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ANTIBIOFILM EFFECT OF COLLAGEN-BASED MATERIAL DEVELOPED FOR WOUND DRESSING

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The ongoing war in Ukraine and the urgent need to equip military personnel with advanced medical supplies has spurred the exploration of innovative next-generation materials for treating wounds. These materials must possess potent antibacterial properties and are likely to be built upon a biopolymeric base. The surplus collagen-rich waste generated by the leather industry offers an opportunity for collagen extraction and repurposing. Collagen, a biopolymer with widespread applications in cosmetics and medicine, plays a crucial role in wound healing products. It acts not only as a protective barrier against external contaminants but also stimulates fibroblast production and acts as a carrier for antimicrobial agents. The aim of our study was to test antimicrobial effectiveness against opportunistic bacteria of collagen-based material obtained from leather industry waste developed for wound dressing. We adapted a sterilization procedure based on a chloroform protocol. Utilizing chloroform sterilization for collagen preparation demonstrated successful reduction in cell attachment for the studied bacterial strains, suggesting a potential anti-fouling approach for preparing wound dressing materials. This innovative collagen-based wound dressing, boasting antimicrobial and antibiofilm properties, holds promise for future clinical studies and potential utilization in wound treatment.

Key words: *collagen dressing, wounds, biofilms, antimicrobials.*

Connection of the publication with planned research works.

This research was performed in the frame of young scientists project «Development of a complex preparation of combined action based on collagen derivatives for the treatment of wound surfaces». State registration number 0120U101290.

Introduction.

The war in Ukraine and the necessity to provide military personnel with advanced first-aid materials create prerequisites for the development of novel next-generation materials for wound treatment. These materials should possess antibacterial and have a biopolymeric basis.

During injury, the first and crucial barrier against infection development is the dressing material that comes into contact with the wound surface within the first 5 seconds. The widespread occurrence of antibiotic-resistant pathogenic microorganism strains in field hospitals complicates the treatment of wounded individuals and may lead to sepsis, further spread of infection, and, consequently, limb amputation or the need for additional surgeries. All of these factors adversely affect the health and combat readiness of the Ukrainian military as a whole. Therefore, the necessity to equip military personnel with advanced first-aid materials during armed conflicts in Ukraine drives the development of innovative next-generation wound treatment materials with antibacterial effect.

For more than 50 years [1], collagen-based products have been widely used in medicine for manufacturing various devices, including wound dressings, tissue growth matrices, and more. The extensive use of collagen can be attributed to several unique biological characteristics that lack synthetic counterparts. These characteristics include high tensile strength, semi-permeable membrane properties, low antigenicity (low likelihood of causing an immune response), hemostatic (blood clotting) properties, and a positive influence on wound healing speed [2]. Currently, numerous collagen-based products are commercially and clinically available. These products have been extracted from various sources, including animal tissues, human and animal cells grown *in vitro*, obtained through recombinant expression, or direct peptide synthesis. Each of these collagen products has its own advantages and disadvantages. The most economically viable method is collagen extraction from animal tissues [3].

In wound healing products, collagen serves not only as a barrier to separate the wound surface from the unfavorable external environment but also plays several other vital roles. It significantly enhances fibroblast production and increases their permeability due to its hydrophilic properties. Additionally, collagen increases the absorption and bioavailability of fibronectin and helps retain leukocytes, macrophages, and epithelial cells [4]. Moreover, collagen can act as a carrier for antimicrobial compounds, preventing bacterial infection at the wound site, and adsorbing wound exudate [5].

The original and natural form of bacterial existence in wounds is biofilms defined as structured consortium attached on a living or inert surface formed by microbial cells and stacked to each other by the self-produced extracellular polymeric matrix [6]. Experimental evidences of biofilms *in vitro* and *in vivo* demonstrated clearly that biofilm bacterial cells are significantly more resistant to antibiotics and host immune defence than their planktonic counterparts [7]. That is why referring to development materials for wound dressing it is important to test antimicrobial part as antibiofilm as well. The most commonly tested agents for wounds treatment among 44 commercially available topical agents were silver, iodine and polyhexamethylene biguanide (PHMB) [8].

The aim of our study.

To test antimicrobial effectiveness against opportunistic bacteria of collagen-based material obtained from leather industry waste developed for wound dressing. Novelty of research is described as study of antibacterial effect of newly developed collagen-based dressing for wound treatment.

Object and research methods.

Collagen was extracted from leather industry waste (delimed pelt) as described previously [9]. The basic chloroform protocol with adaptations [10] was applied for sterilization purposes.

Two model hospital opportunistic microorganisms, *Pseudomonas aeruginosa* ATCC 27853 (PA) and *Staphylococcus aureus* ATCC 25923 (SA), were used to study the effect of antimicrobial part of collagen-based material. Bacterial strains were cultivated aerobically at 37°C with shaking in Luria-Bertani (LB) medium to obtain the inoculum and transferred into 96-well plates covered with collagen-based product (100 µl per well).

Biofilm formation was tested as previously described [11]. The antimicrobial part was represented by the combination of azithromycin (AZM) and chloramphenicol (CAP) in concentrations 32+16 µg/ml. The effectiveness of mentioned concentrations in cells metabolic activity inhibition was previously shown [12].

Statistical analysis. All the experiments were performed in three replicates and are presented as the mean ± standard deviation (SD). One-way ANOVA and Tukey HSD/Tukey Kramer were used to calculate the data variance, and $P<0.05$ represents a significant difference. Normality assumption was checked based on the Shapiro-Wilk Test ($\alpha=0.05$).

Research results and their discussion.

Bacterial infections in wounds often delay the healing process, and may seriously threaten human life [12]. The effectiveness of antimicrobials in wound dressing material is an important part of successful use of that kind of material [13]. In our study we formulated a dressing for wound treatment based on collagen extracted from leather industry waste and antimicrobial part with a combined action of two antibiotics. Sterilization with chloroform was one of the steps to prepare collagen to be used as a base for wound dressing. That is why we tested extracted collagen (control) as well as collagen sterilized with chloroform besides collagen with antimicrobial part itself to check the effect of chloroform residues on biofilms development **(fig. 1-2).**

Antimicrobial part of developed dressing drastically inhibited the growth rate of both studied opportunistic strains. The presence of chosen antimicrobials reduced growth rate more than twice. However, chloroform use had no effect on bacteria population development according to the growth rate. There was no statistically proved difference between extracted collagen washed in distilled water and chloroform-sterilized collagen. Besides, there was no cumulative or additive effect between chloroform and chosen antimicrobials.

However, surprisingly, taking about biofilm formation and cells attachment there was a great difference between extracted and chloroform-sterilized collagen **(fig 2).**

Thus, both Gram-positive and negative cells attachment were 10 times reduced when the surface was represented by chloroform-sterilized collagen. The presence of antimicrobial part reduced the attachment level in the same range. Based on the results it seems that chloroform sterilization may have antibiofilm or antifouling effect as well. It would be interesting to examine collagen samples sterilized with different ways of cold sterilization – electron-beam [14] and ethylene oxide [15] which frequently used for collagen with clinical application.

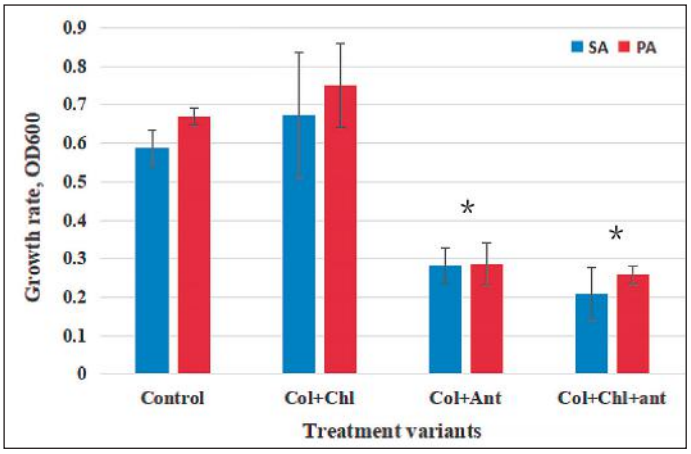

Figure 1 – Bacterial growth rate in different treatment variants. Data are shown as Mean ± SD. Variants with statistically significant difference compare to control are marked with (*).

The interaction of collagen with antibiotics is of significant importance in the treatment of wounds and infections. Collagen, used as a component in wound healing products, can interact with antibiotics at various levels: prolongation of antibiotic action [16], reduction of antibiotic toxicity [17] etc. Collagen can retain antibiotics on the wound surface or release them slowly, extending their action in the tissues. This can be particularly beneficial in the treatment of deep or prolonged wounds, where maintaining a high level of antibiotics over an extended period is necessary. Collagen, as a component of wound healing materials, contributes to optimal tissue regeneration and may provide enhanced infection control, leading to faster recovery.

Conclusions.

Developed wound dressing collagen-based material successfully inhibited biofilm development of Gram-positive and negative infection agents. The use of chloroform sterilization for collagen preparation reduced cell attachment level and could be considered as anti-fouling approach.

Prospects for further research.

Developed collagen-based wound dressing with antimicrobial effect could be used for wound treatment in further clinical studies.

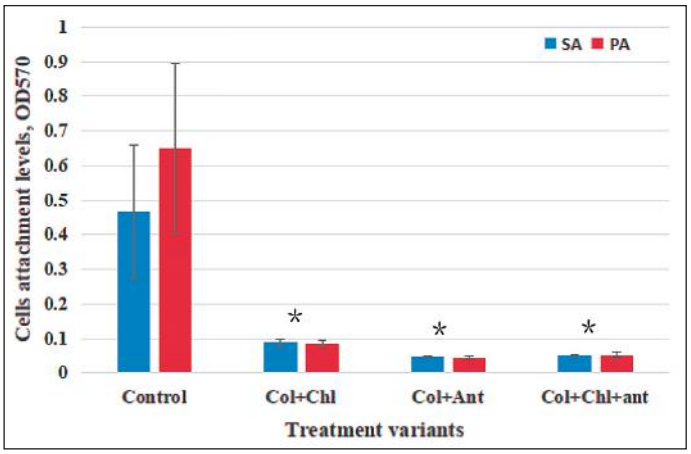

Figure 2 – Cells attachment level in different treatment variants. Data are shown as Mean ± SD. Variants with statistically significant difference compare to control are marked with (*).

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АНТИБІОПЛІВКОВИЙ ЕФЕКТ МАТЕРІАЛУ, РОЗРОБЛЕНОГО НА ОСНОВІ КОЛАГЕНУ, ДЛЯ ЛІКУВАННЯ РАНЕВИХ ПОВЕРХОНЬ

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Резюме. Війна в Україні та необхідність забезпечення військового персоналу передовими матеріалами для першої допомоги створюють передумови для розробки матеріалів нового покоління для лікування ран. Ці матеріали повинні мати антибактеріальні властивості та біополімерну основу. Шкіряна промисловість виробляє величезні кількості відходів, які можуть бути використані для екстракції колагену – біополімеру, що широко використовується в косметології та медицині. У продуктах для загоєння ран, колаген виконує функцію не тільки бар'єру, що відокремлює поверхню рани від небажаного зовнішнього середовища, але й значно сприяє виробленню фібробластів та може діяти як носій антимікробних сполук. Метою нашого дослідження було визначення антимікробної ефективності колагенового матеріалу, отриманого з відходів шкіряної промисловості, розробленого для перев'язування ран. Колаген, вилучений з відходів шкіряної промисловості, був використаний як основа для розробки матеріалу для перев'язування ран. Процедура стерилізації була адаптована протоколу стерилізації хлороформом. Поєднання двох антибіотиків – азитроміцину (AZM) та хлорамфеніколу (CAP) – було протестовано як антимікробну частину матеріалу для перев'язування ран та виявлено її дію щодо попередження росту та утворення біоплівки збудників опортуністичних інфекцій *S. aureus* ATCC 25923 та *P. aeruginosa* PA01. Розроблений колагеновий матеріал для перев'язування ран успішно інгібував утворення біоплівки у Грам-позитивних і Грам-негативних штамів. Використання хлороформу для стерилізації колагену знизило рівень прикріплення клітин обох досліджуваних штамів і може розглядатися як метод боротьби з обростанням поверхні. Розроблений колагеновий матеріал для перев'язування ран з антимікробним ефектом може бути використаний для лікування ран у подальших клінічних дослідженнях.

Ключові слова: колагеновий матеріал, рани, біоплівки, антимікробні речовини.

ANTIBIOFILM EFFECT OF COLLAGEN-BASED MATERIAL DEVELOPED FOR WOUND DRESSING

Maslak V. I., Kalinichenko O. O., Okhmat O. A., Kotlyar M. M., Iungin O. S.

Abstract. The war in Ukraine and the necessity to provide military personnel with advanced first-aid materials create prerequisites for the development of novel next-generation materials for wound treatment. These materials should possess antibacterial effect and likely have a biopolymeric basis. Leather industry produces tremendous amounts of collagen-containing waste that could be recycled for collagen obtaining. Collagen is a biopolymeric substance widely used in cosmetology and for medical purposes as well. In wound healing products, collagen serves not only as a barrier to separate the wound surface from the unfavourable external environment but also significantly enhances fibroblast production, as well as acts as a carrier for antimicrobial compounds. The aim of our study was to test antimicrobial effectiveness against opportunistic bacteria of collagen-based material obtained from leather industry waste developed for wound dressing. Collagen extracted from leather industry waste was used as a base for wound dressing material development. Sterilization procedure was adapted from described chloroform proto-

col. The combination of two antibiotics – azithromycin (AZM) and chloramphenicol (CAP) was tested as antimicrobial part of dressing material for preventing growth and biofilm formation of opportunistic bacteria *S.aureus* ATCC 25923 and *P.aeruginosa* PA01. Developed wound dressing collagen-based material successfully inhibited biofilm development of both Gram-positive and Gram-negative infection agents. The use of chloroform sterilization for collagen preparation successfully reduced cell attachment level of both studied strains and could be considered as anti-fouling approach for wound dressing material preparation. Developed collagen-based wound dressing with antimicrobial and antibiofilm effects could be used for wound treatment in further clinical studies.

Key words: collagen dressing, wounds, biofilms, antimicrobials.

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There is no conflict of interest referring to this research.

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A CLINICAL CASE OF CLUSTER HEADACHE IN A PATIENT WITH RECURRENT RHINOSINUSITIS

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Rhinosinusitis is a widespread disease in most countries of the world, has an annual prevalence of 6-15% and is usually a consequence of Acute Respiratory Viral Infection. The purpose of our work was to study the clinical case of a patient with cluster headaches on the background of recurrent rhinosinusitis.

The article presents a clinical case of cluster headache in a patient with recurrent rhinosinusitis, who, according to the treatment protocol, was prescribed an antibiotic, nasal rinsing with saline solutions, complex herbal preparations with decongestant, anti-inflammatory and secretolytic effects, analgesics and intranasal glucocorticoids.

On the background of the prescribed treatment, inflammation of the mucous membrane of the nasal cavity and paranasal sinuses began to regress; however, the headache intensity did not change. Improvement in the patient's general condition and clinical recovery was achieved only after consultation and treatment correction by a neurologist. This clinical case indicates that patients with rhinosinusitis, in which the nature of the pain is atypical, does not correspond to the severity of inflammation of the nasal mucosa and paranasal sinuses, and is not treatable, should consult related healthcare specialists to determine further treatment tactics.The analysis of clinical cases of patients with sinus pathology and the study of their structural features, innervation and blood supply significantly complement the teaching of human anatomical variability in age, gender and constitutional aspects.

Key words: *rhinosinusitis, frontal sinus, maxillary sinus, cluster headache, nasal septum.*

Connection of the publication with planned research works.

The study was carried out as part of the comprehensive research work of the Department of Normal Anatomy and the Department of Operative Surgery with Topographic Anatomy of Danylo Halytsky Lviv National Medical University «Morphofunctional features of organs in the pre- and postnatal periods of ontogenesis, under the influence of opioids, nutritional supplements, reconstructive surgery and obesity (state registration number 0120U002129).

Introduction.