Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.

Sorptive properties of hydrogel dressings and their antimicrobial action after saturation with tetracyclines

V. Vlizlo*, D. Ostapiv**, N. Kuzmina**, A. Mysak*, N. Nosova***, S. Varvarenko***, V. Samaryk***, V. Stybel****, R. Ostapiv****

*Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, Lviv, Ukraine
**Institute of Animal Biology of the National Academy of Agrarian Sciences of Ukraine, Lviv, Ukraine
***Lviv Polytechnic National University, Lviv, Ukraine
****State Research Control Institute of Veterinary Medicinal Products and Feed Additives, Lviv, Ukraine

Article info

Received 09.04.2025
Received in revised form 29.04.2025
Accepted 12.06.2025

Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, Pekarska st., 50, Lviv, 79010, Ukraine. Tel.: +38-067-675-26-24. E-mail: vasyl.vlizlo@lvet.edu.ua

Institute of Animal Biology of the National Academy of Agrarian Sciences of Ukraine, V. Stusa st., 38, Lviv, 79034, Ukraine. Tel.: +38-063-267-65-21. E-mail: oddost@ukr.net

Lviv Polytechnic National University, S. Bandery st., 12, Lviv, 79000, Ukraine. Tel.: +38-067-674-51-36. E-mail: vjsamaryk@gmail.com

State Research Control Institute of Veterinary Medicinal Products and Feed Additives, Donetska st., 11, Lviv, 79019, Ukraine. Tel.: +380-32-252-33-72. E-mail: vstybel@ukr.net

Vlizlo, V., Ostapiv, D., Kuzmina, N., Mysak, A., Nosova, N., Varvarenko, S., Samaryk, V., Stybel, V., & Ostapiv, R. (2025). Sorptive properties of hydrogel dressings and their antimicrobial action after saturation with tetracyclines. Regulatory Mechanisms in Biosystems, 16(2), e25088. doi:10.15421/0225088

Due to the war in Ukraine and a high number of wounds among combatants, civilians, and also working and domestic animals, the need for high-quality and effective dressing material is extremely relevant. Therefore, the objective of our study was to in vitro examine the sorptive properties of hydrogel dressings that we made and their antimicrobial action after saturation with tetracycline antibiotics. The sorptivity was determined by submerging the hydrogel samples for 1 h in water, wound exudate model, blood, 5% solution of oxytetracycline hydrochloride, and chlortetracycline. The presence of tetracyclines in the samples of hydrogel dressings was studied using high-performance fluid chromatography; the uniformity of their antibiotic saturation was examined under a light microscope; and the antimicrobial properties were studied by incubation with *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The conducted studies revealed that the hydrogel dressings had good sorptivity. During one hour of contact of hydrogel with water, and also blood exudate model, and blood, which are the main components of wounds, the examined samples were actively saturating. After sorption of the dressings with 5% solutions of tetracyclines, we determined a 1.34–1.90 time increase in their mass. Moreover, the content of oxytetracycline hydrochloride and chlortetracycline in the structure of hydrogel was chromatographically confirmed, and histological analysis even revealed that the samples were uniformly filled with antibiotics. The hydrogel dressings loaded with oxytetracycline hydrochloride containing 98.6% and 94.5% active agent and chlortetracycline hydrochloride with an active agent content of 99.9% exerted notable antimicrobial action toward the microorganisms *S. aureus*, *E. coli*, and *P. aeruginosa*. Thus, the hydrogel sheets had good sorptivity and their saturation with tetracyclines led to effective elimination of the microorganisms. Hydrogel dressings with antibacterial drugs can be used to treat wound infections, because they enable a quick application of a necessary therapeutic concentration of medical compounds to the wound surface. Further studies will focus on the efficacy of hydrogel dressings applied directly to animal wounds.

Keywords: hydrogel dressings; sorptivity; tetracycline antibiotics; antimicrobial properties.

Introduction

Over recent years, various countries have seen ongoing development of hydrogel dressings for treating skin injuries (Ghomi et al., 2019; Shi et al., 2020; Moradifar et al., 2025). In Ukraine, various hydrogel means for therapy of wounds of different origin on animals have been developed and broadly approbated (Mysak et al., 2021a; Nosova et al., 2021a; Vlizlo et al., 2022). Once applied to wound region, modern hydrogel means of treatment of skin injuries must perform a number of functions: maintain thermal and moisture balance; provide an influx of oxygen and necessary compounds; protect the wound from mechanical damages; be capable of absorbing and retaining large amount of exudate; and promote proliferation and differentiation of cells, thereby enhancing wound healing (Vlizlo et al., 2021; McGrath et al., 2023; Moradifar et al., 2025). Therefore, a key focus during the development of hydrogel-based means of treatment is the provision of additional functions, particularly the prolonged delivery of therapeutic drugs with different mechanisms of action for effective wound healing. The transdermal pathway of administering medical compounds, which are loaded into wound dressings, allows using medications with a narrow therapeutic index while minimizing variability of therapeutic effects and metabolic changes of the active agent in the liver (Leppert et al., 2018; Lin et al., 2022; Wong et al., 2023). Various therapeutic drugs incorporated into dressings significantly minimize adverse effects on the organism (Kaur et al., 2022; Thang et al., 2023; Zhou et al., 2023). Peroral or parenteral (subcutaneous, intramuscular, intravenous) administrations of different medical compounds that are often used for additional wound treatment can cause a detrimental impact on the vital organs and systems (Ongarora, 2022; Zelenina et al., 2022; Kozak et al., 2023b).

One of the factors hindering the process of wound healing is infection. To eliminate the microorganisms that cause wound infection, antibacterial drugs are employed. Unfortunately, prolonged and non-systemic use of antibiotics leads to development of resistance among microorganisms. Antibiotic resistance is one of the most acute problems of our time and continues to exacerbate (Laxminarayan et al., 2013; Nieuwlaat et al., 2021; Kozak et al., 2023a). Therefore, there should be thoughtful practice of applying antibacterial drugs, in particular, choosing the most effective way of delivery to the organism, minimizing the enteral and parenteral administrations, thereby potentially reducing the risks of development of resistance in microorganisms (Zhao et al., 2023; Karthikeyan & Sivaneswari, 2024).

One of the recommended ways of combating wound infection is the use of dressings containing various antimicrobial drugs (Balcucho et al., 2020; Li et al., 2020; Gadaime et al., 2025). This method is particularly effective when treating purulent wounds (Boateng et al., 2008; Shaprynskyi et al., 2015). In the practice of humane and veterinary medicine, broadly used antibiotics are those of the tetracycline group (Chopra & Roberts, 2001; Granados-Chinchilla & Rodríguez, 2017; Galecio et al., 2022). To treat animals with skin diseases, good results were produced by 5% and 1% oxytetracycline ointments (Yanamoto et al., 2021; Salimiaghdam et al., 2022; Gashaw et al.,

2024). However, applying ointments to wounds is not always recommended to achieve fast healing. Therefore, studies should seek for new methods and means of treating wounds of varying age and origin (firearms, tears, burns, etc.).

The objective of the study was to in vitro study hydrogel sheets made of hydrogel dressings, examine their sorptivity with water, wound exudate model, and blood as the main ingredients of wounds, and also solutions of antibiotics of the tetracycline group (oxytetracycline hydrochloride and chlortetracycline hydrochloride). Furthermore, the hydrogel sheets saturated with these antibacterial drugs were tested against microorganisms.

Materials and methods

For the studies, we used hydrogel dressings made at the Department of National University Lviv Polytechnic. To make hydrogels, we added 15–20 g of 4–10% pectin dispersion, 5–10 g of 3.5% dispersion of sodium alginate, and then added a necessary amount of plastifiers (the ratio of polypropylene glycol PPG 2000 : polyethylene glycol PEG 4500, as 3:1). The obtained mixture was thoroughly mixed, loaded in the forms, and placed in 100–200 mL of 0.2–1.0% calcium chloride solution for 10–20 h. The hydrogels were taken out of the forms, rinsed in water, and stored at a 5 ºC temperature (Dron et al., 2020a; Nosova et al., 2021b).

To study the sorptivity of the hydrogel dressings, strips measuring 0.5 cm in width, 0.3 cm in thickness, and 10 cm in length were cut from them. The obtained samples of the hydrogel sheets were introduced into six test tubes, filled with fluids prepared for the study. The first test tube contained deionized water, obtained using a Milli-Q Synthesis A10 deionizer (Millipore S.A.S., Molsheim, France, 2003). The second test tube contained a wound exudate model − exudate-simulating fluid with pH = 7.4 to 7.5, prepared in accordance with the following procedure: 0.222 g of $CaCl_2$, 2.3376 g of NaCl, 0.968 g of TPIC (2-amino-2-hydroxymethyl-propane-1,3-diol) and 2 g of albumin 5% aqueous solution (BSA) were loaded into a 100 ml flask and diluted to the mark with water (Rezvanian et al., 2017). The third test tube contained whole canine blood, collected from the subcutaneous vein of the forearm in sterile test tubes with heparine (BD Vacutainer; Becton, Dickinson and Company; USA). The following three test tubes individually contained 5% solutions of antibiotics of the tetracycline group (tetracycline drugs manufactured by the company Merck; Darmstadt, Germany): the forth included oxytetracycline hydrochloride containing 98.6% active agent; the fifth contained oxytetracycline hydrochloride with active agent content of 94.5%; and the sixth held chlortetracycline hydrochloride containing 99.9% active agent. The volume of each of the six fluids in the test tubes was 3 mL. The samples of hydrogel were incubated in fluids for one hour. Prior and after incubating the samples in the abovementioned fluids, we used a special device to punch out disks (d = 0.5 cm) from the hydrogel sheets and determined their mass (g) on analytical scales (OHAUS Corporation, Parsippany, New Jersey, USA, 2003).

The uniformity and depth of the sorbed solutions of tetracyclines in the samples of the hydrogel dressings were determined by histological study under a light microscope. The samples were photographed using a digital camera installed in the microscope (Axioskop 40 FL; Serien-Nr.:3311001438; Carl Zeiss, Oberkochen, Germany, 2000).

To determine the content of antibacterial drugs of the tetracycline group in the hydrogel dressings, we used high-performance fluid chromatography. For this purpose, the examined samples of the dressings were cut into 5×5 mm cubes weighing 1 g. They were loaded with 50 mL of solvent (mobile phase; acetonitrile and 0.2% solution of phosphate acid were mixed in the volumetric proportion of 1:1), were kept in an ultrasound bath for 30 min, and mixed in a mixer for one hour. The certified standard samples of oxytetracycline hydrochloride and chlortetracycline hydrochloride (Merck; Darmstadt, Germany) and the extracts obtained from the dressings were diluted in mobile phase until the concentration of operating solutions reached 100 μg/mL. To avoid errors, the experimental and standard samples were filtrated through PTFE syringe filters with a pore size of 0.22 mm (Phenomenex; Torrance, USA). The samples were divided on a liquid chromatograph (Waters Alliance 2690, USA, 1998) with diode array detector. Optimal division was achieved using a chromatographic column Luna C18(2) 250×4.6, mobile phase, consisting of acetonitrile and 0.2% solution of phosphate acid, mixed in a volumetric ratio of 1:1. The speed of the flow of mobile phase was 1.0 mL/min; the samples were detected at 350 nm; and the volume of injection measured 10 μL.

To study the antimicrobial properties of the hydrogel dressings saturated with 5% antibacterial drugs of tetracycline group, we incubated the samples in microorganism cultures. At the same time, after one hour of saturating the sheets with antibiotics, they were applied to the cultivation medium (glucose – 4%, meat-peptone bullion – 1%, agar – 2% in 100 mL of H_2O) with cultured cells of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. According to the growth inhibition zones against the microbial cultures, we determined the effectiveness of antimicrobial action of oxytetracycline hydrochloride containing 98.6% active agent; oxytetracycline hydrochloride containing 94.5% active agent; and chlortetracycline hydrochloride containing 99.9% active agent. The diameter of the growth inhibition zones of the microbial cells (cm^2) was measured using the software TotalLab TL120. The control consisted of the samples of hydrogel dressings loaded with 0.05% chlorhexidine.

The data were analyzed using the software Statistica 6.0 (StatSoft Inc., USA). The results in the tables are presented as x ± SD (mean ± standard deviation). The differences between the values of the control and experimental groups were determined using ANOVA, where they were considered statistically significant at $P < 0.05$ (taking into account the Bonferroni's Correction).

Results

The conducted studies of the fragments of hydrogel-algin sheets, made from the hydrogel dressings, revealed that they were elastic (elasticity module of 650 Pa) and strong. The samples selected for incubation varied little in mass (Table 1). After contact of the hydrogel sheets with the studied solutions, they increased their mass and swelled. Thus, submergence per one hour in water resulted in the greatest swelling of the hydrogel sheets, with preservation of satisfactory mechanical properties. The mass of the dressing sample after incubation in water increased 4.83 times. After hour-long incubation of the hydrogel sheets in the wound exudate model, the mass of the samples increased on average by 3.26 times, and incubation in dog blood increased their mass by 2.70 times. The mechanical properties of the hydrogel dressings did not change, although the sheets increased in volume while maintaining their elasticity.

Table 1
Mass of the samples of the hydrogel dressings after one-hour incubation in water, wound exudate model, and blood (n = 7)

Samples of the hydrogel dressings	Mass of sample, mg	
	x ± SD	lim
Prior to incubation	89 ± 3	72–95
After incubation in water	430 ± 27***	376–494
After incubation in wound exudate model	290 ± 13***	260–342
After incubation in canine blood	240 ± 9***	214–266

Note: in this and subsequent tables, *** indicates significant difference between the parameters prior and after incubation ($P < 0.001$).

After saturating the samples of hydrogel dressings with 5% solutions of tetracycline-group antibiotics for one hour, compared with the values prior to incubation, we determined increase in the mass of the sheets, in particular: by 1.34 times after loading with oxytetracycline hydrochloride containing 98.6% active agent; by 94.5% times after incorporating oxytetracycline hydrochloride containing 94.5% active agent; and by 1.90 times after adding chlortetracycline hydrochloride (Table 2).

According to the results of histological studies of the sections of the hydrogel dressings after incubation in the solutions of tetracycline-group antibiotics, we determined a good and uniform saturation of the sheets (Fig. 1). The capacity of the structure of hydrogel dressings to absorb and become saturated with the solutions of tetracycline antibi-

otics was confirmed by the results of the studies using liquid chromatography (Fig. 2).

Table 2
Mass of the samples of the hydrogel dressings after one-hour incubation in 5% solutions of tetracycline-group antibiotics (n = 5)

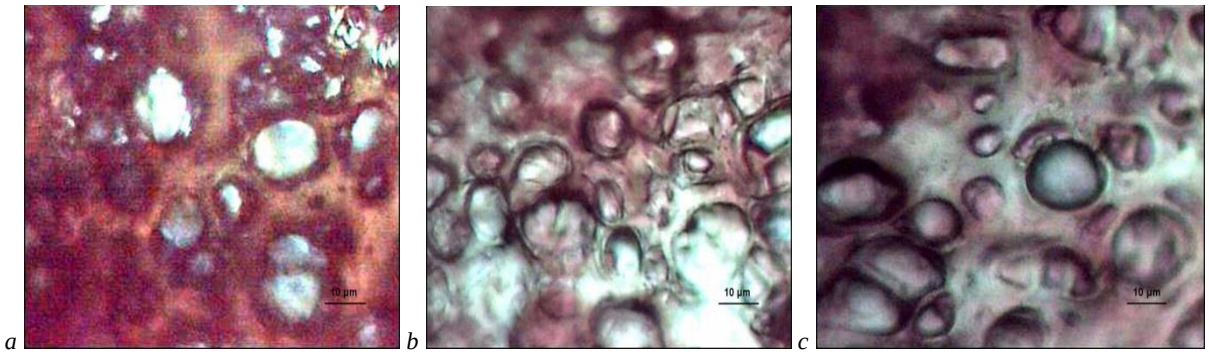
Samples of the hydrogel dressings	Mass of the sample, mg	
	x ± SD	lim
Prior to incubation	89 ± 3	72–95
After incubation in 5% solution of oxytetracycline hydrochloride containing 98.6% active agent	120 ± 10***	100–147
After incubation in 5% solution of oxytetracycline hydrochloride containing 94.5% active agent	125 ± 7***	109–148
After incubation in 5% solution of chlortetracycline hydrochloride containing 99.9% active agent	169 ± 16***	112–204

During the study of the antimicrobial action of the hydrogel dressings loaded with tetracycline-group antibiotics, we determined that the drugs had been effectively released from the samples of the sheets into the environment and inhibited the growth of microorganisms (Fig. 3).

After quantitative measurement of the growth inhibition zones against *S. aureus*, we determined that the maximum action was produced by oxytetracycline hydrochloride containing 98.6% active agent; a 18.4% lower action was displayed by oxytetracycline hydrochloride containing 94.5% active agent, and 38.7% lower was the action of chlortetracycline hydrochloride (Table 3).

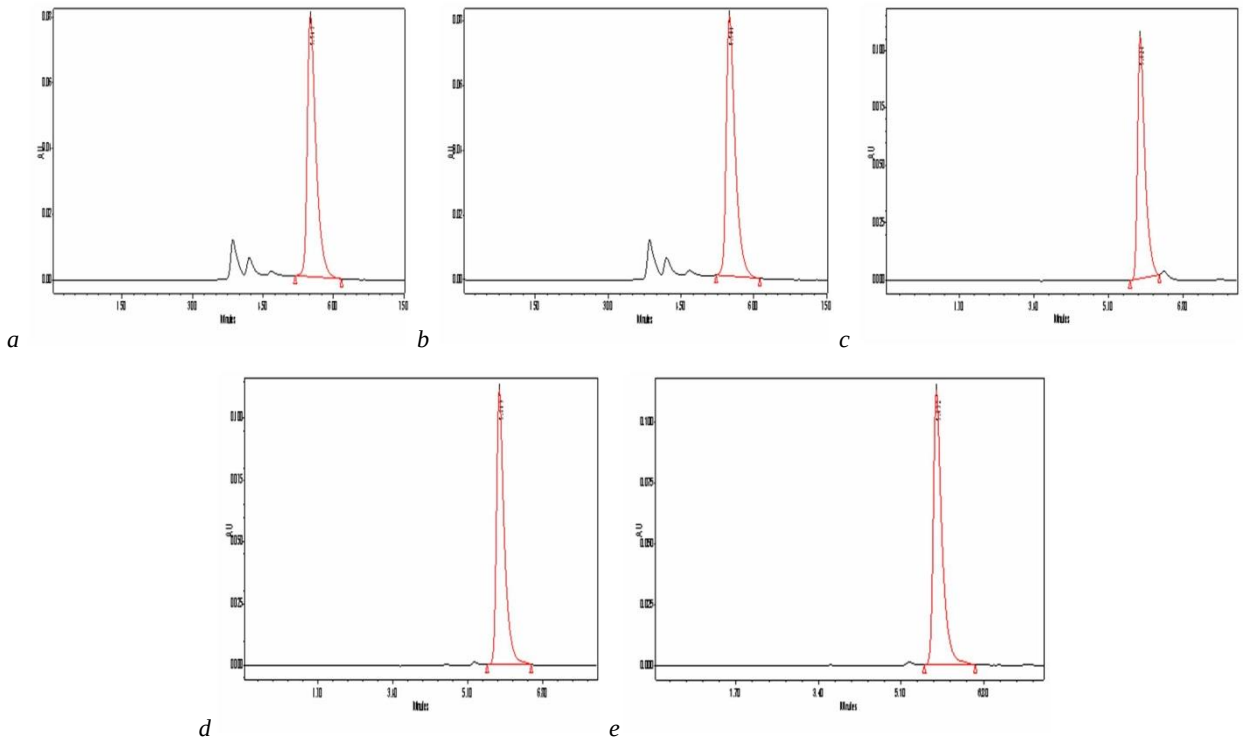
The growth inhibition zone against *E. coli* was maximum after the use of oxytetracycline hydrochloride containing 94.5% active agent, and was almost the same after the actions of oxytetracycline hydrochloride containing 98.6% active agent and chlortetracycline hydrochloride.

The growth inhibition zones of *P. aeruginosa* varied little in the conditions of hydrogel dressing loaded with oxytetracycline hydrochloride containing 98.6% active agent and chlortetracycline hydrochloride, and was lower when using oxytetracycline hydrochloride containing 94.5% active agent.



a b c

Fig. 1. Histological sections of the hydrogel panels based on sodium alginate after saturation with solutions of tetracycline antibiotics: *a* – oxytetracycline hydrochloride containing 98.6% active agent; *b* – oxytetracycline hydrochloride containing 94.5% agent; *c* – chlortetracycline hydrochloride containing 99.9% active agent



a b c d e

Fig. 2. Chromatogram of the solutions of tetracycline samples: *a* – standard sample of oxytetracycline hydrochloride containing 99.9% active agent; *b* – dressing with oxytetracycline hydrochloride containing 98.6% active agent; *c* – dressing with oxytetracycline hydrochloride containing 94.5% active agent; *d* – standard sample of chlortetracycline hydrochloride containing 99.9% active agent; *e* – dressing with chlortetracycline hydrochloride containing 99.9% active agent; ordinate axis: mUA – the magnitude of the signal in millivolts; abscissa axis – time of release of antibiotic, min

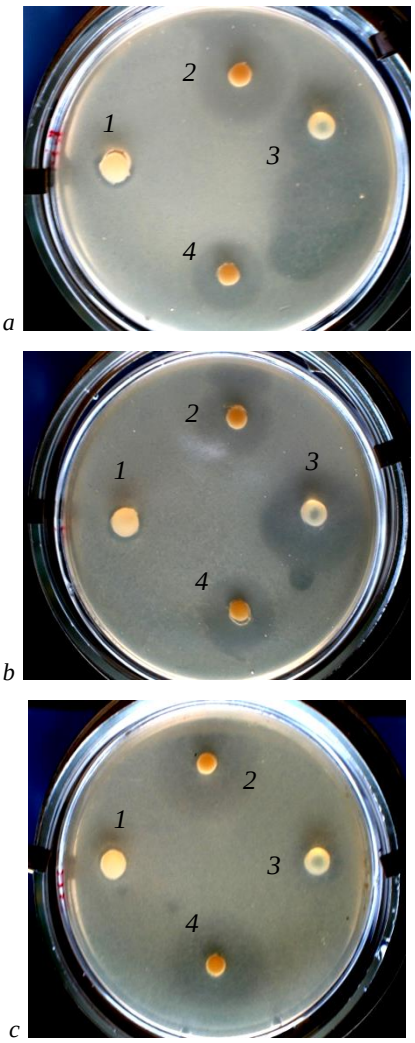


Fig. 3. Growth inhibition zones for the culture cells: *a* – *Staphylococcus aureus*, *b* – *Escherichia coli*, *c* – *Pseudomonas aeruginosa*; *1* – 0.05% chlorhexidine; *2* – oxytetracycline hydrochloride containing 98.6% active agent; *3* – oxytetracycline hydrochloride containing 94.5% active agent; *4* – chlortetracycline hydrochloride containing 99.9% active agent

Table 3
Area of growth inhibition zones against microorganisms produced by chlorhexidine and tetracycline-group antibiotics in the hydrogel dressings (cm^2, n = 7, x ± SD)

Active compound	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
Chlorhexidine, 0.05%	1.36 ± 0.08	1.33 ± 0.05	1.13 ± 0.03
Oxytetracycline hydrochloride containing 98.6% active agent	5.56 ± 0.23***	4.20 ± 0.08***	6.27 ± 0.40***
Oxytetracycline hydrochloride containing 94.5% active agent	4.54 ± 0.13***	4.68 ± 0.10***	5.30 ± 0.12***
Chlortetracycline hydrochloride containing 99.9% active agent	3.41 ± 0.22***	3.97 ± 0.08***	6.11 ± 0.29***

Note: *** – significant difference between the parameters after action of chlorhexidine and tetracyclines, $P < 0.001$.

Discussion

We made hydrogel dressings for treatment of wounds on working dogs sustained during their service in the Armed Forces of Ukraine, Border Patrol, National Guard, National Police, Emergency Service, and Demining Service (Mysak et al., 2021b; Nosova et al., 2021a; Vlizlo et al., 2022). Hydrogel dressings made from bioactive polymers of natural origin (Dron et al., 2020b; Bukartyk et al., 2022), which provide elasticity and strength, are reliably fixated, and also they effectively integrate into various forms of the wound bed. However, the efficacy of treating wounds depends not only on the quality of the created hydrogel, but also on the condition of injured skin, volume of released blood or exudate, and also different complications that emerge during active wound healing process (Ribeiro et al., 2024). Therefore, a good sorptivity of hydrogel dressings is a guarantee of successful wound healing. Already in an hour, our hydrogel dressings actively absorbed water, wound exudate model, canine blood, and solutions of tetracycline-group antibiotics, while not altering their mechanical properties. Such qualities of hydrogels were determined by other researchers (Okeke, & Boateng, 2016; Rezvanian et al., 2017). Active sorption by hydrogel dressings is due to the formation of a macroporous structure within their matrix (Nosova et al., 2020; Bukartyk et al., 2022). Hydrogel dressings not only effectively sorb blood, but can also be able to stop bleeding. The hemostatic action of hydrogel-alginate sheets, used for making dressings, is explained by their content of calcium ions (Boateng et al., 2013). At the same time, gel-like consistency creates an effect of humid environment, which prevents wound from drying, thereby forming a microclimate that promotes the growth of the granulation tissue.

Bacterial wound infection significantly slows skin healing. Since wound surfaces on animals are often compressed, contaminated, and infected, close contact between the hydrogel dressing and the wound allows for the sorption of fluids and the absorption of bacteria present. The studies by Ukrainian researchers (Shaprynskyi et al., 2015; Pidlisny, 2021; Prokopenko et al., 2023) have underscored the utmost gravity of the problem of wound infections against the background of active military actions. Skin infections often occur even due to insignificant injuries, given poor hygiene and weakened immunity (Norman, 2009; Anghel et al., 2012). Since bacterial infection of the skin impedes wound healing, treatment requires application of antimicrobial agents (Mogosanu & Grumezescu, 2014; Mirhaj et al., 2022; Sharon Sofini et al., 2024). This is relevant in case of therapy of purulent wounds when there is a necessity to target the leading factors of pathogenesis, i.e. to inhibit vital wound microflora, decrease inflammatory-infiltrative condition, and enhance reparative processes (Rosa et al., 2018; Rezvani Ghomi et al., 2019).

The study we conducted on saturation of hydrogel dressings with solutions of antibiotics of the tetracycline group revealed their effectiveness against microorganisms. It is related to the fact that antibiotics of the tetracycline group have a broad spectrum of antimicrobial action. They destroy Gram-positive (*Staphylococcus* spp., *Streptococcus* spp., *B. anthracis*, *Listeria monocytogenes*, *Clostridium* spp.) and Gram-negative bacteria (*E. coli*, *Enterobacter* spp., *Klebsiella* spp., *Salmonella* spp., *Yersinia* spp.), intracellular microorganisms (Rickettsiaceae, Chlamidiales, Mycoplasma), and some protozoans (Yanamoto et al., 2021; Galecio et al., 2022; Beganovic et al., 2023). Tetracyclines are able to penetrate the cells of microorganisms through diffusion and active transport and disrupt the synthesis of proteins by blocking the functions of ribosomes (Chopra, & Roberts, 2001). Therefore, these antibacterial drugs, in particular oxytetracycline, are successfully used in veterinary practice to treat infected wounds, exemas, and ulcers, where there is a development of microorganisms that are sensitive to the action of antibiotics of this group (Esposito et al., 2017; Adebowale et al., 2023). The histological studies revealed a uniform saturation of the hydrogel sheets with tetracyclines, and chromatographic study showed a good saturation of their structure.

No less important is the controlled release of antibiotics from hydrogel dressing and effective local action toward the wound infection. The main causes of wound infections were found to be the bacteria *P. aeruginosa*, *E. coli*, and *S. aureus*. They lead to slowed healing of affected skin regions and various complications (Bowler et al., 2001; Gbedema et al., 2010; Balcucho et al., 2020). The study we conducted on the antimicrobial action of hydrogel dressings saturated with tetracycline-group antibiotics – oxytetracycline hydrochloride containing 94.5% and 98.6 % active agent and chlortetracycline hydrochloride containing 99.9% active agent – revealed *in vitro* inhibition of the growth of the microorganisms *S. aureus*, *E. coli*, and *P. aeru-*

ginosa. This indicates that antibiotic-loaded hydrogel dressings effectively release the drugs into the environment and potentially can eliminate bacteria in the wound. Transdermal application of antibiotics can be considered as one of the simplest and safest methods of delivery. All the dose of active agent is beyond the organism, contacting with it only in the wound region. The drugs embedded in dressings promote cleansing of the wound bed from necrotic tissues, remove infection, and ultimately promote tissue regeneration (Boateng et al., 2008; da Silva et al., 2019; Ongarora, 2022). During transdermal administration, the drug at first penetrates the stratum corneum, then the deeper epidermis, until it reaches the dermis. Once in the dermis, it can be absorbed into the systemic bloodstream through the skin's microcirculation (Shaw & Martin, 2009; de Oliveira Gonzalez et al., 2016; Ghomi et al., 2019). The advantages of local application of antibiotics is high concentration in the infected wound, minimal action toward the whole organism, reduced risk of development of resistance among microorganisms, and simplicity of use (Pidlisny, 2021).

Therefore, saturation of hydrogel dressings with tetracycline-group antibiotics will become an important and effective way of combating wound infections, because it allows a quick delivery of necessary therapeutic concentrations of medical agents to the wound surface, and in case of side-effect its application can be terminated.

Further studies can be oriented at hydrogel dressings loaded with antibacterial drugs of the tetracycline group applied directly to wounds of animals. Our objective is to develop an effective dressing material for treating wounds of different origin and severity, which today are unfortunately quite common in our country due to active military actions, attacks on civilian infrastructure, and demining of areas.

Conclusions

Alginate-based hydrogel sheets that are the basis of dressings effectively absorbed water, wound exudate modeled, blood, and 5% solutions of tetracycline-group antibiotics – oxytetracycline hydrochloride and chlortetracycline hydrochloride. The hydrogel dressings saturated with these compounds actively released the antibiotics into the environment and successfully inhibited the growth of the microorganisms *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, which are the main etiological factors of development of wound infection.

The study was conducted within the framework of the Ministry of Education and Science of Ukraine "Development, Approbation, and Introduction of Hydrogel Dressings for Treating Wounds on Working Dogs" (2023–2026), No. 0124U001090.

The authors declared that they have no conflict of interests.

References

Adebowale, O. O., Jimoh, A. B., Adebayo, O. O., Alamu, A. A., Adeleye, A. I., Fasanmi, O. G., Olasoju, M., Olagunju, P. O., & Fasina, F. O. (2023). Evaluation of antimicrobial usage in companion animals at a Veterinary Teaching Hospital in Nigeria. Scientific Reports, 13, 18195.

Anghel, I., Holban, A. M., Grumezescu, A. M., Andronescu, E., Ficai, A., Anghel, A. G., Maganu, M., Lazăr, V., & Chifiriuc, M. C. (2012). Modified wound dressing with phyto-nanostructured coating to prevent staphylococcal and pseudomonal biofilm development. Nanoscale Research Letters, 7, 690.

Balcucho, J., Narváez, D. M., & Castro-Mayorga, J. L. (2020). Antimicrobial and biocompatible polycaprolactone and copper oxide nanoparticle wound dressings against methicillin-resistant *Staphylococcus aureus*. Nanomaterials, 10(9), 1692.

Beganovic, S., Rückert-Reed, C., Sucipto, H., Shu, H. W., Gläser, L., Patschkowski, T., Struck, B., Kalinowski, J., Luzhetskyy, A., & Wittmann, C. (2023). Systems biology of industrial oxytetracycline production in *Streptomyces rimosus*: The secrets of a mutagenized hyperproducer. Microbial Cell Factories, 22, 222.

Boateng, J. S., Matthews, K. H., Stevens, H. N., & Eccleston, G. M. (2008). Wound healing dressings and drug delivery systems: A review. Journal of Pharmaceutical Sciences, 97(8), 2892–2923.

Boateng, J. S., Pawar, H. V., & Tetteh, J. (2013). Polyox and carrageenan based composite film dressing containing anti-microbial and anti-inflammatory drugs for effective wound healing. International Journal of Pharmaceutics, 441(1–2), 181–191.

Bowler, P. G., Duerden, B. I., & Armstrong, D. G. (2001). Wound microbiology and associated approaches to wound management. Clinical Microbiology Reviews, 14(2), 244–269.

Bukartyk, M. M., Nosova, N. G., Maikovych, O. V., Bukartyk, N. M., Stasiuk, A. V., Dron, I. A., Fihurka, N. V., Khomyak, S. V., Ostapiv, D. D., Vlizlo, V. V., Samaryk, V. Y., & Varvarenko, S. M. (2022). Preperation and research of properties of combined alginate/gelatin hydrogels. Journal of Chemistry and Technologies, 30(1), 11–20.

Chopra, I., & Roberts, M. (2001). Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiology and Molecular Biology Reviews, 65(2), 232–260.

da Silva, L. P., Reis, R. L., Correlo, V. M., & Marques, A. P. (2019). Hydrogel-based strategies to advance therapies for chronic skin wounds. Annual Review of Biomedical Engineering, 21, 145–169.

de Oliveira Gonzalez, A. C., Costa, T. F., de Araújo Andrade, Z., & Medrado, A. R. A. P. (2016). Wound healing – a literature review. Anais Brasileiros de Dermatologia, 91(5), 614–620.

Dron, I. A., Nosova, N. H., Yakoviv, M. V., Bukartyk, M. M., Stasyuk, A. V., Varvarenko, S. M., Bordenyuk, O. Y., Maykovych, O. V., Fihurka, N. V., Samaryk, V. Y., & Voronov, S. A. (2020a). Sposib otrymannia antyseptychnoyi hidrohelevoyi pov'yazky [Method of obtaining an antiseptic hydrophilic bandage]. Patent. UA No. 143783 U Byuleten' No. 15, 10.08.2020 (in Ukrainian).

Dron, I. A., Stasyuk, A. V., Bukartyk, M. M., Luhova, Y. R., & Samaryk, V. Y. (2020b). Formuvannia hidroheliv na osnovi pektynu z riznym stupenem esteryfikatsiyi [Formation of hydrogels based on pectin with various degrees of esterification]. Khimiya, Tekhnolohiya Rechovyn ta yikh Zastosuvannya, 3(1), 239–244 (in Ukrainian).

Esposito, M. R., Guillari, A., & Angelillo, I. F. (2017). Knowledge, attitudes, and practice on the prevention of central line-associated bloodstream infections among nurses in oncological care: A cross-sectional study in an area of Southern Italy. PLoS One, 12(6), e0180473.

Gadaime, N. K. R., Mydin, R. B. S. M. N., Govindasamy, G. A., Bustami, Y., & Sreekantan, S. (2025). Advanced hydrogel dressing with zinc oxide-copper oxide nanocomposite for effective wound management: Mechanochemistry, antibacterial efficacy, cytocompatibility and wound healing potentials. Journal of Polymers and the Environment, 33, 1601–1614.

Galecio, J. S., Escudero, E., Corrales, J. C., García-Romero E., de la Fe, C., Hernandis, V., & Marinet, P. (2022). Susceptibility of caprine mastitis pathogens to tildipirosin, gamithromycin, oxytetracycline, and danofloxacin: Effect of serum on the *in vitro* potency of current macrolides. World Journal of Microbiology and Biotechnology, 38, 221.

Gashaw, B., Yizengaw, E., Nibret, E., Workineh, A., & Tilahun, F. (2024). Case notification of cutaneous leishmaniasis at different elevations in the North-Central Ethiopia from 2018 to 2022. Iranian Journal of Parasitology, 19(3), 349–357.

Gbedema, S., Emelia, K., Francis, A., Kofi, A., & Eric, W. (2010). Wound healing properties and kill kinetics of *Clerodendron splendens* G. Don, a Ghanaian wound healing plant. Pharmacognosy Research, 2(2), 63–68.

Ghomi, E. R., Khalili, S., Khorasani, S. N., Neisiany, R. E., & Ramakrishna, S. (2019). Wound dressings: Current advances and future directions. Journal of Applied Polymer Science, 136, 47738.

Granados-Chinchilla, F., & Rodríguez, C. (2017). Tetracyclines in food and feedingstuffs: From regulation to analytical methods, bacterial resistance, and environmental and health implications. Journal of Analytical Methods in Chemistry, 2017, 1315497.

Karthikeyan, E., & Sivaneswari, S. (2024). Advancements in transdermal drug delivery systems: Enhancing medicine with pain-free and controlled drug release. Intelligent Pharmacy, 2024, in press.

Kaur, G., Narayanan, G., Garg, D., Sachdev, A., & Matai, I. (2022). Biomaterials-based regenerative strategies for skin tissue wound healing. ACS Applied Bio Materials, 5(5), 2069–2106.

Kozak, M., Stasiuk, A., Vlizlo, V., Ostapiv, D., Bodnar, Y., Kuzmina, N., Figurka, N., Nosova, N., Ostapiv, R., Kotsumbas, I., Varvarenko, S., & Samaryk, V. (2023a). Polyphosphate ester-type transporters improve antimicrobial properties of oxytetracycline. Antibiotics, 12(3), 616.

Kozak, M., Zelenina, O., Ostapiv, D., Skrypka, M., Samaryk, V., & Vlizlo, V. (2023b). Blood creatinine content and rat kidney structure after intramuscular injection of pegylated antibiotic enrofloxacin. Studia Biologica, 17(3), 47–56.

Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A. K., Wertheim, H. F., Sumpradit, N., Vlieghe, E., Hara, G. L., Gould, I. M., Goossens, H., Greko, C., So, A. D., Bigdeli, M., Tomson, G., Woodhouse, W., Ombaka, E., Peralta, A. Q., Qamar, F. N., Mir, F., Kariuki, S., Bhutta, Z. A., Coates, A., Bergstrom, R., Wright, G. D., Brown, E. D., & Cars, O. (2013). Antibiotic resistance – the need for global solutions. The Lancet Infectious Diseases, 13(12), 1057–1098.

Leppert, W., Malec-Milewska, M., Zajaczkowska, R., & Wordliczek, J. (2018). Transdermal and topical drug administration in the treatment of pain. Molecules, 23(3), 681.
Li, W., Wang, B., Zhang, M., Wu, Z., & Wei, J. (2020). All-natural injectable hydrogel with self-healing and antibacterial properties for wound dressing. Cellulose, 27, 2637–2650.
Lin, Y., Chen, Z., Liu, Y., Wang, J., Lv, W., & Peng, R. (2022). Recent advances in nano-formulations for skin wound repair applications. Drug Design, Development and Therapy, 16, 2707–2728.
McGrath, M., Zimkowska, K., Genoud, K. J., Maughan, J., Gonzalez, J. G., Browne, S., & O'Brien, F. J. (2023). A biomimetic, bilayered antimicrobial collagen-based scaffold for enhanced healing of complex wound conditions. ACS Applied Materials and Interfaces, 15(14), 17444–17458.
Mirhaj, M., Labbaf, S., Tavakoli, M., & Seifalian, A. (2022). An overview on the recent advances in the treatment of infected wounds: Antibacterial Wound Dressings. Macromolecular Bioscience, 22, 2200014.
Mogosanu, G. D., & Grumezescu, A. M. (2014). Natural and synthetic polymers for wounds and burns dressing. International Journal of Pharmaceutics, 463(2), 127–136.
Moradifar, F., Sepahdoost, N., Tavakoli, P., & Mirzapoor, A. (2025). Multifunctional dressings for recovery and screenable treatment of wounds: A review. Heliyon, 11(1), e41465.
Mysak, A., Ostapiv, D., Dron, I., Bukartyk, N., Nosova, N., Varvarenko, S., Samaryk, V., & Vlizlo, V. (2021a). Stvorennia i aprobuvannia hidrohelevykh pektynovykh povyazok dlia likuvannia ran u tvaryn [Creation and approval of hydrogel pectin dressings for wound healing in animals]. In: International Scientific and Practical Conference "Veterinary preparations: Development, quality control and use". Lviv. P. 68 (in Ukrainian).
Mysak, A., Ostapiv, D., Kuzmina, N., Kozak, M., Lukashchuk, B., Dron, I., Bukartyk, N., Bukartyk, M., & Vlizlo, V. (2021b). Application of dressings based on pectin hydrogel plates for healing aseptic wounds. In: Proceedings of the XX Middle European Buiatric Congress. Ptuj, Slovenia. P. 49.
Nieuwlaat, R., Mbuagbaw, L., Mertz, D., Burrows, L. L., Bowdish, D. M. E., Moja, L., Wright, G. D., & Schünemann, H. J. (2021). Coronavirus disease 2019 and antimicrobial resistance: Parallel and interacting health emergencies. Clinical Infectious Diseases, 72(9), 1657–1659.
Norman, D. C. (2009). Factors predisposing to infection. In: Norman, D., Yoshikawa, T. (Eds.). Infectious disease in the aging. Infectious disease. Humana Press. VA Greater Los Angeles Healthcare System, Los Angeles. P. 11–18.
Nosova, N. H., Maykovych, O. V., Bordenyuk, O. Y., Yakoviv, M. V., & Varvarenko, S. M. (2020). Armuvannya al'hinat-zhelatynovoho hidroheliu funktsionalizovanym polipropilenovym mikrovoloknom [Reinforcement of alginate-gelatin hydrogel with functionalized polypropylene microfibers]. Chemistry, Speech Technology and Their use, 3(1), 232–238 (in Ukrainian).
Nosova, N., Bukartyk, M., Dron, I., Bukartyk, N., Ostapiv, D., Vlizlo, V., Mysak, A., & Samaryk, V. (2021a). Antiseptic hydrogel bandages for use in veterinary medicine. In: First Ukrainian-Polish Scientific Forum agrobioperspectives. Lviv. P. 84.
Nosova, N., Dron, I., Bukartyk, M., Figurka, N., Varvarenko, S., & Samaryk, V. (2021b). Sposib otrymannia alhinatnoyi hidrohelevoyi plastyny [Method for producing an alginate hydrogel plate]. Patent UA No. 149411u. Byuleten No. 46, 17.11.2021 (in Ukrainian).
Okeke, O. C., & Boateng, J. S. (2016). Composite HPMC and sodium alginate based buccal formulations for nicotine replacement therapy. International Journal of Biological Macromolecules, 91, 31–44.
Ongarora, B. G. (2022). Recent technological advances in the management of chronic wounds: A literature review. Health Science Reports, 5(3), e641.
Pidlisny, O. (2021). Modern approaches to multimodality therapy of purulent wounds (literature review). Health of Society, 9(2), 46–51.
Prokopenko, K., Parkhomenko, K., Dudchenko, M., Kravtsiv, M., & Drozdova, A. (2023). Improving surgical strategies for treating extensive soft tissue wounds resulting from mine and explosive trauma. Bulletin of Ukrainian Medical Stomatological Academy, 23(4), 159–162.
Rezvani Ghomi, E., Khalili, S., Nouri Khorasani, S., Esmaeely Neisiany, R., & Ramakrishna, S. (2019). Wound dressings: Current advances and future directions. Journal of Applied Polymer Science, 136, 47738.
Rezvanian, M., Ahmad, N., Amin, M. C. I. M., & Ng, S.-F. (2017). Optimization, characterization, and *in vitro* assessment of alginate-pectin ionic cross-linked hydrogel film for wound dressing applications. International Journal of Biological Macromolecules, 97, 131–140.
Ribeiro, M., Simes, M., Vitorino, C., & Mascarenhas-Melo, F. (2024). Hydrogels in cutaneous wound healing: Insights into characterization, properties, formulation and therapeutic potential. Gels, 10(3), 188.
Rosa, J. M., Bonato, L. B., Mancuso, C. B., Martinelli, L., Okura, M. H., Malpass, G. R. P., & Granato, A. C. (2018). Antimicrobial wound dressing films containing essential oils and oleoresins of pepper encapsulated in sodium alginate films. Microbiology, Ciencia Rural, 48(3), e20170740.
Salimiaghdam, N., Singh, L., Schneider, K., Nalbandian, A., Chwa, M., Atilano, S. R., Bao, A., & Kenney, M. C. (2020). Potential adverse effects of ciprofloxacin and tetracycline on ARPE-19 cell lines. BMJ Open Ophthalmology, 5, e000458.
Shaprynskyi, V. O., Skalskyi, S. S., Palamarchuk, S. V., & Shaprynskyi, Y. V. (2015). Modern approaches to treatment of purulent wounds. Unsolved problems. Hospital Surgery, 3, 70–73.
Sharon Sofini, P. S., Biswas, K., Mercy, D. J., Girigoswami, A., & Girigoswami, K. (2024). Nanostructure-assisted wound dressing materials: A literature review. Macromolecular Research, 32, 1065–1087.
Shaw, T. J., & Martin, P. (2009). Wound repair at a glance. Journal of Cell Science, 122(18), 3209–3213.
Shi, C., Wang, C., Liu, H., Li, Q., Li, R., Zhang, Y., Liu, Y., Shao, Y., & Wang, J. (2020). Selection of appropriate wound dressing for various wounds. Frontiers in Bioengineering and Biotechnology, 8, 182.
Thang, N. H., Chien, T. B., & Cuong, D. X. (2023). Polymer-based hydrogels applied in drug delivery: An overview. Gels, 9(7), 523.
Vlizlo, V., Mysak, A., Leno, Y., Slivinska, L., Lukashuk, B., Ostapiv, D., Kuzmina, N., Bodnar, Y., Samaryk, V., Varvarenko, S., Nosova, N., Dron, I., & Bukartyk, N. (2022). Armovana aseptychna hidroheleva povjazka [Reinforced aseptic hydrogel dressing]. Patent UA No. 151071, 01.06.2022 (in Ukrainian).
Vlizlo, V., Mysak, A., Stybel, V., Voloshyn, R., Leno, Yu., Kuzmina, N., Kozak, M., Bodnar, Y., Petpuh, I., Ostapiv, D., Dron, I., Samaryk, V., Bukartyk, N., Nosova, N., & Varvarenko, S. (2021). Treatment of postoperative wounds using pectin-based reinforced dressings and their antimicrobial activity. Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies, Series: Veterinary Sciences, 23(104), 41–46.
Wong, W. F., Ang, K. P., Sethi, G., & Looi, C. Y. (2023). Recent advancement of medical patch for transdermal drug delivery. Medicina, 59(4), 778.
Yanamoto, S., Soutome, S., Tsuda, S., Morishita, K., Hayashida, S., Harata, S., Murata, M., Omori, K., Rokutanda, S., & Umeda, M. (2021). Inhibitory effect of topical antibiotics/antiseptics administration on bacterial growth in the open wound of the jawbone surgery: Randomized controlled, preliminary study. Journal of Dental Sciences, 16(1), 154–159.
Zelenina, O., Vlizlo, V., Kozak, M., Ostapiv, D., Samaryk, V., Dron, I., Stetsko, T., Skrypka, M., Tomchuk, V., Danchuk, O., & Levchenko, A. (2022). Antimicrobial activity of the PEGylated antibiotic enrofloxacin and its functional and structural effect on the liver in rats. Journal of Applied Pharmaceutical Science, 12(6), 68–75.
Zhao, L., Vora, L. K., Kelly, S. A., Li, L., Larrañeta, E., McCarthy, H. O., & Donnelly, R. F. (2023). Hydrogel-forming microarray patch mediated transdermal delivery of tetracycline hydrochloride. Journal of Controlled Release, 356, 196–204.
Zhou, R., Zhou, Q., Ling, G., & Zhang, P. (2023). A cross-linked hydrogel of bismuth sulfide nanoparticles with excellent photothermal antibacterial and mechanical properties to combat bacterial infection and prompt wound healing. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 660, 130832.