds were applied once, and were washed off with isotonic saline when examining the wound progression, by day 3. Subsequent wound healing occurred under the same conditions, without the use of drugs.
The wound healing rate was assessed on days 0, 3, 7, 11, 15, 19, and 21 using digital images. The camera was set at the same distance from the skin surface. The lens was placed perpendicular to the wound. A metric graduated ruler was placed next to the wound to assess its size. A metric scale was used as a standard. Wound defect areas were determined on each image using the ImageJ software (U.S. National Institutes of Health, Bethesda, MD, USA). The wound surface area change was calculated with the formula:

$$\Delta S = \frac{(S_0 - S_n)}{S_0} * 100\%,$$

where S_0 – initial wound area, S_n – wound area on day n.

A histological examination was performed on days 1 and 7 after the start of the experiment. Animals were sacrificed by Zoletil overdose (100 mg/kg, Virbac). Then, tissues were excised for a subsequent histological examination. The tissues including the experimental wound area and fragments of scaffolds implanted were fixed in a 10% buffered formalin solution for 24 hours. Histological preparation, embedding, and microtomy at a slice thickness of 4 µm were performed according to the standard technique. Tissue specimens were stained with hematoxylin and eosin. The quantification of inflammatory infiltration involved the morphometry of digital histotopograms (high-resolution images) of longitudinal sections of the tissue samples obtained. The entire section area was recorded using a high-performance scanner Aperio AT2 (Leica Biosystems, Germany) at ×20 magnification. Out of 30 visual fields (VFs) with an area of 1 mm^2 randomly selected on each histotopogram 10 VFs were located above the skin muscle, 10 VFs being at the level of the muscle, and 10 Fs located under the muscle, respectively. Then, segmented WBCs were counted on the selected VFs.

A microbial examination of the wound surface was performed in 0, 48, 96 and 168 hours after beginning the experiment. A sterile 1.0 × 1.0 cm gauze was placed on the wound surface with a sterile instrument, and slightly pressed to the wound bed. The gauze soaked with a wound exudate was placed in a test tube containing 1.0 ml of sterile saline. A washout diluted as 1:100 and 1:1,000 (0.1 ml each) was inoculated on a plain dense medium BrainHeartInfusionAgar (Himedia). Culture plates were incubated at 37°C for 48 hours, then grown microorganism colonies were counted. A microbial content was estimated as a number of colony-forming units in 1.0 ml of the washout - CFU/ml taking into account the dilutions and the washout volume. Colonies of different morphological types were examined in Gram-stained smears with light microscopy.

Statistical processing of the results obtained was performed on a personal computer using the software such as Microsoft Office Excel 2019, Jamovi 1.0.1.9, and IBM SPSS Statistics 26. A comprehensive multivariate ANOVA with repeated measurements was performed to analyze quantitative characteristics. *F*- and *t*-tests were performed. The Post Hoc Tests procedure with the Bonferroni correction was applied for paired comparisons. The 0.95 probability (95% confidence interval or $p < 0.05$) was taken as the confidence level.

Results

Purulent wounds formed in 48 hours (the initial point of data recording) and had classic signs of inflammation. Wound edges were necrotic, slightly hyperemic, and pale. The wound bed was moist, yellow-green and burgundy-bluish to black with areas of necrosis and fibrin overlay. There was a purulent exudate in a moderate amount from 0.5 to 1.0 ml, yellow-green, hemorrhagic, turbid, with malodor. On experiment day 3 wounds in the control animals were characterized by purulent-necrotic inflammation with a destruction within the skin superficial muscle. In the control group purulent exudate persisted up to 11-13 days. On day 3 Group 1 animals had wounds characterized by a pronounced exudative inflammation, with scaffold remains on its surface. By day 15 a dense secondary slough was formed in Groups 1 animals and the controls, with a zone of epithelialization when sloughing (by day 21 in the control group and by day 19 in Group 1). It should be noted that the wound process occurred more favorably, with less pronounced symptoms of purulent inflammation in Group 1, as compared to the controls. On day 3 of the experiment, a whitish scaffold-based film was formed on the wound defect surface in Group 2. No secondary slough formed in animals of this Group, the wound rearrangement and epithelialization occurred by day 19 (**Fig. 1**).

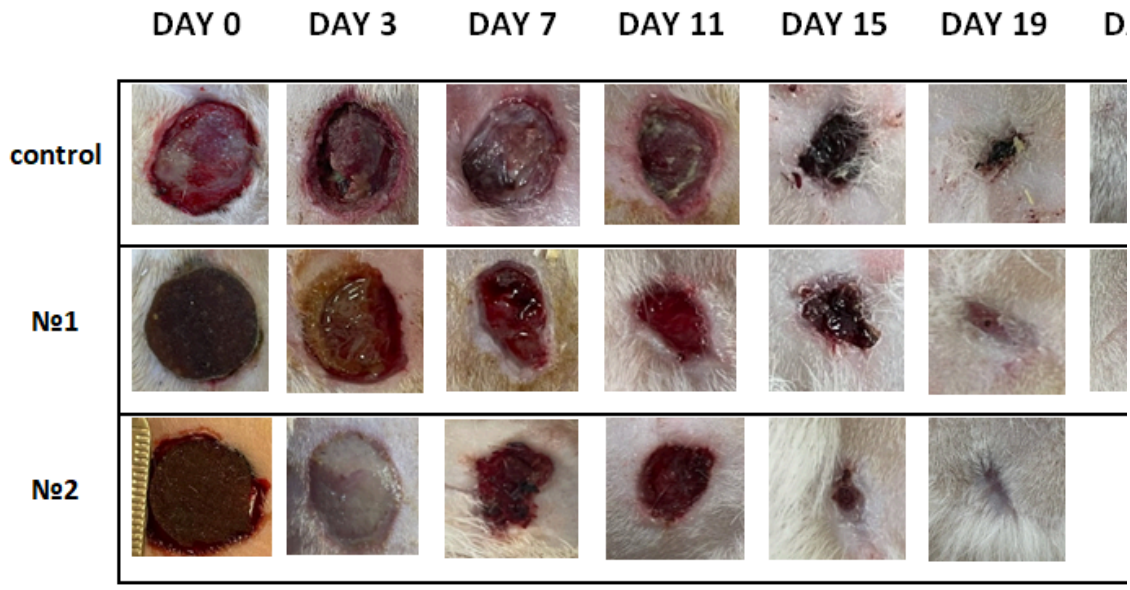


Fig. 1. Skin wound healing in an experiment.

A decrease of the wound surface area was generally higher in Group 2 than in Group 1 and the controls. For example, by day 19, it was 98.4±1.34% in Group 2 which is 21.13±0.9 % and 6.25±0.31% (both $p<0.05$) more than in the controls and Group 1, respectively (**Table 1**).

Table 1. Changes in the skin wound area.

	Wound surface area (% decrease from baseline)		
Observation Day	**Control group**	**Group 1**	**Group 2**
3	5.22 ±0.24*	19.6 ±4.6*	26.6 ±3.41*
7	15.22 ±1.12*	33.06 ±2.4*	48.63 ±2.08*
11	25.2 ±3.67*	47.95 ±1.78*	57.95 ±3.66*
15	56.8 ±12.42	63.4 ±5.1*	82.15 ±6,71*
19	77.27 ±2.24*	92.15 ±1.65*	98.4±1.34*
21	95.64 ±3.88*	97.9 ±1.05*	

*Note: *p <0.05*

The wound defect area was lower in Group 1 when compared with Group 1 and the controls. On observation day 11 it was 25.2 ± 3.67% in the control group, that is 1.95 times less than in Group 1 (47.95 ±1.78, $p<0.05$).

A histological examination of tissues, including the experimental wound, skin defect edges, the skin muscle and subcutaneous adipose tissue, demonstrated typical stages of necrosis and suppurative exudative inflammation. However, during the first week its severity varied in animals of different groups. A day later, changes in the tissues were typical and consisted of interstitial edema, hemorrhagic impregnation as a result of mechanical injury. Inflammation was the main pathomorphological phenomenon, with paravulnar tissues excessively infiltrated with white blood cells such as neutrophils, macrophages, lymphocytes and mast cells and all tissues swollen. The skin muscle structure nearby the defect was mainly maintained, however, there was fraying of fibers and swelling. Inflammatory infiltration appeared to be less intense in Groups 1 and 2 than in the controls, as confirmed by the morphometry ($p<0.05$, Table 2). In Group 2 animals

there was an early formed torus demarcationis, separating a zone of irreversible damage (Fig. 2). At the wound bed there was tissue detritus, densely infiltrated with polymorphonuclear WBCs. The superficial dorsal muscles forming the defect bed were swollen within the wound bed, the endomysium diffusely infiltrated with neutrophilic granulocytes. There was a protrusion of swollen subcutaneous adipose tissue into the wound in animals of all groups.

On day 7 of the experiment, the wound area in the control group was filled with a mass of granulation tissue penetrated by full-blooded blood vessels. There was a slough on the superficial wound part located between its edges, preventing the effective growth of the stratified epithelium regenerating from the edges. In Group 1 there were signs of the active granulation tissue formation. Its stroma consisted of thin chaotically arranged bundles of collagen fibers produced by connective tissue cells such as poorly differentiated fibroblasts. In addition, histiocytes and endotheliocytes were detected as part of thin-walled newly formed blood vessels. Also, there were single mast cells and lymphocytes. In Group 2 animals the granulation tissue was formed in place of the superficial muscle. There were single degrading muscle fibers, as well as newly formed blood vessels in its structure., Intact muscle fibers were visualized closer to the wound edges with minimal leukocyte infiltration and the swollen stroma. An epithelial layer actively grew from the wound edges.

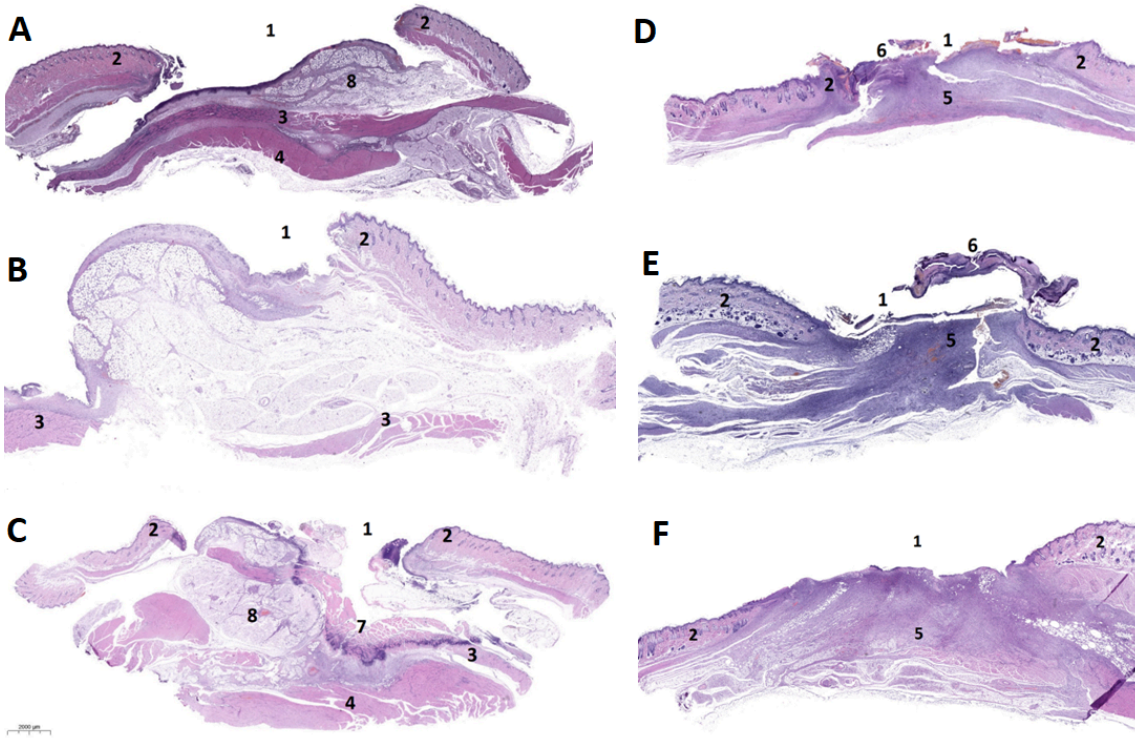


Figure 2. Histotopograms of wound skin defects. A – 1 day, control group; B - 1 day, group №1; C - 1 day, group №2; D - 7 day, control group; E - 7 day, group №1; F - 7 day, group №2. 1 – defect area; 2 - wound edges; 3, 4 - superficial and deep spinal muscles; 5 - granulation tissue; 6 - slough; 7 - torus demarcationis; 8 - adipose tissue. Staining: hematoxylin and eosin. Scale bar - 2000 µm.

The morphometry results (**Table 2**) demonstrated leukocyte infiltration above the muscle to be more intense in the controls than in Groups 1 and 2 ($p = 0.004$) on day 1. On day 7

the leukocyte infiltration index was higher in all layers of the wound of Group 1 animals than in the control and Group 2 ones ($p<0.001$).

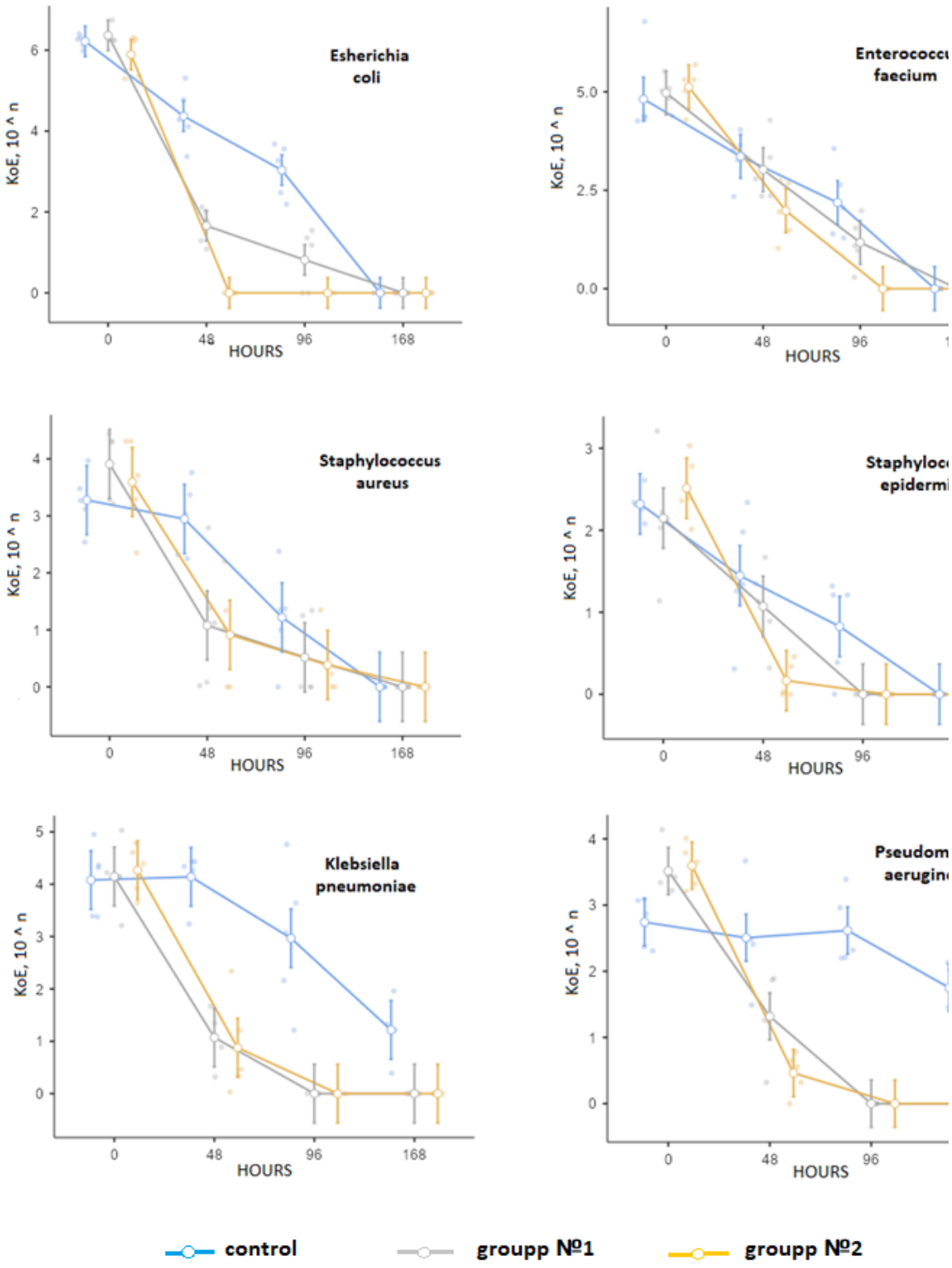
Table 2. Leukocyte infiltration of the wound at different time points of observation.

	Day 1			**Day 7**		
Group	**Infiltration above the muscle**	**Infiltration of the muscle**	**Infiltration beneath the muscle**	**Infiltration above the muscle**	**Infiltration of the muscle**	**Infiltration beneath the muscle**
Control	**405±78.4***	269.3±114.7	279.4±63.8	550±198.3	501±51.5	490±131.9
Group 1	322.2±109.2	273±110.5	167.8±25.3	**623±197.9***	**567±289.21***	**615±77.6***
Group 2	122.7±28.2	135.5±97.7	295.5±104.1	405±52.7	218±74.65	186±60.25

Note: Skin muscle was examined to evaluate muscle tissue infiltration.

A bacteriologic culture test of the wound exudate at the initial stage of the experiment demonstrated a polymicrobial content. Staphylococcus epidermidis, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus faecalis, and Escherichia coli were grown. A rate of bacterial contamination was lowest in Group 2. For example, by 48 hours from the start of recording the experiment data, E.Coli was not detected in the wound treated with the use of the scaffold "CM" 2 ($p<0.05$), S. epidermidis and K.pneumoniae counts were minimal ($p<0.05$). In 96 hours, no E. faecalis was detected ($p<0.05$). Bacterial contamination was also lower in Group 1, when compared with the controls; however, there was no statistically significant difference in E. faecalis between the two groups ($p = 0.13$). In 168 hours, no bacterial growth was detected in the groups of animals treated with the scaffolds. In the control group, of K.pneumoniae and P.aeruginosa persisted during the abovementioned period (**Table 3**).

Table 3. Bacterial contamination of the wound in dynamics.



Discussion

Properties of the organic biodegradable scaffold “CM” in two modifications (1 and 2, respectively) were studied in a model of an infected skin wound. The scaffold is gelatin-based, which is a natural polypeptide, produced in collagen hydrolysis. Gelatin-based dressings have been widely used in the management of chronic skin wounds. Note that gelatin is biocompatible, easily biodegradable, non-immunogenic, as

well as easily produced, available and cost-effective. Porous gelatin composites absorb wound exudate and retain moisture, thereby facilitating the process of wound healing. A gelatin base acts as a matrix for cell migration and provides mechanical and structural support for the growth of new tissue [17].

The disadvantage of using gelatin-based dressings in the treatment of chronic skin wounds is their low antimicrobial activity. The integration of antibacterial agents into a gelatin matrix, whose positive effect has been demonstrated in a number of clinical trials can possibly overcome this disadvantage. Despite this, developing, or existent antibiotic resistance can neutralize the effect of an antibiotic used.

To date, the integration of non-specific antimicrobial medicinal components with diverse action into a wound dressing is considered as an important tool in the management of persistent wound infection [29, 30, 31]. Taking it into account, a combination of antimicrobial agents was incorporated into the scaffolds "CM", including both natural components such as herbal extracts of St. John's wort, sage, yarrow, and gentamicin, a broad-spectrum biosynthetic antibiotic.

St. John's wort extract *in vitro* is reported to have a pronounced antimicrobial effect [32, 33] against gram-positive bacteria [34, 35]. Some authors demonstrated a yarrow extract to possess good antioxidant properties, evident anti-fungal and bactericidal effects against B. cereus.

A number of researchers have described the antibacterial activity of a yarrow extract against S. aureus [37, 38]. Team of authors provided the latest data on sage extract properties [44]. Essential oil and an alcohol solution of sage extract exhibit strong antibacterial and bacterial growth-inhibitory activity against both Gram(+) and Gram (-) bacteria. Bacillus cereus, Bacillus megaterium, Bacillus subtilis, E. faecalis, Listeria monocytogenes and Staphylococcus epidermidis have high sensitivity to different herbal agents including a sage extract. Sage essential oil has a significant growth-inhibitory effect on the of E. coli, Klebsiella oxytoca, K. pneumonia, Pseudomonas morgani, Salmonella enteritidis, and so on. In addition, a sage extract has evident antioxidant, antinociceptive and anti-inflammatory properties. Moreover, triterpenoids, oleanolic and ursolic acids [42, 43] have a growth-inhibitory activity against multidrug-resistant bacteria such as vancomycin-resistant enterococci, penicillin-resistant Streptococcus pneumonia, and methicillin-resistant S. aureus [44, 45, 46].

We studied properties of scaffolds “CM” 1 and 2 in a model of a polymicrobial infected wound. The wound microbial content was demonstrated in the experiment to be significantly lower in groups of animals treated with the scaffolds as compared to the controls. At the same time, there was a highest decrease in the microbial content in Groups 1 and 2 within the first 48 hours from the beginning of the experiment when scaffolds were in direct contact with wounds. In 168 hours since the data recording, K.pneumoniae and P.aeruginosa persisted in the control group, while no bacterial growth detected in cultures from Group 1 and 2 wound samples (**Table 3)**. In general, the antibacterial effect of scaffolds was in moderate and profound in Groups 1 and 2, respectively.

That a physical examination of wounds in animals treated with scaffolds showed a more favorable course of the wound process was to be expected. For example, by days 3-5 there were no signs of active inflammation in Groups 1 and 2, with the exudate being serous and hemorrhagic. On day 7 the wound defect area decreased by 48.63 ± 2.08% of the baseline in Group 2, with the value being 33.06 ± 2.4% in Group 1. The wound process demonstrated signs of a pronounced exudative inflammation up to 11-13 days in the control group. Wound edges were swollen and hyperemic with the presence of

purulent exudate. By day 7 the wound area decreased by 15.22 ± 1.12% of the baseline. The duration of complete layer-by-layer wound healing was 21-22 days in the control group, and 19 days in the experimental groups.

Leukocyte infiltration was assessed in three areas such as above the muscle, in the muscle (which is the wound bed) and beneath the muscle based on full-thickness wound histotopograms. By day 7 it showed a two-fold decrease in wounds treated with the use of scaffold “CM” 2 as compared to the control group. The use of the scaffold “CM” 1 resulted in increased leukocyte infiltration of the wound by day 7. Its value was higher than in the controls and Group 2, that gives evidence to a less intense inflammatory process and the end of exudating when passing to the third phase of an inflammatory response.

Scaffolds “CM” have demonstrated good performance in the treatment of infected skin wounds and can potentially be used as an all-purpose dressing for persistent, chronic infected skin wounds. The data obtained should be confirmed in non-clinical studies.

Conclusions

1. Scaffolds “CM” promote more rapid wound healing in the model of an infected skin wound. The duration of complete wound healing was 19 days in the experimental group, and 21-22 days in the control one.
2. Scaffolds “CM” *in vivo* have a good antibacterial effect, against P. aeruginosa as well.
3. Scaffolds “CM” can be potentially used as a tool to manage a wound infection in persistent, chronic skin wounds, as well as in those with a possibly high risk of infection.

References

[1] Sen CK. Human Wounds and Its Burden: An Updated Compendium of Estimates. Adv Wound Care (New Rochelle). 2019;8(2):39-48. doi:10.1089/wound.2019.0946

[2] Nussbaum SR, Carter MJ, Fife CE, DaVanzo J, Haught R, An economic evaluation of the impact, cost, and medicare policy implications of chronic nonhealing wounds. Value in Health. 2018; 21(1):27-32.

[3] Raziyeva K, Kim Y, Zharkinbekov Z, Kassymbek K, Jimi S. Immunology of acute and chronic wound healing. Biomolecules. 2021; 11(5): 700.

[4] Maheswary T, Nurul AA, Fauzi MB. The Insights of Microbes’ Roles in Wound Healing: A Comprehensive Review. Pharmaceutics. 2021; 13(7):981

[5] Potekaev NN, Frigo NV, Michenko AV, Lvov AN, Panteleev AA, Kitaeva NV. Chronic indolent ulcers and wounds of the skin and subcutaneous tissue. Russian Journal of Clinical Dermatology and Venereology=Klinicheskaya dematologiya i venerologiya. 2018;17(6):7-12. (In Russ.). https://doi.org/10.17116/klinderma2018170617)

[6] Stone RC., Stojadinovic O, Rosa AM, Ramirez HA, Badiavas E. A bioengineered living cell construct activates an acute wound healing response in venous leg ulcers. Science translational medicine.2017; 9(371), eaaf8611.

[7] Theocharidis G, Thomas BE., Sarkar D, Mumme HL., Pilcher WJ. Single cell transcriptomic landscape of diabetic foot ulcers. Nature communications. 2022; 13(1): 1-17.

[8] Lindholm C, Searle R. Wound management for the 21st century: combining effectiveness and efficiency. Int Wound J. 2016;13 Suppl 2(Suppl 2):5-15. doi:10.1111/iwj.12623

[9] Enste P, Kucharski AB. Social, Legal and Economic Implications for the Implementation of an Intelligent Wound Plaster in Outpatient Care. Frontiers in Political Science. 2021; 73.
[10] Tiscar-González V, Menor-Rodríguez MJ, Rabadán-Sainz C, Fraile-Bravo M, Group TL. Clinical and economic impact of wound care using a polyurethane foam multilayer Dressing. Advances in Skin & Wound Care. 2021; 34(1): 23.
[11] Sen CK, Gordillo GM, Roy S, et al. Human skin wounds: a major and snowballing threat to public health and the economy. Wound Repair Regen. 2009;17(6):763-771. doi:10.1111/j.1524-475X.2009.00543.x
[12] Robert G. Frykberg and Jaminelli Banks. Advances in Wound Care. Sep 2015.560-582
[13] Nunan R, Harding K, Martin P. Clinical challenges of chronic wounds: searching for an optimal animal model to recapitulate their complexity. Disease models & mechanisms. 2014; 7(11): 1205-1213.
[14] Saleh, K & Sönnergren, HH 2016, Control and treatment of infected wounds. in Functional Biomaterials. vol. 2, Elsevier, pp. 107-115. https://doi.org/10.1016/B978-1-78242-456-7.00005-2
[15] Joshi M, Purwar R. (2019). Composite dressings for wound care. In Advanced textiles for wound care 2019. p.313-327. Woodhead Publishing.
[16] Miranda, OJ, Srinivasan G. Advanced trends in treatment of wounds. Current Science, 2016: 641-647.
[17] Ndlovu SP, Ngece K, Alven S, Aderibigbe BA. Gelatin-Based Hybrid Scaffolds: Promising Wound Dressings. Polymers (Basel). 2021;13(17):2959. Published 2021 Aug 31. doi:10.3390/polym13172959
[18] Wiwatwongwana F, Surin P. In Vitro Degradation of Gelatin/carboxymethylcellulose Scaffolds for Skin Tissue Regeneration. Chemical Engineering Transactions, 2019;74: 1555-1560.
[19] Alven S, Aderibigbe BA. Hyaluronic Acid-Based Scaffolds as Potential Bioactive Wound Dressings. Polymers. 2021; 13(13): 2102.
[20] Negut I, Grumezescu V, Grumezescu AM. Treatment Strategies for Infected Wounds. Molecules. 2018; 23(9):2392. Published 2018 Sep 18. doi:10.3390/molecules23092392
[21] Rezvani Ghomi E, Khalili S, Nouri Khorasani S, Esmaeely Neisiany R, Ramakrishna S. Wound dressings: Current advances and future directions. Journal of Applied Polymer Science 2019; 136(27): 47738
[22] Ghomi ER, Khalili S, Khorasani SN, Neisiany RE, Ramakrishna S. Wound dressings: Current advances and future directions. Journal of Applied Polymer Science. 2019; 136(27).
[23] Ahmed A, Boateng J. Treatment of mixed infections in wounds. Therapeutic Dressings and Wound Healing Applications 2020; 91-113.
[24] Ng SF. Freeze‐dried wafers for wound healing. Therapeutic Dressings and Wound Healing Applications. 2020; 137-155
[25] Ahmed A, Getti G, Boateng J. Medicated multi-targeted alginate-based dressings for potential treatment of mixed bacterial-fungal infections in diabetic foot ulcers. International Journal of Pharmaceutics. 2021; 606: 120903-120903.
[26] Pivovarov N.A., Drobyshev A.Yu., Manuilov B.M., Romaschenko V.V., Grechishnikova O.G. Clinical and Microbiological Evaluation of the Efficacy of Modern Herbal and Antibacterial Agents in Patients in Early Post-Surgery Period after Implant

Dentistry.// Vestnik of Novel Medical Technologies. E-zine, No.4, 2015. Publication 7-3 [in Russian]
[27] Pozdnyakov D.A. Innovative Herbal Medicines in Complex Treatment of Periodontal Inflammations. /Pozdnyakov D.A., Sokolova O.R. Siberian Dental Forum. Innovative Approaches to Education, Science and Practice in Dentistry: proceedings of the XI All-Russia Research-to-Practice Conference devoted to the 75-th Anniversary of Krasnoyarsk State Medical University n.a. Professor V.F. Voino-Yasenetsky, Krasnoyarsk, March 01-03, 2017.//Editor-in-Chief V.V. Alyamovsky; ed. by S.A. Ovchinnikova. – Krasnoyarsk, Znak Ltd., 2017. pp. 73-75. [in Russian]
[28] Shomurodova B.S. The Use of Scaffolds CM-2 and Helium-Neon Laser in Complex Treatment of Periodontal Inflammations./ Shomurova B.S., Tairov U.T.// Reports of the Academy of Sciences, Republic of Tajikistan. – 2014. -Vol. 57, Is. 11-12. pp. 876-881. [in Russian]
[29] Gaspar-Pintiliescu A, Stanciuc AM, Craciunescu O. Natural composite dressings based on collagen, gelatin and plant bioactive compounds for wound healing: A review. Int J Biol Macromol. 2019;138:854-865. doi:10.1016/j.ijbiomac.2019.07.155
[30] Mariia K, Arif M, Shi J, Song F, Liu C. Novel chitosan-ulvan hydrogel reinforcement by cellulose nanocrystals with epidermal growth factor for enhanced wound healing: In vitro and in vivo analysis. International Journal of Biological Macromolecules. 2021;183: 435-446.
[31] Fatima A, Yasir S, Ul-Islam M, Kamal T, Ahmad M. Ex situ development and characterization of green antibacterial bacterial cellulose-based composites for potential biomedical applications. Advanced Composites and Hybrid Materials, 2021; 1-15.
[32] Akşit NN, Gürdap S, İşoğlu SD, İşoğlu İA. Preparation of antibacterial electrospun poly (D, L-lactide-co-glycolide)/gelatin blend membranes containing Hypericum capitatum var. capitatum. International Journal of Polymeric Materials and Polymeric Biomaterials 2021;70(11): 797-809.
[33] Li T, Sun M, Wu S. State-of-the-Art Review of Electrospun Gelatin-Based Nanofiber Dressings for Wound Healing Applications. Nanomaterials, 2022;12(5): 784.
[34] Pakolpakçıl A, Draczyński Z, Szulc J, Stawski D, Tarzyńska N. An In Vitro Study of Antibacterial Properties of Electrospun Hypericum perforatum Oil-Loaded Poly (lactic Acid) Nonwovens for Potential Biomedical Applications. Applied Sciences, 2021;11(17): 8219.
[35] Lyles JT, Kim A, Nelson K, et al. The Chemical and Antibacterial Evaluation of St. John's Wort Oil Macerates Used in Kosovar Traditional Medicine. Front Microbiol. 2017;8:1639. Published 2017 Sep 8. doi:10.3389/fmicb.2017.01639
[36] Sadgrove NJ, Jones GL. From petri dish to patient: Bioavailability estimation and mechanism of action for antimicrobial and immunomodulatory natural products. Frontiers in Microbiology, 2019; 2470
[37] Ashayerizadeh O, Dastar B, Pourashouri P. Study of antioxidant and antibacterial activities of depolymerized fucoidans extracted from Sargassum tenerrimum. International Journal of Biological Macromolecules. 2020;151:1259-1266.
[38] El-Kalamouni C, Venskutonis PR, Zebib B, Merah O, Raynaud C, Talou T. Antioxidant and Antimicrobial Activities of the Essential Oil of Achillea millefolium L. Grown in France. Medicines (Basel). 2017;4(2):30. Published 2017 May 19. doi:10.3390/medicines4020030
[39] Aćimović M, Zorić M, Zheljazkov VD, Pezo L, Čabarkapa I. Chemical characterization and antibacterial activity of essential oil of medicinal plants from Eastern Serbia. Molecules, 2020; 25(22): 5482.

[40] Salehi B, Selamoglu Z, Sevindik M, Fahmy NM, Al-Sayed E. Achillea spp.: A comprehensive review on its ethnobotany, phytochemistry, phytopharmacology and industrial applications. Cellular and Molecular Biology, 2020; 66(4): 78-103.
[42] Albayrak S, Silahtarlıoğlu N. Determination of biological activities of essential oil and extract obtained from Achillea coarctata Poir. Advances in Traditional Medicine. 2020; 20(1):77-88.
[43] Drobac MM, Kukić-Marković JM, Milenković MT, Niketić MS, Petrović SD. The chemical composition, antimicrobial and antiradical properties of the essential oil of Achillea grandifolia aerial parts from Serbia. Botanica Serbica. 2021; 45(2): 233-240.
[44] Ghorbani A, Esmaeilizadeh M. Pharmacological properties of Salvia officinalis and its components. J Tradit Complement Med. 2017;7(4):433-440. Published 2017 Jan 13. doi:10.1016/j.jtcme.2016.12.014
[45] Russo C, Edwards KD, Margetts G, Kleidonas S, Zaibi NS. Effects of Salvia officinalis L. and Chamaemelum nobile (L.) extracts on inflammatory responses in two models of human cells: Primary subcutaneous adipocytes and neuroblastoma cell line (SK-N-SH). Journal of Ethnopharmacology. 2021; 268: 113614.
[46] Gupta M, Singh N, Gulati M, Gupta R, Sudhakar K. Herbal bioactives in treatment of inflammation: An overview. South African Journal of Botany. 2021; 143: 205-225

Conception and design of study: E.M. Trunin, B.M. Manuylov,
V.V. Tatarkin, Yu.L. Vasiliev;
acquisition of data: S.Yu. Rakita, A.V. Demitrichenko,
S. Pod'ezzhikh, A.M. Emelin, E.V. Presnyakov, Zh.B. Aidemirova,
M.V. Tolgsky;
analysis and/or interpretation of data: S.Yu. Rakita, B.M. Manuylov,
R.V. Deev,
Drafting the manuscript: E.V. Presnyakov, A.M. Emelin, S.Yu. Rakita,
A.V. Demitrichenko, A.D. Solovieva, S. Pod'ezzhikh, M.V. Tolgsky, A. F. Bikmeeva
Zh.B. Aidemirova;
revising the manuscript critically for important intellectual content:
E.M. Trunin, B.M. Manuylov, R.V. Deev, V.V. Tatarkin, Yu.L. Vasiliev,
E.Yu. Diachkova, A.I. Schegolev.
Approval of the version of the manuscript to be published (the names of all authors must be listed):
E.M. Trunin, B.M. Manuylov, R.V. Deev, V.V. Tatarkin, Yu.L. Vasiliev,
E.Yu. Diachkova, A.I. Schegolev, E.V. Presnyakov, A.M. Emelin, S.Yu. Rakita,
A.V. Demitrichenko, A.D. Solovieva, S. Pod'ezzhikh, M.V. Tolgsky, A. F. Bikmeeva,
Zh.B. Aidemirova.

Acknowledgments
All persons who have made substantial contributions to the work reported in the manuscript (e.g., technical help, writing and editing assistance, general support), but who do not meet the criteria for authorship, are named in the Acknowledgments and have given us their written permission to be named. If we have not included an Acknowledgments, then that indicates that we have not received substantial contributions from non-authors.