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46001, майдан Волі, 1, м. Тернопіль, Україна  
E-mail: [info@bmbr.com.ua](mailto:info@bmbr.com.ua)  
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**Editors office address:**

I. Horbachevsky Ternopil National Medical University  
46001, 1 Maidan Voli, Ternopil, Ukraine  
E-mail: [info@bibr.com.ua](mailto:info@bibr.com.ua)  
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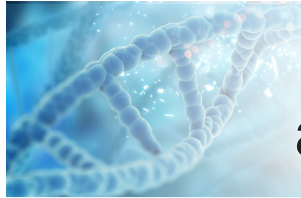
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## Morphometric assessment of age-related structural changes in the vessels of the microcirculatory bed of the prostate gland under conditions of ethanol intoxication

### Mykhailo Hnatjuk\*

Doctor of Medical Sciences, Head of the Department  
I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine  
46001, 1 Maidan Voli, Ternopil, Ukraine  
<https://orcid.org/0000-0002-4110-5568>

### Serhii Nesteruk

PhD in Medical Sciences, Associate Professor  
I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine  
46001, 1 Maidan Voli, Ternopil, Ukraine  
<https://orcid.org/0000-0003-1573-4480>

### Liudmyla Tatarchuk

Doctor of Medical Sciences, Associate Professor  
I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine  
46001, 1 Maidan Voli, Ternopil, Ukraine  
<https://orcid.org/0000-0002-4678-4205>

### Nataliia Monastyrskya

PhD, Assistant Professor  
I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine  
46001, 1 Maidan Voli, Ternopil, Ukraine  
<https://orcid.org/0000-0003-2799-0895>

**Abstract.** Vessels of the microcirculatory bed ensure the full trophism of the body at the level of capillary-tissue relations and are the first to respond to various functional and pathological conditions of organs. At the same time, morphological changes in prostate microvasculature with age under conditions of prolonged ethanol poisoning have been understudied. The purpose of this study was to determine the age-related remodelling of the prostate vessels of the microcirculatory bed in case of prolonged alcohol intoxication. Injection, histological, morphometric, and statistical methods were used. The microvasculature of the prostate gland of 80 sexually mature white male rats of different ages was studied, 40 animals served as controls, and 40 rats were injected daily for 28 days with a 30% ethanol solution at a dose of 20 ml/kg intragastrically. Morphometric analysis indicated that chronic ethanol poisoning in white rats significantly reduces the lumens of arterial microvessels and hemocapillaries, while expanding the venous vessels in the microcirculatory bed of the prostate gland. When venous congestion occurs, the density of microvessels decreases, and microcirculation bed is disturbed. This disruption is accompanied by atrophic, dystrophic, and necrobiotic changes in endotheliocytes, epithelial cells, muscle cells, stromal structures, infiltration, and sclerosing. Intragastric 28-day administration of a 30% ethanol solution at a dose of 20 ml/kg to laboratory mature white male rats leads to pronounced structural changes in the microvasculature of the prostate microcirculatory bed: constriction of arterioles, precapillary arterioles and hemocapillaries, dilation of the capillary venules and venules, which is complicated by significant venous haemorrhage, development of atrophy, dystrophy, necrosis of vascular endothelial cells, glandular epithelial cells, muscle cells, connective tissue structures,

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\*Corresponding author



cellular infiltration, and sclerosis. Vessels of the microcirculatory bed play a leading role in ethanol-induced damage of prostate structures, which dominate in 24-month-old experimental animals

**Keywords:** morphological parameters; vascular remodelling; alcohol; age

## ◆ INTRODUCTION

Ethanol and its metabolites have a powerful membranotropic effect, complicated by a pronounced increase in endogenous intoxication, increased lipoperoxidation, decreased antioxidant defence, deterioration of microcirculation bed, metabolic disorders in the body, structural and functional changes in all organs and systems [1-3]. Modern sources of medical and biological literature indicate that prolonged and systematic use of ethanol leads to damage and dysfunction of the cardiovascular, digestive, nervous, endocrine, and immune systems [4-6]. Thus, J. Fernández-Solà [5] found that cardiovascular damage caused by alcohol is often complicated by cardiomyopathy, which mainly leads to disability and mortality. O.A. Kostiuk & O.V. Denefil [2], studying ethanol cirrhosis and liver fibrosis in high and low emotional rats, found that the intensity of lipid peroxidation dominated in liver cirrhosis in rats with high emotional reactivity. S.O. Nesteruk & I.M. Klishch [1] investigated changes in endogenous intoxication indicators by the level of average mass molecules and erythrocyte index in rats of different sexes with chronic ethanol poisoning. The studies and the results obtained showed that under conditions of prolonged ethanol intoxication of animals, endotoxicity indicators increased, which were dominant in females. The growth of endogenous intoxication in rats under the influence of ethanol adversely affects the state of membrane structures, which is complicated by impaired function of various organs and body systems.

The microcirculatory system is directly important for the full life support of cells and tissue structures of the body. Unlike arteries and veins, microvessels form an orderly system of microcirculation pathways, facilitating continuous blood flow near cells and tissues of organs, carrying out various metabolic processes and ensuring the full functioning of organs and body systems. The microcirculatory system of organs, consisting of arterioles, precapillary arterioles, hemocapillaries, postcapillary venules, venules and arteriolo-venular anastomoses, plays a major role in the development of such general pathological processes as hypoxia, inflammation, degeneration, necrobiosis. Microvessels are the first to react to various changes in hemodynamics and the effects of negative endogenous and exogenous factors on the body [7-9]. Despite numerous studies investigating the structural and functional restructuring of the microcirculatory system in various physiological and pathological conditions, the patterns of reactions to these processes of the microcirculatory system links: arterial (arterioles and precapillary arterioles), metabolic vessels (hemocapillaries), and its venous part (postcapillary venules and venules) are still unresolved [7, 10].

Age-related changes in the structures and vascular bed of the prostate gland have been investigated by experimentalists and clinicians [3-5]. With age, profound structural and functional changes occur in the epithelial, muscular, stromal components and vascular bed of the prostate gland, which can lead to pathological damage to

this organ, which often occurs under various effects of negative endogenous and exogenous factors on the body [1]. The number of pathological conditions of this organ (inflammatory processes, hyperplasia, adenoma, benign and malignant tumours) is also increasing. In their morphogenesis, the leading role is played by the microcirculatory system, which is a prominent issue in gerontology and urology, as it adversely affects the reproductive function and performance of men [11]. The microcirculatory system in the prostate gland functions most optimally during puberty, adequately and fully meeting the needs of this organ and its tissue homeostasis. At various stages of ontogenesis, the structure and function of the prostate microvasculature change. Quantitative morphological methods (morphometry) are widely used to investigate the angioarchitectonics of the intra-organ vascular bed of intact organs and in various pathological conditions. Complex processes of blood, tissue, and cell interactions are mainly localised in blood vessels. As noted by M.S. Hnatjuk *et al.* [7], the microcirculatory system plays a significant role in the blood supply to organs and metabolic processes. This system not only reacts sensitively to various physiological and pathological conditions, but also substantially changes the structure and function of cells, tissues, organs, and systems. Therewith, the age-related features of the remodelling of the prostate vessels of the microcirculatory bed in the case of prolonged ethanol poisoning have been understudied. Comprehensive morphometry enables the quantitative characterisation of patterns of age-related morphogenesis and adaptive morphology in ethanol poisoning. This approach provides an objective interpretation of processes, significantly enhances researcher capabilities, predicts complications and develops adequate methods for correcting this pathology. The purpose of this study was to investigate the effect of prolonged ethanol intoxication on age-related vascular remodelling of the prostate vessels of the microcirculatory bed.

## ◆ MATERIALS AND METHODS

The experiment was carried out in September 2021 on 4 groups of laboratory mature white male rats. Group 1 included 20 control intact healthy experimental rats aged 8 months, Group 2 – 20 similar white male rats aged 24 months, Group 3 – 20 rats aged 8 months with ethanol intoxication, Group 4 – 20 animals aged 24 months rats with the indicated modelled pathology. Ethanol intoxication was modelled by intragastric administration of a 30% ethanol solution at a dose of 20 ml/kg once daily for 28 days [1]. The experimental animals were of the same weight: 8-month-old rats – 166-170 g, 24-month-old rats – 295-300 g. The dose of ethanol was calculated for each animal separately according to its weight. According to laboratory practice, intragastric administration of drugs to experimental animals does not require anaesthesia. The euthanasia of the rats was performed by bloodletting under

general thiopental anesthesia on day 28 from the beginning of the experiment. The rats were from the vivarium of the I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine. The vivarium room was kept at a constant humidity and air temperature, with daily fluctuations in day and night lighting, and the vivarium's diet. The experimental animals were constantly monitored, and sick rats were culled and not used in the experiment.

The vascular bed of the prostate gland was filled with a carcass-gelatin mixture [10]. The excised pieces of the organ under study were fixed in Buen's solution, passed through ethyl alcohols of increasing concentration, and embedded in paraffin to form paraffin blocks. Microtome sections (5-6  $\mu\text{m}$  thick) were deparaffinised and stained with haematoxylin and eosin, a mixture of acid fuchsin and picric acid, Mallory's mixture (including three working solutions: Acid fuchsin solution, phosphoric-molybdenum acid solution, a mixture of aniline blue, orange, and oxalic acid), Masson's trichrome method, toluidine blue, and silver nitrate impregnation [12, 13] and studied light-optically and morphometrically.

The diameters of arterioles, precapillary arterioles, hemocapillaries, postcapillary venules, and venules were measured on the obtained microslides of the prostate gland, and the number of microvessels per 1  $\text{mm}^2$  of tissue of the organ under study was determined [10]. Fifty measurements were made on each microslide. For the morphometry of prostate microvessels, an Olympus light

microscope (Japan) with a digital video camera and the Video Size 5.0 and Video Test 5.0 software packages were used. The obtained morphometric parameters were statistically processed using the Statsoft Statistica software package (licence No. VXXR303F737429FA-8). The reliability of the difference between the comparative characteristics was determined according to Student's t-test [14, 15].

The rats were kept and all manipulations on them were carried out in strict accordance with the "General Ethical Principles for Animal Experiments" adopted by the Fifth National Congress on Bioethics [16], which is consistent with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [17], the Law of Ukraine № 3447-IV "On the Protection of Animals from Cruelty" [18] and met the requirements of the Bioethics Commission of the I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine.

## RESULTS

Quantitative morphological characteristics of the vessels of the microcirculatory bed of the investigated organ of laboratory mature white male rats, obtained as a result of the study, are presented in Table 1. A comparative assessment of the obtained quantitative indicators of prostate microvessels revealed that they all changed significantly with age and under conditions of chronic alcohol intoxication.

**Table 1.** Morphometric characteristics of prostate microvessels of mature white laboratory male rats of different ages ( $M \pm m$ )

Morphometric parameter	Animal group under study			
	1	2	3	4
Diameter of arterioles, $\mu\text{m}$	15.86 $\pm$ 0.12	15.16 $\pm$ 0.12**	13.40 $\pm$ 0.12***	12.70 $\pm$ 0.09***
Diameter of precapillary arterioles, $\mu\text{m}$	10.14 $\pm$ 0.11	9.35 $\pm$ 0.09**	8.90 $\pm$ 0.09***	8.10 $\pm$ 0.09***
Hemocapillary diameter, $\mu\text{m}$	4.82 $\pm$ 0.04	4.60 $\pm$ 0.03**	4.18 $\pm$ 0.04***	3.95 $\pm$ 0.03***
Diameter of postcapillary venules, $\mu\text{m}$	12.85 $\pm$ 0.06	13.45 $\pm$ 0.06**	14.50 $\pm$ 0.09***	15.40 $\pm$ 0.12***
Diameter of venules, $\mu\text{m}$	26.90 $\pm$ 0.18	28.10 $\pm$ 0.21**	30.15 $\pm$ 0.21***	32.10 $\pm$ 0.24***
Number of microvessels	3820.5 $\pm$ 27.3	3810.4 $\pm$ 26.4	3550.2 $\pm$ 30.3**	3460.8 $\pm$ 31.2***

**Notes:** \*\* –  $p < 0.01$ ; \*\*\* –  $p < 0.001$  relative to the first group of animals

**Source:** compiled by the authors

It was found that the diameter of the arterioles of the intact prostate gland in 24-month-old laboratory sexually mature white male rats statistically significantly ( $p < 0.01$ ) decreased from (15.86  $\pm$  0.12)  $\mu\text{m}$  (young animals) to (15.16  $\pm$  0.12)  $\mu\text{m}$ , i.e., by 4.4%. With age, the diameter of the precapillary arterioles changed similarly. Thus, this quantitative morphological index of the prostate gland in 8-month-old laboratory mature white male rats was (10.14  $\pm$  0.11)  $\mu\text{m}$ , and in the second group of observations (24-month-old experimental animals) – (9.35  $\pm$  0.09)  $\mu\text{m}$ . These morphometric parameters were statistically significantly different ( $p < 0.01$ ). Therewith, the diameter of precapillary arterioles in white rats of the second group of observations was 7.8% smaller compared to the same quantitative morphological index of the prostate gland of the first group of animals.

Age also influenced the metabolic link of the microcirculatory bed (hemocapillaries) of the prostate gland of laboratory sexually mature white male rats. It was found that in the 1st group of observations (8-month-old white rats) the diameter of the hemocapillaries of the organ under study was (4.82  $\pm$  0.04)  $\mu\text{m}$ , and in the 2nd group (24-month-old experimental animals) – (4.60  $\pm$  0.03)  $\mu\text{m}$ . The latter morphometric parameter showed that the lumen of prostate hemocapillaries in laboratory mature white male rats of the older age group was smaller by 4.56% compared to 8-month-old animals.

The obtained and analysed morphometric parameters of the vessels of the prostate microcirculatory bed in laboratory mature white male rats indicated that venous microvessels (postcapillary venules and venules) expanded with age. Thus, the diameter of the postcapillary venules

of the organ under study in 24-month-old animals was  $(13.45 \pm 0.06) \mu\text{m}$ . This quantitative morphological parameter with a statistically significant difference ( $p < 0.01$ ) was 4.66% higher than the same morphometric parameter of the prostate gland ( $12.85 \pm 0.06$ ) of micrometre white rats of the younger age group.

The structural age-related changes in the prostate venules of laboratory mature white male rats were similar to the changes in the postcapillary venules described above. In 8-month-old animals, the diameter of the venules of the organ under study reached  $(26.90 \pm 0.18) \mu\text{m}$ , and in 24-month-old laboratory mature white male rats –  $(28.10 \pm 0.21) \mu\text{m}$ . A statistically significant difference was found between the above morphometric parameters ( $p < 0.001$ ), and the latter quantitative morphological index exceeded the previous one by 4.46%. The number of microvessels on the area of prostate tissue under study in 8-month-old laboratory mature white male rats was  $(3820.5 \pm 27.3)$ , and in 24-month-old animals –  $(3810.4 \pm 26.4)$ . The above morphometric parameters did not differ significantly from each other, i.e., the state of prostate microcirculation bed did not change substantially with age.

Light-optimally, the structure of the prostate gland in 24-month-old laboratory sexually mature white male rats was normal. It contained all the structural components of the organ under study. In some venous vessels of the prostate gland, moderate haemorrhage was noted, and single apoptotically altered glandular epithelial cells, muscle cells, and endothelial cells were found. It was found that the diameter of prostate arterioles in the 3<sup>rd</sup> group (8-month-old animals) under ethanol intoxication statistically significantly decreased from  $(15.86 \pm 0.12) \mu\text{m}$  to  $(13.40 \pm 0.12) \mu\text{m}$ , i.e., by 15.5%. In 24-month-old laboratory sexually mature white male rats, the morphometric parameter under the influence of ethanol was found to be reduced by 16.2% with a statistically significant difference.

The diameter of the precapillary arterioles of the prostate gland under conditions of prolonged ethanol intoxication also changed markedly. Thus, the studied morphometric parameter of the intact prostate gland in the 1<sup>st</sup> group of (young laboratory sexually mature white male rats) was  $(10.14 \pm 0.11) \mu\text{m}$ , and under the influence of ethanol –  $(8.90 \pm 0.09) \mu\text{m}$ . A statistically significant difference was found between these morphometric parameters ( $p < 0.001$ ). Therewith, the diameter of precapillary arterioles in 8-month-old animals under the influence of ethanol decreased by 12.2% compared with the control group. In animals of the older age group, this quantitative morphological index decreased by 13.3% compared to the same control value.

The diameters of hemocapillaries of the prostate microcirculatory bed also decreased with a high degree of statistically significant difference in prolonged ethanol intoxication. In young animals (8-month-old rats), this parameter was reduced by 13.2%, and in 24-month-old laboratory mature white male rats – by 14.1%. It was found that the venous microvessels of the prostate hemomicrocirculatory bed in the modelled ethanol poisoning tended to dilate. The lumen of the postcapillary venules in young animals under the simulated experimental conditions increased significantly by 12.84%, in 24-month-old white rats – by 14.50% ( $p < 0.001$ ), and similar morphometric

parameters of the venules of the prostate microcirculatory bed also expanded by 12.1% and 14.2%, respectively. The number of microvessels per  $1 \text{ mm}^2$  of prostate tissue decreased under the influence of prolonged ethanol poisoning. In young animals, this morphometric index statistically significantly decreased by 7.1%, and in rats of the 4<sup>th</sup> group (24-month-old animals) – by 9.2% ( $p < 0.001$ ). The dynamics of the number of microvessels in the prostate gland indicated a marked deterioration in microcirculation bed, blood supply, and metabolic processes in the organ under study [7].

The light-optical examination of prostate microdissections in case of prolonged ethanol intoxication revealed vascular disorders, haemorrhage, dilation of mainly venous vessels, perivascular and stromal oedema, foci of dystrophically, necrobiotically, apoptotically altered endothelial cells, glandular epithelial cells, muscle cells, focal infiltrates and connective tissue proliferation. Vessels of the prostate microcirculatory bed with uneven lumen, arterioles, precapillary arterioles, hemocapillaries, spasmodic, their lumen is narrowed, the wall was thickened, the lumen of the postcapillary venules, and venules was markedly enlarged, these vessels were tortuous, full of blood with varicose dilations and numerous sacculations, stasis, thrombosis foci, and diapedesis haemorrhages. Plasmorrhagia occurred in the wall of these vessels and perivascular space. The paravascular spaces were enlarged due to oedema, their stroma was distinctly disorganised with disorganisation and sclerosis. The tortuosity of almost all microvessels was noted, which was pronouncedly prevalent in the postcapillary venules and venules of the prostate gland of experimental animals of the older age group. Swollen endothelial cells with processes of atrophy, mainly protein dystrophy, necrosis, foci of desquamation and proliferation were detected. Proliferative processes indicated ischemia and hypoxia of the organ [19]. In some venous microvessels, the absence of endothelial lining was observed. The decrease in the number of microvessels in the prostate tissue was partly due to their reduction, which was visualised in the form of fibrous formations with signs of hyalinosis and incomplete or total obliteration of microvascular lumens on histological sections. Haemolysed red blood cells and hyalinised thrombi were found in the lumen of some microvessels. Basal membranes in arterial microvessels were thickened, often homogeneous with foci of hyalinosis, and there was multiplication and fragmentation of elastic fibres. In the areas of necrotic changes in cells and tissues, cellular infiltration appeared, followed by connective tissue proliferation, i.e., the development of sclerosis. Foci of sclerosis led to a marked reduction in the glandular structures of the prostate gland. The above morphological changes prevailed in the prostate gland of 24-month-old laboratory sexually mature white male rats.

The findings and their comprehensive evaluation prove that prolonged ethanol poisoning of laboratory mature white male rats was complicated by spasm and narrowing of resistive microvessels, which was confirmed by their quantitative morphological characteristics presented in Table 1. The venous microvessels of the microcirculatory bed (postcapillary venules and venules) of the prostate gland under simulated experimental conditions (prolonged ethanol poisoning of animals) were markedly dilated, which

was complicated by venous haemorrhage and hypoxia. The expansion of paravascular spaces with plasmorrhagia, connective tissue proliferation, stasis, thrombosis in the postcapillary venules and venules of the prostate gland significantly increased hypoxia, which disrupted oxygen homeostasis, the full functioning of oxidative enzymes, energy synthesis and all energy-dependent and synthetic processes.

## ◆ DISCUSSION

In the context of comparable studies, scientists have also previously investigated essential aspects of the microcirculatory system, including its structural changes under different conditions, but there are certain aspects that distinguish the current study from others. It is crucial for the conducted experiment that all links of the prostate gland's microcirculatory channel are comprehensively examined concerning age and long-term ethanol intoxication of experimental animals. This approach allows for an adequate response of the microcirculation bed to the specified exogenous negative factor. Scientists often investigate only a part of the vessels of the microcirculatory system, not covering all its links. Y.V. Silkina *et al.* [3] studied the dynamics of morphometric parameters of the resistive link (arterioles) of the microcirculatory bed of the submandibular gland in chronic alcohol intoxication. The authors considered the outer and inner diameters of the arterioles and their wall thickness and found that ethanol induced spasm of the studied vessels and narrowed their lumen, which is also confirmed by the results of this experiment. These authors investigated only changes in arterioles under the influence of ethanol, i.e., a part of resistive vessels, without examining the long-term effect of ethanol on the patterns of microvascular remodelling of all links of the microcirculatory system, which was supplemented by the present study. Y.V. Silkina *et al.* [3], having conducted a morphometric study of only salivary gland arterioles in chronic ethanol intoxication, indicate that these resistive vessels provide a full blood supply to the organs and adequate oxygenation. The resistive link of the microcirculatory system also includes the pre-capillary arterioles, which play a significant role in the blood supply to organs. These authors did not investigate the metabolic vessels (hemocapillaries), the structure of which determines the fullness of transcappillary metabolism, as well as venous vessels (postcapillary venules and venules), the change in structure and venous fullness of which substantially impairs the oxygenation of cells and tissues, complicated by hypoxia [7].

V.I. Babenko *et al.* [10], studying the long-term effect of various food additives on the microcirculatory system of the gums of rats, histologically, morphometrically, and statistically examined only hemocapillaries, i.e., only the metabolic link of the microcirculation bed, without showing structural changes in arterial and venous microvessels. It is known that the study of the vessels of one link of the microcirculation bed cannot adequately and fully elucidate the patterns of reactions of the microcirculatory system of organs in various physiological and pathological conditions [7, 9].

The analysis of the quantitative morphological studies and the data obtained showed that in healthy 24-month-old laboratory mature white male rats, arterioles, precapillary arterioles, hemocapillaries were moderately narrowed

and postcapillary venules and venules of the prostate gland were dilated. The age-related changes in the vessels of all links of the microcirculatory bed of the intact prostate gland were objectively and adequately obtained. The study was conducted on healthy laboratory mature white male rats, excluding any adverse effects on this organ, which cannot always be fully investigated in the human body that develops various comorbidities with age [1, 11]. The detected age-related changes in the vessels of the prostate microcirculatory bed were substantially enhanced by ethanol, which led to a pronounced disturbance of microcirculation bed and morphological changes in the structural components of the organ under study.

The obtained and analysed results of the study indicate that the effect of ethanol on the body of experimental animals leads to unequal severity of remodelling of arterial, metabolic and venous vessels of the prostate microcirculatory bed, i.e., these microvessels are characterised by different reactions to the action of negative endogenous and exogenous factors. J. Paloczi *et al.* [4] also agree with this statement. The main properties of arterioles, precapillary arterioles, and hemocapillaries are mobility, permeability, and plasticity [7]. These features of these microvessels are different. These vessels of the microcirculatory bed, in the wall of which smooth muscle cells are localised, can change the lumen to the greatest extent, which correlates with the morphometric parameters obtained. The above is adequately confirmed by the pronounced narrowing of arterioles, precapillary arterioles, hemocapillaries and dilation of the postcapillary venules and venules of the prostate gland of laboratory mature white male rats of different ages under prolonged ethanol intoxication.

Most authors [3, 7, 9] assert that a significant constriction of arterioles, i.e., a reduction in the luminal space within arterial vessels of the microcirculatory bed due to adverse endogenous and exogenous factors, impedes organ blood supply, exacerbates major pathologies, and elevates complication rates. A.V. Omelchenko-Seliukova *et al.* [20], having investigated the frequency and nature of complications in patients with polytrauma who consumed alcohol, found a substantial increase in the frequency of various complications in them compared to patients with polytrauma who did not consume alcohol. The current study fully confirms the above statement.

For the first time, the number of microvessels in prostate tissue was studied. It was found that this morphometric parameter decreased slightly with age in the intact organ, with ethanol intoxication in 8-month-old rats – by 7.1%, in 24-month-old animals – by 9.4%. The detected decrease in the number of microvessels of the microcirculatory bed in the prostate tissue in ethanol poisoning leads to a deterioration in microcirculation, blood supply, and metabolic processes in the organ under study. The established tortuosity of the venous microvessels of the prostate microcirculatory bed in case of prolonged ethanol intoxication indicates a compensatory restructuring of the microcirculatory system and an increase in vascular resistance and impaired venous blood drainage [7].

Vessels of microcirculatory bed of the prostate gland exhibits expanded, full-blooded, dilated microvessels in ethanol intoxication. This condition is further complicated by venous congestion, plasmorrhagia and hypoxia, which

causes cell and tissue atrophy, dystrophy, and necrobiosis. Infiltration and sclerosis foci are also observed. The latter leads to a pronounced reduction in the glandular structures of the organ under study and impairment of its function. The described changes in the structure of microvessels cause a marked decrease in the size of the glandular structures of the organ under study and impairment of its functions. The findings confirm that prolonged ethanol intoxication of laboratory mature white male rats leads to a pronounced structural alteration of microvessels in all parts of the prostate microcirculatory system. This is especially pronounced in 24-month-old experimental animals.

## ★ CONCLUSIONS

The study is aimed at revealing the effect of long-term alcohol intoxication on the remodelling of the prostate vessels of the microcirculatory bed. The main purpose of this study was to determine structural changes in the vessels of the organ under the influence of ethanol and to determine the effect of age on this process. To fulfil this purpose, a range of morphological research methods was utilised, enabling the acquisition of the most objective and appropriate evaluation of structural alterations in the microvasculature of the prostate gland. Specifically, it was found that morphometric methods of research allow for an accurate assessment of changes in the diameters of arterioles, precapillary arterioles, hemocapillaries, and venules. It was also discovered that alcohol intoxication impacts various segments of the prostate microcirculatory system in distinct ways. The experiment revealed that arterioles of the prostate gland constrict under the influence of ethanol, and this process depends on the age of the animals. Morphometrically, it was found that under prolonged alcohol poisoning in 8-month-

old experimental animals, prostate arterioles were narrowed by 15.5%, precapillary arterioles – by 12.2%, hemocapillaries – by 13.2%, in 24-month-old laboratory mature white male rats – by 16.2%, 13.3%, and 14.1%, respectively. It was found that the venous vessels of the microcirculatory bed (postcapillary venules and venules) dilated under prolonged exposure to ethanol. Quantitative morphology revealed that in the modelled pathology in 8-month-old rats, the lumen of venules increased by 12.84%, venules – by 12.1%, in 24-month-old experimental animals – by 14.5% and 14.2%, respectively. Increased venous vessel diameters led to venous haemorrhage, hypoxia, which was complicated by atrophy, dystrophy, necrosis of endothelial cells, glandular epithelial cells, muscle cells, prostate stromal structures, and the emergence of cellular infiltration and sclerosis. The detected morphological changes in the organ under study were dominant in 24-month-old rats. The potential for additional research lies in an exhaustive and thorough examination of the quantitative morphology of structural changes in the vessels of all components of the prostate microcirculatory system while under the influence of ethanol. Such a study would greatly enhance the ability to diagnose, correct and prevent related pathologies.

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## ★ CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## Морфометрична оцінка вікових структурних змін гемомікроциркуляторного русла передміхурової залози в умовах етанолової інтоксикації

### Михайло Степанович Гнатюк

Доктор медичних наук, завідувач кафедри  
Тернопільський національний медичний університет імені І. Я. Горбачевського МОЗ України  
46001, майдан Волі, 1, м. Тернопіль, Україна  
<https://orcid.org/0000-0002-4110-5568>

### Сергій Олександрович Нестерук

Кандидат медичних наук, доцент  
Тернопільський національний медичний університет імені І. Я. Горбачевського МОЗ України  
46001, майдан Волі, 1, м. Тернопіль, Україна  
<https://orcid.org/0000-0003-1573-4480>

### Людмила Василівна Татарчук

Доктор медичних наук, доцент  
Тернопільський національний медичний університет імені І. Я. Горбачевського МОЗ України  
46001, майдан Волі, 1, м. Тернопіль, Україна  
<https://orcid.org/0000-0002-4678-4205>

### Наталія Ярославівна Монастирська

Доктор філософії, асистент  
Тернопільський національний медичний університет імені І. Я. Горбачевського МОЗ України  
46001, майдан Волі, 1, м. Тернопіль, Україна  
<https://orcid.org/0000-0003-2799-0895>

**Анотація.** Гемомікроциркуляторні судини забезпечують повноцінну трофіку організму на рівні капілярно-тканинних відношень та першими реагують на різні функціональні та патологічні стани органів. В той же час морфологічні зміни мікросудин передміхурової залози з віком в умовах тривалого етанолового отруєння повністю не вивчені. Мета дослідження полягала у з'ясуванні вікових ремодельовань судин гемомікроциркуляторного русла передміхурової залози при тривалій алкогольній інтоксикації. Використані ін'єкційні, гістологічні, морфометричні, статистичні методи. Вивчено мікросудини передміхурової залози 80 статевозрілих білих щурів-самців різного віку, 40 тварин слугували контролем, а 40 шурам щоденно протягом 28 днів внутрішньошлунково вводили 30 % розчин етанолу в дозі 20 мл/кг. Морфометрично виявлено, що під впливом тривалого отруєння білих щурів етанолом виражено зменшуються просвіти артеріальних мікросудин та гемокапілярів, значно розширюються венозні судини мікрогемодинамічного русла передміхурової залози, виникає венозне повнокрів'я, зменшується щільність мікросудин, порушується гемодинаміка, що супроводжується атрофічними, дистрофічними та некробіотичними змінами ендотеліоцитів, епітеліоцитів, міоцитів, стромальних структур, інфільтрацією та склерозуванням. Внутрішньошлункове 28 добове введення лабораторним статевозрілим білим шурам-самцям 30 % розчину етанолу в дозі 20 мл/кг призводить до виражених структурних змін мікросудин гемодинамічного русла передміхурової залози: звуження артеріол, передкапілярних артеріол і гемокапілярів, розширення закапілярних венул та венул, що ускладнюється значним венозним повнокров'ям, розвитком атрофії, дистрофії, некробіозу ендотеліоцитів судинного русла, залозистих епітеліоцитів, міоцитів, сполучнотканинних структур, осередків клітинної інфільтрації та склерозу. Судинам гемодинамічного русла належить провідна роль в етаноловому пошкодженні структур передміхурової залози, що домінують у 24-місячних експериментальних тварин

**Ключові слова:** морфологічні параметри; ремодельовання судин; алкоголь; вік



## Biophysical evaluation of the effectiveness of high-frequency bipolar electric welding for closing defects in the *dura mater* in frontal sinus tumours with intracranial spread

**Dmytro Zabolotnyi\***

Doctor of Medical Sciences, Director  
State institution "O. S. Kolomyichenko Institute of Otolaryngology  
of the National Academy of Medical Sciences of Ukraine"  
03680, 3 Zoolohichna Str., Kyiv, Ukraine  
<https://orcid.org/0000-0001-9429-4414>

**Olena Kvasha**

Aspirant  
State institution "O. S. Kolomyichenko Institute of Otolaryngology  
of the National Academy of Medical Sciences of Ukraine"  
03680, 3 Zoolohichna Str., Kyiv, Ukraine  
<https://orcid.org/0000-0003-2904-8748>

**Abstract.** One of the main tasks of surgical treatment of patients with frontal sinus neoplasms with intracranial spread is to seal the subdural space, which is achieved by hermetically connected *dura mater*. The purpose of this study was to investigate the structure of the tissues of the *dura mater* and *dura mater*-fascia junction using bipolar electric welding. The methods of light microscopy and infrared spectroscopy were used. When evaluating the results, no  $\beta$ -structured aggregates were observed in the tissue samples under study. There was minimal traumatic damage to the adjacent *dura mater* tissue and minimal thermal damage from vaporisation. Pacchioni granulations, venous pial, and cortical vessels of the *dura mater* were preserved. The study observed complete identity of the wave values of amide A, B, amide I, II spectra; preservation of amide A, B peaks. Preservation and curvature of the N-H arm and O=C-N band were changed within acceptable limits. The findings indicate the absence of  $\beta$ -structured protein aggregates in the junction area, which excludes the possibility of fibrous structures and, as a result, does not contribute to the formation of a meningeal scar. The structure of the collagen protein in the junction area is normal, altered but intact, with preservation of its functions. This indicates the feasibility of using the method of high-frequency bipolar electric welding for surgical closure of defects of the *dura mater*, as an alternative to closure with suture material

**Keywords:** electrosurgery; infrared spectroscopy; *dura mater* defects; cerebrospinal fluid leakage; cerebrospinal fluid effusion; liquorrhea

### ✦ INTRODUCTION

One of the most frequent complications of radical removal of frontal sinus tumours with intracranial growth is a disruption of the closed physiological circulation of cerebrospinal fluid (CSF), i.e., the emergence of external and occult CSF liquorrhea. This is caused by insufficient tightness of the closure of defects in the *dura mater* (DM) at the final stage of surgery. These complications can be prevented by

using a special method of joining *dura mater* defects using bipolar high-frequency electric welding (HFEW) developed jointly with the E.O. Paton Institute of Electric Welding of the National Academy of Sciences of Ukraine (NAS) [1]. Protein-associated electrothermal tissue adhesion ensures hermetic binding of the tissues of the DM without the use of foreign materials and the formation of a coagulation

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\*Corresponding author



scab. To evaluate the new method of DM connection, special attention should be paid to the structure of the zone of direct tissue connection, its similarity or difference from the surrounding tissues. The answer to this question can be obtained using histological and biophysical methods (confocal infrared spectroscopy).

O.I. Palamar *et al.* [2] found that a violation of the integrity of the *dura mater* during elective neurosurgical and otorhinolaryngological operations causes a risk of developing CSF liquorrhea in the postoperative period with a frequency of 15.9–27.5%. Insufficient sealing of the DM, according to V.V. Kishchuk *et al.* [3], contributes to the leakage of CSF outside the subdural space, resulting in the emergence of external CSF liquorrhea through the surgical wound and other natural openings (otoliquorrhea, rhinorrhea), as well as latent CSF in the form of subaponeurotic accumulation of cerebrospinal fluid, which may stay undiagnosed for some time. One of the main tasks in the surgical treatment of patients with this pathology, according to multiple studies by B. Marlier *et al.* [4], is the sealing of the subdural space, which is achieved by a hermetically sealed *dura mater*. However, if the defect in the *dura mater* is not linear and, when stretched, leads to overstretching of the *dura mater* and compression of the brain tissue, *dura mater* plastics material is required. According to comparative studies by B. Coucke *et al.* [5], autografts are considered to be the ideal material for DM plastics: periosteum, broad fascia of the thigh, temporalis muscle aponeurosis, and only then – synthetic artificial materials.

The newest types of sutures, staplers, shape memory compression devices, diathermocoagulation, biological adhesives, artificial *dura mater* are used to connect *dura mater* defects, but P. Nicolai *et al.* [6] and L. Häni *et al.* [7] found that they cause several complications: persistent infection, focal intense cellular inflammatory reaction, dense encapsulation of foreign material, cerebrospinal fluid, haematomas, rejection reactions, and Creutzfeldt-Jakob disease. A. Kinaci & T.P.C. Van Doormaal [8] found that the negative effects of using sutures to connect the *dura mater* are as follows: leaving foreign material in the tissues, squeezing the tissues that are sutured, which substantially affects the healing process of the wound surface and often causes chronic inflammation in the tissues, allergic reactions to tissues of biological sutures, resulting in wound suppuration, repeated needle trauma, and potentiation of the wound surface by sutures.

Based on the research data of M.M. El-Sayed & E. Sarioglan [9], thermal denaturation of proteins – coagulation – occurs when they are heated above 450°C. Coagulation is an inertial process that absorbs energy. The coagulation rate depends on the temperature. T.L. Smith, & J.M. Smith [10] found that living soft tissue should be heated to a temperature at which protein coagulation should end in a few seconds, and the tissue should be clamped with a certain defined pressure. S.S. Podpriatov *et al.* [11] found that with an increase in voltage, the quality of the connection increases, but at the same time, the temperature of the surface layer of living soft tissue between the electrodes increases, which leads to unacceptable thermal damage. In another study, the authors concluded that the reason for this phenomenon is that protein coagulation in cells occurs mainly before the destruction of lipid membranes, i.e.,

before the formation of a single protein space for the protein to be joined [12]. Thus, an effective technical solution was proposed, which is defined by the fact that to destroy cell membranes before the start of protein coagulation, it is necessary to modulate high-frequency stress with rectangular pulses with a frequency of several thousand hertz.

The purpose of this study was to compare the collagen capacity in the area of the *dura mater* and broad fascia of the thigh after high-frequency bipolar electric welding with the normal collagen structure of the intact *dura mater* and to assess its ability to regenerate in the weld zone.

## ✦ MATERIALS AND METHODS

The study was conducted in 2018–2022 in the experimental research laboratories of the State Institution “Academic A. Romodanov Institute of Neurosurgery of the National Academy of Medical Sciences of Ukraine”. The experiment was performed on 18 porcine cadaveric *dura mater* and 8 porcine cadaveric broad fasciae of the thigh. Immediately after collection, the porcine tissue samples were placed in sterile dishes and thermal bags to preserve the physiological properties of the tissue and delivered to the State Institution (SI) “E.O. Paton Institute of Electric Welding”. Then the material was subjected to selected modes of high-frequency coagulation for biological tissue welding using a hardware welding complex in automatic and semi-automatic modes. The study using infrared spectroscopy (IR spectroscopy) was performed jointly with the SI “Institute of Physics of the NAS of Ukraine”, Department of Physics of Biological Systems, Kyiv. Histological studies were conducted according to the generally accepted review method of staining histological sections with haematoxylin and eosin.

Four rectangular pieces of 4 × 3 cm were prepared from each porcine *dura mater*. From each porcine broad fascia of the thigh, 6 pieces of rectangular shape 6 × 4 cm were harvested. The animal material was divided into 3 groups (2 experimental groups and 1 control group): the first group was a simulation of high-frequency bipolar electric welding of porcine *dura mater* with porcine *dura mater* (n = 25). The second group was modelling of HFEW of porcine *dura mater* with porcine broad femoral fascia (n = 25). The third group consisted of samples of intact porcine cadaveric *dura mater* (control) (n = 30). In Groups 1 and 2, the samples were joined using high-frequency bipolar electric welding in automated modes of the hardware welding complex PATONMED EC 300 M1 (Ukraine) [11].

The obtained samples were immediately fixed in a solution of neutral formalin (sequentially 5–7–10%, 24 hours), dehydrated in ethanol, toluene, and sealed in paraffin (Thermo Scientific Richard-Allan Scientific, Paraffin type 6 REE 8336, Kalamazoo, USA). Sections of 7 μm thickness were made on a HM430 microtome (Microm, Germany), stained with haematoxylin and eosin, picrosirius red (Direct Red 80, Magnacol Ltd, UK) and embedded in balm (Thermo Scientific Richard-Allan Scientific Cytoseal 60, REF 8310-16, Kalamazoo, USA). Microsections were examined using an Axiophot microscope (Carl Zeiss, Germany). The morphometric study of the thickness of the *dura mater* was performed using Carl Zeiss software (AxioVision SE64 Rel.4.9.1, Germany). Tissue samples of 3 groups with a volume of up to 0.5 mm<sup>3</sup> were examined by infrared spectroscopy at 5 points from each compound sample.

To analyse the infrared absorption spectra, thin sections of the post-manipulation tissue were made and dried at room temperature between two CaF<sub>2</sub> plates (fluorite glass, transparent in the infrared region). Next, the films on the substrates were placed in the cuvette chamber of the device to record the absorption spectra. The spectra were recorded on a Bruker INVENIO-R spectrometer (Germany) in a wide spectral range (from 3800 to 800 cm<sup>-1</sup>) at room temperature and humidity of about 60% in a cuvette chamber. The sample under study was placed in one of the interferometer arms or at its outlet in mixed beams.

To obtain the transmission spectrum, the following steps were performed: 1) the interferogram of the free channel (without sample) was recorded. After the Fourier transform, the comparison spectrum R( $\nu$ ) was obtained; 2) the test sample was placed in the channel and the interferogram with the sample was recorded. After the Fourier transform, the spectrum of the sample S( $\nu$ ) was obtained.

It was similar to R( $\nu$ ), but with a lower intensity in the absorption regions of the sample; 3) the original transmission spectrum T( $\nu$ ) was defined as the ratio T( $\nu$ ) = S( $\nu$ )/R( $\nu$ ). OPUS 4.2 software (Bruker Corp., Germany) was used to analyse the spectral data. All spectra were corrected for the baseline according to the broken line method using 10-15 points, as well as normalised by absorption in the region of the amide bands A, B, I-VII.

The in-plane spectral imaging modes depend on C=O stretching, C-N stretching, N-H stretching, and O-C-N bending, and the out-of-plane mode is conditioned upon the C-N torsion. The characteristic bands of the amide groups of protein chains are similar to the absorption bands of secondary amides in general and are designated as amide bands. There are nine such groups of amides, namely amide A, amide B, and amides I-VII, which are summarised in the table below in descending order of wavenumber (Table 1).

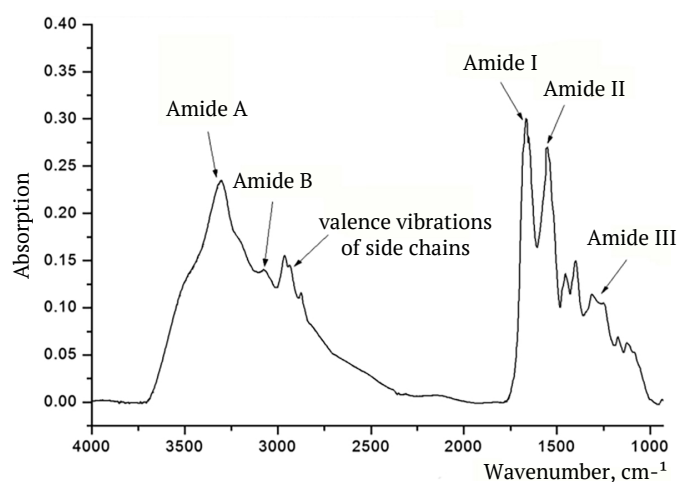
**Table 1.** Characteristic infrared absorption bands of protein amides

Designation	Wave number (cm <sup>-1</sup> )	Value
A	3300	N-H stretching in resonance with an overtone (2 × amide II)
B	3110	
I	1653	80% C=O stretching; 10% C-N stretching; 10% N-H bending
II	1567	60% N-H bending; 40% C-N stretching
III	1299	30% C-N stretching; 30% N-H bending; 10% C=O stretching; 10% O=C-N bending; 20% other
IV	627	40% O=C-N bending; 60% other
V	725	N-H bending
VI	600	C=O bending
VII	200	C-N torsion

Source: [13]

The amide I and amide II bands are particularly informative for studying protein conformation [13]. Amide I has characteristics including 80% C=O stretching, 10% C-N stretching and 10% N-H bending. Amide II represents 60% N-H bending and 40% C-N stretching.

According to the studies, the characteristic absorption bands of the protein of normal unchanged *dura mater* in the infrared range were determined (Fig. 1), which were used during the study as data for comparison with the indicators obtained during the joining using HFEW [14].



**Figure 1.** Characteristic absorption bands of normal *dura mater* protein in the infrared range

Source: [14]

Statistical data processing was performed using Stat-Plus 5.9.9.4/Core v6.7.3 software (AnalystSoft Inc., USA).

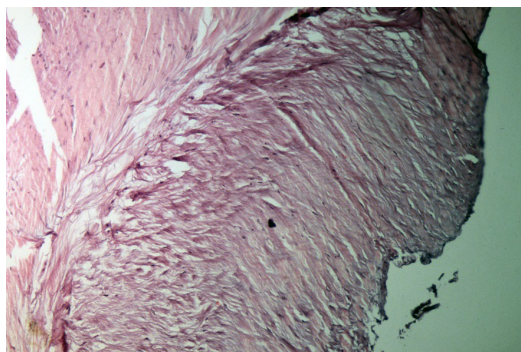
The hypothesis 76 of a normal distribution in the data samples was tested using the Kolmogorov-Smirnov test.

Between-group differences in data were investigated using one-way analysis of variance followed by the Bonferroni post-hoc test and the non-parametric Mann-Whitney U test. The Pearson linear correlation method was used. All data are presented as mean  $\pm$  standard error of the mean ( $M \pm m$ ). The difference was considered significant at  $P < 0.05$ .

The experiment was approved by the Bioethics Committee of the SI "Institute of otolaryngology named after Prof. O. Kolomiychenko of the National Academy of Medical Sciences of Ukraine". Therewith, the principles of bioethics regulated by the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" [15] and Directive 2010/63/EU "On the Protection of Animals Used for Scientific Purposes" [16] were upheld.

## RESULTS

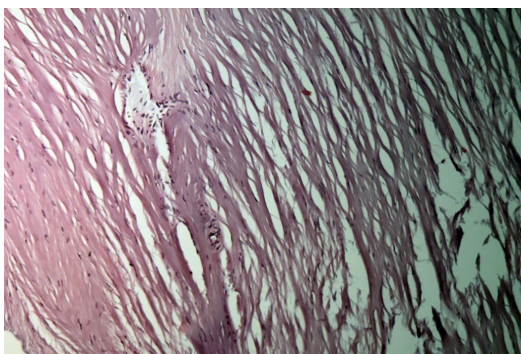
When evaluating the results of a morphological study performed using light microscopy at 400x magnification and haematoxylin-eosin staining, important structural features of the joining of the *dura mater* with the broad fascia of the thigh (Fig. 2) revealed the interweaving of collagen fibres with the fibrillar structures of the broad fascia of the thigh, which creates a stable weld, and complete preservation of the orientation of collagen fibres, which form the basis of the extracellular matrix skeleton and are the basis of hermetic state. There was no dry coagulation necrosis, which indicates a better prognostic variant of the course of *dura mater* defect healing.



**Figure 2.** The area of the "welded" *dura mater* suture with the broad fascia of the thigh, haematoxylin-eosin stain, x400  
**Source:** photographed by the authors

The morphological study of the joining of the *dura mater* with the *dura mater* (Fig. 3) revealed heterogeneity in the density of collagen fibres, looseness of the collagen structure, areas of tissue oedema, areas of fibroblast accumulation, and complete preservation of the orientation of the *dura mater* collagen fibres. There is minimal traumatic

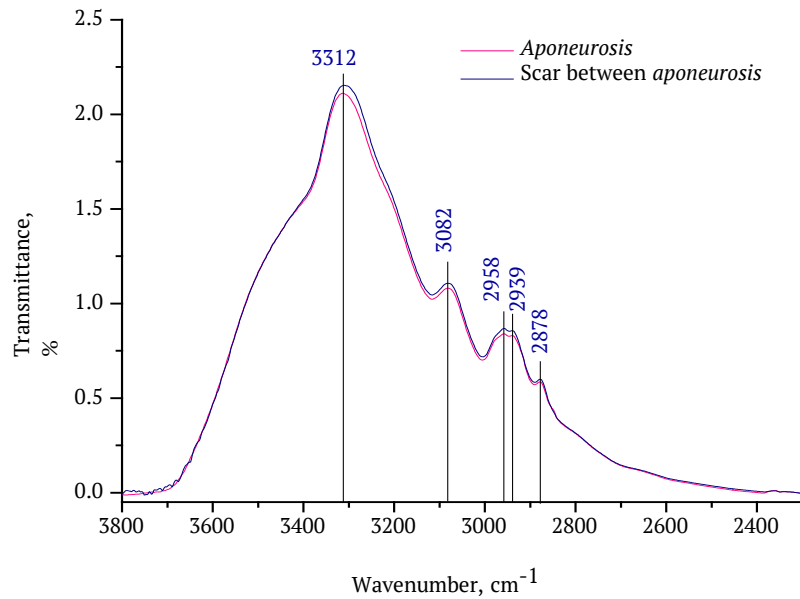
damage to the adjacent *dura mater* tissue, indicating minimal thermal injury due to vaporisation. The preservation of Pacchioni granulations, venous pial and cortical vessels in the brain tissue indicates the absence of coagulation necrosis. These structures are actively involved in fluid resorption and maintaining the balance of intracranial pressure.



**Figure 3.** Section of the "welded" suture between *dura mater* and *dura mater*  
**Source:** photographed by the authors

The technique of confocal infrared spectroscopy has made it possible to control the optical properties of biological substances and objects to increase sensitivity and increase the penetration path of visible and near-infrared light photons into deeper regions, which improves the recognition of the molecular structure of the sample under study. When using computer analysis to calculate the relative area of the bands of the components depicted

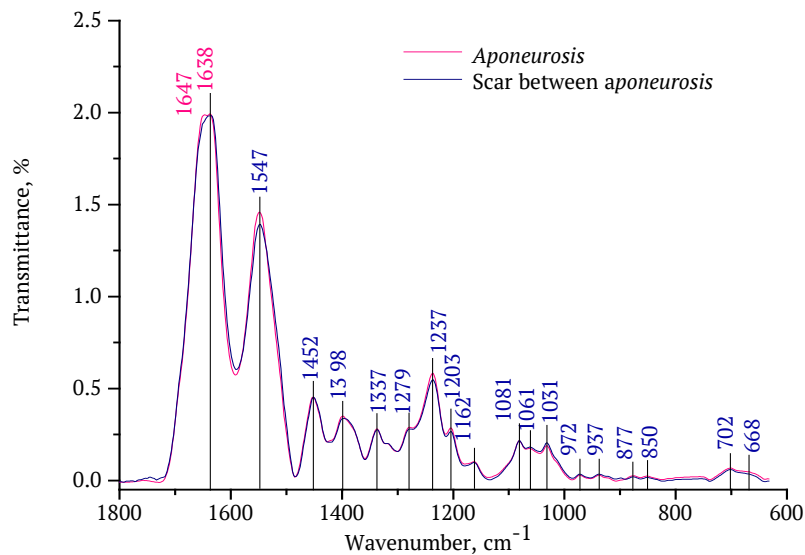
in the amide A and B spectrum (Fig. 4), the results presented indicate that the protein of the thigh broad fascia tissue, connected by HFEW, is almost completely identical to the normal thigh broad fascia tissue. The complete identity of the wave values of the amide A and B spectra, the preservation of the amide A and B peak, the preservation and bending of the N-H arm and the O=C-N band are observed.



**Figure 4.** Characteristic absorption bands of amide I and amide II waves of protein of intact broad femoral fascia and broad femoral fascia protein joined by bipolar high-frequency electric welding in the infrared range  
**Source:** compiled by the authors

When using computer analysis to calculate the relative area of the bands of the components depicted in the amide I and II spectrum (Fig. 5), the results presented indicate that the peptides and polypeptides of the broad fascia of the thigh tissue joined by HFEW are almost completely

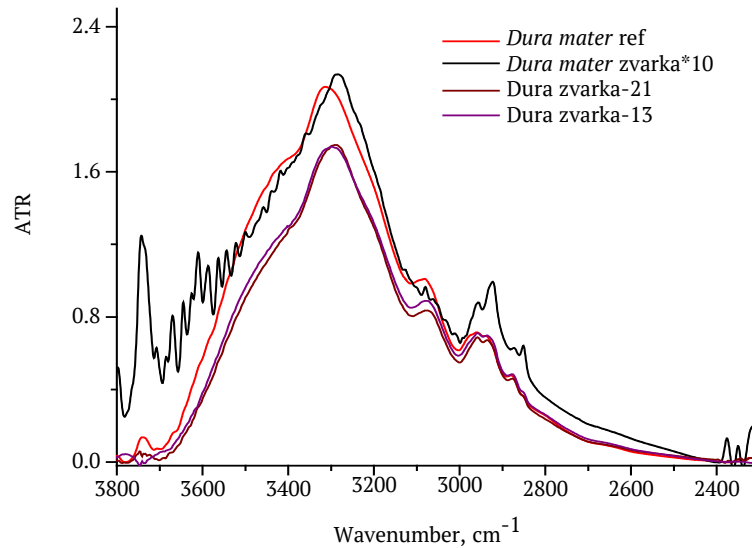
identical to normal broad fascia of the thigh tissue. The complete identity of the wave values of the amide A and B spectra, the preservation of the amide A and B peak, the preservation and bending of the N-H arm and the O=C-N band are observed.



**Figure 5.** Characteristic absorption bands of amide III waves protein of intact broad femoral fascia and broad femoral fascia protein connected by bipolar high-frequency electric welding in the infrared range  
**Source:** compiled by the authors

The computer analysis used to calculate the relative area of the bands of the components depicted in the spectrum of amide A and B (Fig. 6) indicates that the tissue in the area of HFEW compared to healthy *dura mater* is characterized by a bending of the N-H arm and oscillations of the amide A band (by 0.6 ATR (attenuated total reflectivity)),

the characteristic presence of the C=O band of oscillations at 3732  $\text{cm}^{-1}$  with the preservation of the amide A peak, the presence of valence vibrations of the side chains shifted by 0.4-0.1 ATR, the presence of overstretching of the  $\text{CH}_2$  band in the valence vibrations of the side chains at wave values of 2960-2850  $\text{cm}^{-1}$ .

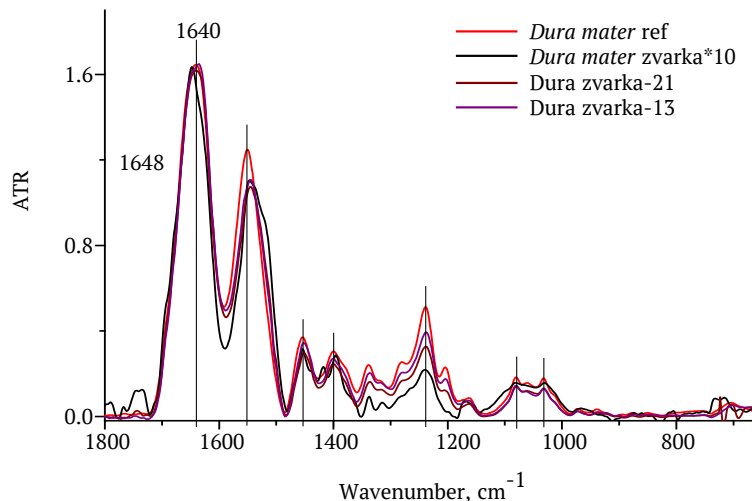


**Figure 6.** Characteristic protein absorption bands of amide I and amide II waves of intact *dura mater* and *dura mater* protein connected by bipolar high-frequency electric welding in the infrared range

**Source:** compiled by the authors

In the spectrum of amide I and II for the tissue in the area of the HFEW (Fig. 7) in comparison with healthy *dura mater*, there was a characteristic bend of the  $\text{NH}_3^+$  arm (amino group) in the region of the wave value  $1648\text{ cm}^{-1}$  (compared to  $1640\text{ cm}^{-1}$  of normal *dura mater* tissue), the absence of an arm in the region of  $1640\text{ cm}^{-1}$ , due to which the Amide I band is slightly narrowed and a low-frequency shift of the Amide II band (by  $0.4\text{ ATR}$ ) is recorded. In the wavenumber region at  $1575\text{ cm}^{-1}$ , a low-frequency shift of the Amide II band is observed with no change in the peak, an N-H bending and vibrations in the C-N band (by  $0.4\text{ ATR}$ ).

In the region of the wave value at  $1450\text{ cm}^{-1}$ , an asymmetric bending of the  $\text{CH}_3$  arm is observed with preservation of its value, in the region of the wave value at  $1400\text{ cm}^{-1}$ , deformation vibrations of the C=O band and symmetric stretching of the  $\text{CO}_2$  band are observed. In the region of the wave value at  $1230\text{ cm}^{-1}$ , a bending of the O=C-N arm (by  $0.42\text{ ATR}$ ) and a bending of the N-H band with the preservation of the Amide III band peak were detected. In the wavenumber region at  $1020\text{ cm}^{-1}$  and  $1090\text{ cm}^{-1}$ , a  $0.01\text{ (ATR)}$  bending of the N-H band is observed with the preservation of the Amide III band peak.



**Figure 7.** Characteristic absorption bands of amide III waves protein of intact *dura mater* and *dura mater* protein connected by bipolar high-frequency electric welding in the infrared range

**Source:** compiled by the authors

The results obtained indicate that the regenerative process occurs in the area of joining the *dura mater*, *dura mater* with the *aponeurosis*, and *dura mater* with the broad fascia of the thigh using HFEW instead of charring or coagulation necrosis. When using HFEW in the joint area,

the collagen structure and the fibrous structure of the broad fascia of the thigh were changed, but not damaged, and retained their function. Infrared spectroscopy studies showed that the peptides and polypeptides of the *dura mater* associated with HFEW were almost identical to normal

intact *dura mater* tissue. The complete identity of the spectral values of the amide A and B waves, the preservation of the amide A and B peaks, the preservation and curvature of the N-H arms and the O=C-N bands were observed.

When comparing tissue samples from the junction of the *dura mater* with the broad fascia of the thigh, joined by HFEW, the identity in the fluctuations of the amide bands A, B is preserved, the maximum spread allowed for the amide peak A, III. Fluctuations in the valence state of the side chains are allowed, and therefore it can be concluded that the structures of proteins (collagen and glycosaminoglycans) in the samples under study are alive, have minor signs of coagulation necrosis, and are capable of regeneration.

## ◆ DISCUSSION

A. Panteleichuk *et al.* [14] found in their experiment that the presence of the C=O vibration band in the region of  $1739\text{ cm}^{-1}$ , the absence of the arm in the region of  $1,667\text{ cm}^{-1}$  is characteristic of the *dura mater* scar tissue, due to which the Amide I band in the scar is significantly narrowed and a low-frequency shift of the Amide II band was detected. When evaluating the results of the study, it was found that in the area of the HFEW joining, the C=O vibration band at the joining of the *dura mater* with the *dura mater* shows a arm deformation of  $1,400\text{ cm}^{-1}$ , but there are no changes at the above frequencies, which means that there is no deformable scar in the joining area. When assessing the joining between the *dura mater* and the broad femoral fascia, there were no bendings or deformities of the C=O bands compared to the intact broad femoral fascia, indicating absolute elasticity of the connection using the HFEW and no scarring changes.

In the studies of A. Kinaci *et al.* [17], the Amide II wave value in the regions of  $1400\text{ cm}^{-1}$  –  $1600\text{ cm}^{-1}$  and the fluctuations of the N-H and C-N bands in the limit values from 0.1 to 0.7 ATR are acceptable and do not affect the hermetic state of the tissue. The results obtained during this study show that in the wavenumber region at  $1575\text{ cm}^{-1}$  a low-frequency shift of the Amide II band was detected with no change in the peak, N-H bending, and C-N band oscillations (by 0.4 ATR) were observed. An asymmetric bending of the CH<sub>3</sub> arm occurred in the region of the wave value at  $1450\text{ cm}^{-1}$  with its value preserved, and deformation vibrations of the C=O band and symmetric stretching of the CO<sub>2</sub> band occurred in the region of the wave value at  $1400\text{ cm}^{-1}$ , which are permissible according to the authors' studies. Thus, the changes in the C-N, N-H, and CH<sub>3</sub> bands obtained in this study are within the permissible range, and therefore the amides of the *dura mater* protein and the broad fascia of the thigh in the area of the junction are biologically active and perform their support functions without the formation of scar tissue.

According to P. Charalampaki *et al.* [18], when examining live tissue samples and studying the wave values of Amide III bands, scar tissue changes and coagulation necrosis of proteins are observed when the O=C-N arm appears at an ATR value above 1.0. For ATR values below 0.9, the O=C-N arm bend is an acceptable variation. The data obtained in this study indicate the presence of a bending of the O=C-N arm (by 0.42 ATR), a bending of the N-H band with the preservation of the Amide III band peak in the region of the wave value at  $1230\text{ cm}^{-1}$ . In other words, the

wave value describing the state of amide III at the joining using the HFEW is within the permissible limit values and indicates the absence of signs of scarring and vaporisation.

N. Ajayan *et al.* [19] investigated the fluctuations in the spectra of amide A and B. It was found that the fluctuations of the N-H arm, C=O, CH<sub>2</sub> bands in living tissues capable of regeneration should fluctuate within 0.65-0.1 ATR, and when this threshold is exceeded, fibroblasts cannot perform their functions and are prone to the formation of  $\beta$ -aggregates. The results obtained in this study indicate that in the HFEW region, compared to healthy *dura mater*, there is a characteristic bending of the N-H arm and fluctuations in the amide A band (by 0.6 ATR), the presence of valence vibrations of side chains shifted by 0.4-0.1 ATR, the presence of CH<sub>2</sub> band overstretching (by 0.3 ATR), i.e., in the area of the HFEW joining, proteins are capable of regeneration and there is no formation of scar tissue (namely,  $\beta$ -aggregates).

H. Mehidine *et al.* [20] found that when describing the results of the wave values of Amide III, the vibration of the N-H and O=C-N bands, while maintaining the support function of the amide and providing its basis for the extracellular matrix, the wave value of the bands should not exceed 0.53 ATR. According to the results of this experiment, in the area of the HFEW joining, the O=C-N arm bending (by 0.42 ATR), the N-H band bending with the preservation of the Amide III band peak by 0.01 (ATR), and the preservation of the Amide III band peak were observed at  $1230\text{ cm}^{-1}$ . Thus, the data obtained prove that the joining of the *dura mater* with the *dura mater*, *dura mater* with the broad fascia of the thigh, performed using HFEW, is strong, and the protein in this area is the functioning basis of the extracellular matrix skeleton, which is the basis of hermetic state.

According to the results of the study by A. Jaafar *et al.* [21], an important indicator in assessing the regenerative capacity of tissue is the presence of the NH<sub>3</sub><sup>+</sup> arm in the spectrum of amide I and II, for sufficient regenerative capacity of globular proteins in the area of the arm emergence, the arm should not exceed 0.5 ATR, which was confirmed by D. DePaoli *et al.* [22]. According to the findings of this study, in the area of HFEW, compared to healthy *dura mater*, there is a characteristic bending of the NH<sub>3</sub><sup>+</sup> arm in the region of the wave value of  $1648\text{ cm}^{-1}$  and in the region of  $1640\text{ cm}^{-1}$  with an ATR value of 0.4. In other words, in the area of the joining using HFEW, globular amide I and II proteins are regeneratively capable, which predicts a better course of tissue healing in the postoperative period. Thus, the new method of joining *dura mater* fabrics using high-frequency bipolar electric welding is an effective alternative to joining with suture material (polypropylene).

## ◆ CONCLUSIONS

Morphological data indicate that regeneration processes occur in the area of joining the *dura mater*, the *dura mater* and the broad fascia of the thigh, performed using HFEW, and not charring or the formation of coagulation necrosis. The structure of the collagen and fibrillar structures of the broad fascia of the thigh after the use of HFEW in the joining area is normal, altered but not damaged, with preservation of its functions. Studies using infrared spectroscopy indicate that the peptides and polypeptides of

the *dura mater* connected by HFEW are almost completely identical to normal tissue of the intact *dura mater*. A complete correspondence of the wave values of the spectra of amide A and B was observed, as well as the preservation of the peak for both amides, and the preservation and bending of the N-H arm and the O=C-N band. When comparing tissue samples of the *dura mater* and broad fascia of the thigh, joined by HFEW, the identity in the vibrations of the amide bands A, B is preserved, the permissible maximum change in the peaks of amides A, III, there are vibrations of the valence side chains that are permissible. Thus, it can be concluded that the structure of proteins (collagen and glycosaminoglycans) in the samples under study is alive, with virtually no signs of coagulation necrosis, and capable of regeneration.

The ability of high-frequency bipolar electric welding to connect the *dura mater* tissue and the broad fascia of the thigh, forming a connection that is alive, elastic, tight, and contains collagen that is fully capable of regeneration, was substantiated. Infrared spectroscopy and morphological data indicate that regeneration processes occur in the area of the *dura mater* joining made using high-frequency

bipolar electric welding, rather than charring or coagulation necrosis. The structure of the collagen protein in the area of this connection is normal, altered but not damaged, with preservation of its functions.

Future research should aim to develop a thermal resistor that will automatically turn off the bipolar high-frequency welding clamp when the temperature rises above 40 degrees Celsius and causes the threat of thermal damage and vaporisation around the brain tissue.

#### ◆ ACKNOWLEDGEMENTS

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#### ◆ CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## Біофізична оцінка ефективності застосування височастотного біполярного електрозварювання для закриття дефектів твердої мозкової оболонки при пухлинах лобних пазух з інтракраніальним поширенням

### Дмитро Ілліч Заболотний

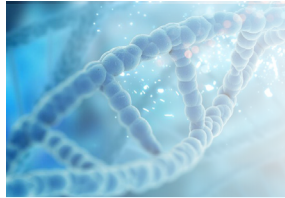
Доктор медичних наук, директор  
Державна установа «Інститут отоларингології ім. проф. О. С. Коломійченка  
Національної академії медичних наук України»  
03057, вул. Зоологічна, 3, м. Київ, Україна  
<https://orcid.org/0000-0001-9429-4414>

### Олена Михайлівна Кваша

Аспірант  
Державна установа «Інститут отоларингології ім. проф. О. С. Коломійченка  
Національної академії медичних наук України»  
03057, вул. Зоологічна, 3, м. Київ, Україна  
<https://orcid.org/0000-0003-2904-8748>

**Анотація.** Одним із основних завдань хірургічного лікування хворих з новоутвореннями лобної пазухи з внутрішньочерепним поширенням є непроникне сполучення субдурального простору, яке досягається герметично з'єднаною твердою мозковою оболонкою. Мета дослідження полягала у вивченні будови тканин з'єднання твердої мозкової оболонки та твердої оболонки з фасцією за допомогою біполярного електрозварювання. Були використані методи світлової мікроскопії та інфрачервоної спектроскопії. При оцінці результатів в досліджуваних тканинних зразках  $\beta$ -структурованих агрегатів не спостерігалось, отримано мінімальне травматичне пошкодження прилеглої тканини твердої мозкової оболонки, мінімальне термічне ураження від вапоризації, збереження грануляцій Паккіоні, венозних піальних і коркових судин твердої оболонки; повна ідентичність хвильових значень спектрів аміду А, В; аміду I, II; збереження піків аміду А, В; збереження та викривлення плеча N-H і смуги O=C-N в допустимих межах були змінені. Результати вказують на відсутність  $\beta$ -структурованих білкових агрегатів у зоні з'єднання, що виключає можливість утворення фіброзних структур і, як наслідок, не сприяє утворенню оболонково-мозкового рубця. Структура білка колагену в зоні з'єднання нормальна, змінена, але інтактна, зі збереженням його функцій. Це свідчить про доцільність використання методу височастотного біполярного електрозварювання для хірургічного закриття дефектів твердої мозкової оболонки, як альтернативи закриттю шовним матеріалом

**Ключові слова:** електрохірургія; інфрачервона спектроскопія; дефекти твердої оболонки; витік спинномозкової рідини; лікворея



## Organizational and methodological principles of teaching the elective discipline “Modern Aspects of Medical Parasitology” for students of the Educational and Research Institute of Nursing

### Larysa Fedoniuk

Doctor of Medical Sciences, Professor, Head of Department  
I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine  
46001, 1 Maidan Voli, Ternopil, Ukraine  
<https://orcid.org/0000-0003-4910-6888>

### Nelia Hlyvka\*

PhD in Biological Sciences, Assistant Professor  
I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine  
46001, 1 Maidan Voli, Ternopil, Ukraine  
<https://orcid.org/0000-0002-8568-088X>

### Yaroslav Stravskyy

Doctor of Veterinary Sciences, Senior Researcher, Senior Lecturer  
I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine  
46001, 1 Maidan Voli, Ternopil, Ukraine  
<https://orcid.org/0000-0001-6541-9097>

**Abstract.** Based on the training of nurses according to the generally accepted international educational standards, the most effective way to attain a higher standard of education for future specialists is to incorporate optional courses that provide a novel approach to learning. The purpose of this study was to present the experience of the Department of Medical Biology of the I. Horbachevsky Ternopil National Medical University in the development and implementation of the elective discipline “Modern Aspects of Medical Parasitology” in the educational programme “Nursing” of the first (bachelor’s) level of higher education in the branch of knowledge 22 Healthcare, specialty 223 Nursing. The study analysed the theoretical and methodological foundations of conducting classes in the elective discipline “Modern Aspects of Medical Parasitology”. It was found that the educational and methodological complex, which corresponds to the curriculum and is developed based on the regulatory documents of the educational programme “Nursing”, enables students to master, expand, and improve the level of mastery of the initial material, develop an individual approach to solving the tasks according to the goals and programme results. The paper points out the importance of forming a holistic understanding of parasitism as a form of relationship between organisms in nature. The study covered the teaching methods employed for the elective course “Modern Aspects of Medical Parasitology” in a higher education institution. It analysed how using teaching aids, including museum exhibits in the educational process and the role of the Department of Medical Biology, enhances the effectiveness of students’ learning activities in studying the fundamentals of medical parasitology. The incorporation of highly visible means, information and communication technologies, well-designed educational and methodological support, and impeccably organised classroom activities will enhance the quality, content, and principles of educational services offered in the educational programme “Nursing”, speciality 223 Nursing

**Keywords:** educational process; medical biology; medical parasitology; speciality 223 Nursing; nursing

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\*Corresponding author



## ◆ INTRODUCTION

Modern nursing education requires improvement of the quality, content, and principles of educational services, which determine the complex of professionalism, modern technical means of education, pedagogical teaching technologies and educational standards. The best strategy for achieving a conceptually new level of quality of training of future specialists based on the training of nurses according to generally accepted international educational standards is the introduction of elective disciplines that provide a new approach to teaching.

I. Dobroskok *et al.* [1] believe that the reform of higher education in Ukraine requires a unified methodological approach to different disciplines, such as medicine, technology, and pedagogy. S. Gordiyuk [2] notes that education underlies Ukraine's competitiveness, and its political and economic independence reflects an important trend in the modern world. It is education that enables the continuous development of abilities and shapes the life aspirations of the population. The higher the level of education a person has, the more opportunities they have to create better living and working conditions.

Parasitic illnesses are still prevalent worldwide, particularly in less developed nations. Helminth infections constitute 89% of all parasitic infections, posing significant public health threats [3]. According to the World Health Organization (WHO), one in four people is infected with parasitic worms [4]. N.V. Dovganych & N.I. Frych [5] note that over 300 species of helminths and 18 species of protozoa cause diseases in most tropical countries and rank fourth among other diseases in all parts of the world, behind only coronary heart disease, tuberculosis, and diabetes mellitus. They affect billions of people, killing millions every year and causing debilitating injuries. Parasitic diseases can manifest themselves with various clinical symptoms, depending on the tissue affected. Therefore, as the occurrence of parasitic diseases in the field of nursing, both therapeutic and surgical, increases, it is vital to elevate the standard of education and preparedness within the educational system for their professional duties [6].

In 2008, Ukraine implemented a nursing education system that aligns with the educational structure of numerous countries worldwide, including Canada, England, and the United States of America. The system features training at international standards levels. The USA has implemented novel training and development initiatives. These include certifying nurse managers, licensing nursing practices, providing fundamental and advanced nursing education, conferring the academic rank of professor, devising nursing master's programmes and doctoral studies in the same field. In 1973, the American Nurses Association established the National Academy of Nursing Sciences, which has taken a leadership position in the organisation of nursing. Nursing education in the United States is considered one of the best in terms of quality and effectiveness. The training is based on a phased system leading to the licensing exam. Graduates of nursing colleges have a wide range of career opportunities, including independent practice and working with patients. Having analysed, for example, the English and American models of nursing education separately, it can be noted that they are fundamentally different, but equally

important for the formation of nursing in the national system of many countries [7]. Nursing education in European countries is provided in nursing schools and colleges in the UK, Hungary, Sweden, and higher education in nursing – in institutes, universities, and academies in Denmark, Greece, Ireland, Spain, Estonia, and Poland [7, 8]. Nursing students in Ukraine are trained at Bukovinian State Medical University, Kharkiv National Medical University, and Zhytomyr Institute of Nursing [9].

With the increasing prevalence of parasitic diseases in nursing, it is crucial to improve education and training in both therapeutic and surgical settings. Therefore, searching for standards in medical education is the best approach to attain a conceptually modernised level of training quality. This is based on training medical personnel in line with widely recognised global educational standards. The study of the discipline "Medical Parasitology" is based on the mastery of parasitological concepts, theories, and a review of scientific evidence and facts, which forms the basis for the training of highly qualified specialists and the formation of a scientific worldview. Therefore, the quality of diagnostics and the ability to conduct it substantially affects the tactics of sampling, research methods and principles of further treatment and care of patients [10].

The growing importance of parasitic diseases in the overall structure of morbidity in the population necessitates the improvement of the relevant training of health-care professionals. Therefore, the problem of forming professional competences of nursing specialists is a priority task of many academic disciplines, including the elective discipline "Modern Aspects of Medical Parasitology", which is designed to develop professional and communicative competences. The purpose of this study was to cover the specific features of acquiring professional skills and abilities of future nurses in studying the elective discipline "Modern Aspects of Medical Parasitology".

## ◆ MATERIALS AND METHODS

The programme in the discipline "Modern Aspects of Medical Parasitology" is designed for higher education students at the I. Horbachevsky Ternopil National Medical University (TNMU) of the Ministry of Healthcare of Ukraine under the educational programme 223 Nursing, according to the working curriculum, which is annually discussed and approved at a meeting of the Academic Council of the University. The purpose of the elective is to help students understand the fundamental concepts of development and systematic knowledge of living organisms, their biotic relationships in nature, as well as the life cycles of parasites and parasitic diseases that affect humans.

"Modern Aspects of Medical Parasitology" is an educational subject that builds upon the fundamental knowledge acquired in elementary and high school's biological component of the educational field "Natural Science". It provides relevant knowledge, competencies, and program learning outcomes for further assimilation by students in higher education, specializing in theoretical and clinical disciplines such as microbiology with immunology basics, pharmacy hygiene, and ecology.

According to the curriculum of Ternopil National Medical University, the elective discipline "Modern Aspects

of Medical Parasitology”, which is studied by students of speciality 223 Nursing in the first year of the Educational and Research Institute of Nursing at TNMU, includes 90 hours, of which 16 are lectures, 24 are practical classes, and 50 hours are for independent study. The subject “Medical

Parasitology” is studied in the 1st year, in the second semester. Completion implies a type of control: credit.

The programme of the elective discipline “Modern Aspects of Medical Parasitology” includes 6 topics that fully reflect the content of the discipline’s sections (Table 1).

**Table 1.** Structure of the elective discipline “Modern Aspects of Medical Parasitology”

Topic titles	Lectures	Practical sessions	Independent work
1. The phenomenon of parasitism, its forms, distribution and role in nature.	2.0	0	10.0
2. Medical Protozoology. Kingdom <i>Protozoa</i> . Type <i>Sarcomastigophora</i> . Type <i>Ciliophora</i> . Class ( <i>Zoomastigophora</i> ). Type <i>Apicomplexa</i> . Class <i>Sporozoea</i> .	2.0	6.0	5.0
3. Medical Helminthology. Type <i>Plathelminthes</i> . Class <i>Trematoda</i> . Class <i>Cestoidea</i> .	4.0	4.0	15.0
4. Medical Helminthology. Type <i>Nemathelminthes</i> . Class <i>Nematoda</i> .	2.0	6.0	0
5. Methods of laboratory diagnostics of helminthic diseases.	2.0	2.0	10.0
6. Medical Arachnoentomology. Type <i>Arthropoda</i> . Class <i>Arachnoidea</i> . Class <i>Insecta</i> .	4.0	6.0	10.0
<b>TOTAL</b>	<b>16 hours</b>	<b>24 hours</b>	<b>50 hours</b>

Source: [11]

The curriculum includes the main types of classes: lectures; practical classes; independent work of students and consultations. The work programme includes 12 practical sessions. The following teaching methods are used in the study of the discipline “Modern Aspects of Medical Parasitology”: verbal (explanation, conversation, narration); practical (experiment, practical work); explanatory and illustrative (natural objects, educational literature, lectures, videos, etc.); reproductive; analytical; productive; problem-based presentation of material; research.

## ◆ RESULTS

Since 2015, the I. Horbachevsky Ternopil National Medical University has started training higher education students in the speciality Nursing. Over 4000 people have received specialised education at the TNMU. Since 2022, several academic disciplines have been added to the curriculum, including an elective discipline at the Department of Medical Biology – “Medical Parasitology” [11]. The educational process for the implementation of this discipline is based on the following three main principles that ensure an optimal approach to teaching and learning: what needs to be learned; how to learn; why it needs to be learned.

The principle “What needs to be learned” determines the scope and content of the discipline and its integration with other disciplines of biomedical and clinical orientation. For this, the department’s teachers have developed a working curriculum and a syllabus for the discipline, which clearly structured thematic plans for lectures, practical classes, and topics for independent study of the discipline [11]. The curriculum includes the main types of classes: lectures; practical classes; independent work of students and consultations.

The lectures cover the key issues encountered in the discipline, focusing on the theoretical aspects of one or

multiple topics. Specifically, the lecturers provide a detailed account of the general principles pertaining to the organisation and biology of protozoa, helminths, and arthropods. They also analyse the morphophysiological characteristics, patterns of parasite life, ecological and ethological features, and life cycle. Additionally, the main methods adopted for the laboratory diagnosis of parasites are discussed. The training material is presented through multimedia presentations, which helps to improve the assimilation of information.

During practical classes, students, together with their teachers, study theoretical concepts in detail and develop skills and abilities to apply them through individually defined tasks and solving situational problems. Students study the biological basis of parasitism and the role of parasites in the emergence of vector-borne and naturally occurring diseases. They also examine the influence of environmental factors on the evolution of members of the Kingdom *Protozoa*, Types Flatworms and Roundworms, and Type *Arthropoda*. Additionally, students explore the pathological effects of parasites on the human body and acquire knowledge on the effectiveness of preventing parasitic diseases based on the route of infection. In the study of medical parasitology, the teachers pay special attention to the principles of laboratory diagnosis of human parasitic infections, the understanding of which is based on the students’ knowledge of the structure of parasites of various systematic groups and their life cycles, the regularities of the components of the parasite-host system, as well as methods of preventing parasitic diseases.

Students’ independent work involves studying certain topics of the educational material in the hours free from primary classes and includes preparation for all types of control tasks. Specifically, students study the biology of pathogens of human parasitic infections; the basics of

classification of parasites and hosts; ways, mechanisms of transmission of pathogens, and principles of their entry into the human body; biological features of vectors and pathogens of natural environment diseases; structural components of the natural environment; medical significance of representatives of the Arthropoda as carriers of pathogens of human infectious diseases; representatives of certain groups of poisonous plants, animals, and fungi. The material studied in the areas defined by the curriculum, which students master in independent work, is submitted for final control in the form of a test together with the material learned in classroom classes. Tutorials are conducted to assist students in comprehending and elucidating challenging topics, resolving intricate issues encountered while studying educational materials, in readiness for practical lessons or assessments.

The principle "How to learn" is based on certain principles of teaching and learning, using which teachers convey educational and cognitive information to students in the form of individual and collective constructive tasks and practical skills, considering the individual characteristics of students. The main forms of this principle consist of natural object visualisation, which demonstrates the structural organisation of parasites on macro preparations and their morphological features on micro preparations, and artificial visualisation, which involves displaying videos on the life cycles of helminths, existing models of helminth strobe structures, as well as diagrams, graphs, and tables.

However, the success of the lesson relies greatly on the students' preparation for it. In this regard, one of the key parts of the educational and methodological work is the organisation of students' self-preparation for classes, which is ensured by the availability of teaching materials, consultative assistance from the teacher, and the possibility of using technical learning tools. To create the most complete picture of the content of the wildlife around us and the interrelationships of individuals among themselves, the Department of Medical Biology has an exhibition "Medical and Biological Aspects of Parasitism", which was created in the I. Yaremko Educational and Biological Museum. The museum exposition is based on the principle of interaction and biotic relations between parasite and host at the level of parasitic relationships between individuals. The exhibition materials demonstrate the natural occurrence of parasitic diseases and infestations, presenting a clear picture of the connection between an animal's lifestyle and the manifestation of parasitic infestation. The display cases of this exhibition contain educational information about 13 diseases caused, transmitted or hosted by animals, which are located in special containers on the walls of the educational museum. The exposition also illustrates the systematic position, structural features, developmental cycles of parasites, ways of infection and entry of the parasite into the human body, clinical picture, laboratory diagnostics and methods of prevention of 7 infectious and 6 invasive diseases.

Every year, the department's educators analyse the impact of the visual material in the department's educational museum on the quality of students' learning. They also determine how audience-teacher interactions can contribute to excellent learning and better assimilation of theoretical material. The expositions of the Educational and Biological Museum play a significant role in the study of the structural

organisation of the human body, the structure of multicellular individuals, and the specific features of their vital activity. The students take part in thematic excursions, which aim to provide a comprehensive and in-depth understanding of the materials in the natural history exhibition. The presentations and videos about intracellular parasites and their harmful impact on human health merit a favourable evaluation. The museum's exhibits are of great importance in practical classes in medical parasitology. They elucidate the principles of parasite-host interaction, furnishing a foundation for comprehending the fundamental tenets of parasite prevention in the examination of venomous creatures. Additionally, it sheds light on the anthropogenic impact on natural ecosystems. Students positively evaluate schemes that contain information about clinical symptoms and syndromes of parasitic diseases, as well as methods of laboratory diagnosis of infections. This aids in the comprehension of parasite life cycles, their means of transmission, and preventive measures for students. As a result, students develop a more profound understanding of these diseases. Furthermore, thanks to the museum exhibits, students gained a better insight into micro- and macroevolutionary processes.

The principle "Why it need to be learned" is based on motivation: when mastering each topic or part of the section, not only the purpose of certain biological facts in the system of science is indicated, but also their place in the life and activities of the student as a future doctor is revealed.

To implement the above principles of teaching, the team of teachers prepared a textbook "Medical Parasitology: Laboratory Guide", which reflects the latest achievements in the study of certain issues in medical protozoology, helminthology and arachnoentomology [11]. The laboratory guide comprises 12 practical classes with a logical progression of materials. Students are given the chance to illustrate the physical characteristics and internal structure of human parasitic pathogens, draw developmental cycle diagrams for various classes of parasites, complete tables outlining pathogen morphology and functional characteristics, pathogenic effects and infection methods. The manual also contains didactic material for self-study, a list of theoretical questions for knowledge control, test tasks for self-checking, a dictionary of fundamental concepts and terms, which ensures the activation of students' learning activities during practical classes. The use of protocols is a methodological technique that activates the student's attention and develops their observation skills.

For better learning of the educational material, using pedagogical and methodological techniques that improve the level of learning, the teachers of the department use a system of visual analysis of micropreparations. This system transmits the image of a specimen from a light microscope through a video camera to a TV screen, where, according to the set magnification of the microscope, the structures in the field of view can be studied. This system enables comprehensive and simultaneous analysis of images in the presence of a large audience of students. It facilitates detailed study without subjective evaluations. The use of the visual analysis system is widely used by teachers when students perform practical work. With this system, teachers also conduct oral and written tests to determine students' knowledge of the structural organisation of parasites. The use of interactive technologies for collective group learn-

ing (case-method, brainstorming, general circle); technologies for processing discussion issues (defining a position, defending it, changing a position, debating); elements of distance learning (MS Teams, Google Meet, etc.) also contribute to more effective learning.

Mastering practical skills is compulsory for students majoring in 223 Nursing during the academic year. Matriculation of practical skills, which students complete upon finishing the study of the discipline, includes the acquisition of practical skills, some of which are incorporated into the curriculum of the respective program. Compilation of skills, in addition to practical implementation, requires the student to possess thorough knowledge and understanding of their theoretical aspects at a suitable level of mastery. At the Department of Medical Biology, students of the Educational and Research Institute of Nursing learn three practical skills when studying medical parasitology: how to use a light microscope; microscopic analysis of permanent micro preparations of protozoa with the determination of the type of parasite and its medical significance; identification of arthropods using a magnifying glass or light microscope and indicating their medical significance.

According to the working curriculum for the elective discipline "Modern Aspects of Medical Parasitology", students can receive additional points for their individual work at the Department of Medical Biology. This work involves the development of students' abilities in an individually directed way and realises their creative potential through research and creative activity. Students can get additional points for completing biological crosswords in the section of medical parasitology, selecting video and audio materials, and selecting exhibits for the I. Yaremenko Educational and Biological Museum.

The Department of Medical Biology of TNMU annually involves students at the Institute of Nursing in the work of scientific forums held at the Department of Medical Biology and the University [12]. Scientific and practical conferences highlight the main results of the student's own research under the guidance of a supervisor, thereby developing the ability to solve creative and innovative problems.

Thus, developing reputation, raising the rating and competitiveness are key tasks necessary to maintain professional competence. The motivation of teachers and the effectiveness of pedagogical work reflects the attitude of students to education and the intensity of the educational process. The formation of professionalism is an important quality of the educational process, especially when it comes to higher medical and nursing education. The Department of Medical Biology has developed a methodological complex to enhance the standard of training in the speciality 223 Nursing. However, the level of organisation of the educational process relies on several factors like students' interest, cognitive abilities, skill formation, and practical expertise.

## ◆ DISCUSSION

The emergence of the Coordination Council and working groups on curriculum development, including WHO experts, has led to significant changes in the content and new conditions of nursing education in Ukraine and Europe. This has resulted in revised requirements, the development of applied research in nursing education, and improvement of curricula and programmes [13].

Nursing education in European countries is carried out pursuant to regulations and with the innovative processes taking place in the healthcare system. In most European countries, namely in Spain, Greece, Sweden, and Estonia, the period of study to obtain a professional level of a registered nurse is 3 years, in Belgium, Austria, Denmark, the UK, and the Netherlands – 3.5 years [9]. Unlike European countries, the American model of nursing education is comprehensive and differs substantially in its structure from the one adopted in Ukraine and Europe. Given the legislative requirements, there are several educational degrees in the field of nursing education in the United States: Licensed Practical Nurse (LPN), Associate Specialist Nurse (ASN), Bachelor of Nursing (RN to BSN), Master of Nursing (MSN), Doctor of Nursing (DNS) [8]. In Ukraine, there is a graduate nursing education: junior specialist (accreditation level I) and bachelor's degree – nurse (accreditation level II) [14].

The examination of research conducted by V.P. Pishak & Y.I. Bazhora [15], S.K. Sharma *et al.* [16] suggests that elements which contribute to the standard of education comprise of not solely the professional preparation and personal traits of teachers, but also the educational and methodological support during the teaching process. The inclusion of optional subjects in higher education curricula is due to contemporary teaching methods and the aim of students gaining competent knowledge and skills. This position is supported by S. Gordiychuk [2] and O.S. Isaieva & I.R. Khmiliar [17].

The Department of Medical Biology employs diverse methodological tools and training complexes to facilitate the development of both general and specialised competencies in students. These competencies encompass knowledge, practical skills, and the ability to effectively utilise information, communication, and modern educational technologies. The study of elective disciplines by students, their involvement in the educational process and research activities contributes to the training of qualified medical specialists. The analysis of sources by O.M. Khrystenko *et al.* [18] and J.H. Xu [19] complement the results on improving the forms and methods of training qualified specialists and improving the educational process in higher education institutions.

The implementation and research data correspond to the findings of C.H. Rushton *et al.* [20] concerning the development of higher education students' capacity to work accurately with diverse information sources and adhere to scientific ethical norms. This is of utmost importance for teachers in shaping a high-quality education. It is important to promote true moral and ethical values, including academic integrity, among the student community, especially first-year students [21, 22].

To improve the perception of educational and methodological material, educational museums are being created in higher education institutions to help students understand the laws of science by demonstrating natural objects and preserving their appearance in everyday life. Museums, as an educational system, reveal the role and functions of science in a new way, emphasise its importance in the modern world, and shape the worldview of visitors and understanding of society as a whole [23].

According to L.Y. Fedoniuk *et al.* [24], among the various visualisations, the first place should be taken by natural objects that create an idea of the content of the

surrounding wildlife. The museum's exhibits also reflect the relationship between organisms and the environment, flora and fauna, form an idea of the structure of multicellular organisms, and also form a body of knowledge about the laws of nature. Thus, they provide the basis for learning the basics of human parasites and parasitic infections, while developing students' ability to think abstractly.

To deliver superior healthcare and attain expertise in the field, higher education students must possess the ability to evaluate parasitic diseases, demonstrate the natural focal and transmissible categorization of human illnesses, and recognize pathogens and vectors through macro- and micropreparations for diagnosing parasitic diseases. Students must be competent in identifying the different stages of the life cycle of human parasites, substantiating the key laboratory diagnostic methods, utilizing laboratory techniques for diagnosing infestations, elucidating the morpho-functional characteristics of parasites and their developmental cycles, ascertaining modes of infection by parasites, methods of laboratory diagnosis of parasitic diseases, and preventive measures. The acquired knowledge lays the foundation for attaining a proficient level of job readiness within the chosen field [25]. According to O.P. Kravchenko [26], the introduction of auxiliary nurse training in Ukraine will improve the quality of medical services for patients in need of skilled care in hospices, nursing homes and at home, which will be cost-effective.

Thus, the study found that the use of methodological materials of the Department of Medical Biology allows not only to improve the cognitive activity of students in solving educational tasks, but also to improve the quality of professional orientation and independence in combination with an individual approach to learning.

## ◆ CONCLUSIONS

The implementation of the elective course "Modern Aspects of Medical Parasitology" in the "Nursing" programme for students pursuing a Bachelor's degree in the branch 22 Healthcare (speciality 223 Nursing) at the I. Horbachevsky Ternopil National Medical University, and the subsequent analysis of the curriculum revealed that independent cognitive activity is the primary mode of education and plays a crucial role in fostering students'

## ◆ INTRODUCTION

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motivation, sense of responsibility, and their ability to apply theoretical knowledge in practical settings. Therefore, the training of future nurses in higher education medical institutions should contribute to the reform and modernisation of the higher nursing education system, since the quality of education is determined by educational standards and includes a set of modern technical means of teaching, pedagogical technologies and quality of learning.

The incorporation of contemporary pedagogical approaches, combined with a cohesive integration of theoretical and practical aspects within the curriculum, is particularly beneficial in the field of medical parasitology. Such measures optimise the learning process, resulting in enhanced comprehension and retention of the subject matter, thereby facilitating its practical application in future practice. The I. Yaremenko Educational and Biological Museum plays a significant role in the educational process of the I. Horbachevsky Ternopil National Medical University of the Ministry of Healthcare of Ukraine. The museum's expositions exhibit the principles of interaction between parasites and hosts, which creates a positive influence on the professional training of nurses by enabling students to understand the basic methods of preventing parasitic diseases.

Prospects for further research are to implement the elective discipline "Modern Aspects of Medical Parasitology" in the educational process at the Department of Medical Biology of the I. Horbachevsky Ternopil National Medical University for applicants for the second (master's) level of higher education in the branch 22 Healthcare, speciality 223 Nursing. Also promising is the advancement of global collaboration in nursing, including the creation of workable curricula and the adjustment of educational material to meet international standards. Such a process will be essential for enhancing the training of competent nurses and effectively integrating the Ukrainian higher nursing school into the wider European community.

## ◆ ACKNOWLEDGEMENTS

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## ◆ CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## Організаційні та методичні засади викладання вибіркової дисципліни «Сучасні аспекти медичної паразитології» для студентів навчально-наукового інституту медсестринства

### Лариса Ярославівна Федонюк

Доктор медичних наук, професор, завідувач кафедри  
Тернопільський національний медичний університет імені І. Я. Горбачевського МОЗ України  
46001, майдан Волі, 1, м. Тернопіль, Україна  
<https://orcid.org/0000-0003-4910-6888>

### Неля Богданівна Глипка

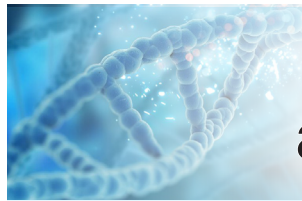
Кандидат біологічних наук, асистент  
Тернопільський національний медичний університет імені І. Я. Горбачевського МОЗ України  
46001, майдан Волі, 1, м. Тернопіль, Україна  
<https://orcid.org/0000-0002-8568-088X>

### Ярослав Степанович Стравський

Доктор ветеринарних наук, старший науковий співробітник, старший викладач  
Тернопільський національний медичний університет імені І. Я. Горбачевського МОЗ України  
46001, майдан Волі, 1, м. Тернопіль, Україна  
<https://orcid.org/0000-0001-6541-9097>

**Анотація.** Базуючись на підготовці медичних сестер, відповідно до загальноприйнятих міжнародних освітніх стандартів, найкращою стратегією для досягнення якості підготовки майбутніх спеціалістів на новому рівні є запровадження факультативів, які пропонують новий підхід до навчання. Метою роботи було представити досвід кафедри медичної біології Тернопільського національного медичного університету імені І. Я. Горбачевського щодо розробки та впровадження у навчальний процес вибіркової дисципліни «Сучасні аспекти медичної паразитології» за освітньою програмою «Сестринська справа» першого (бакалаврського) рівня вищої освіти галузі знань 22 Охорона здоров'я, спеціальності 223 Медсестринство. В статті проаналізовано теоретико-методологічні засади проведення занять з вибіркової дисципліни «Сучасні аспекти медичної паразитології». Встановлено, що навчально-методичний комплекс, який відповідає навчальному плану та розроблений на основі нормативних документів освітньої програми «Сестринська справа», дає можливість здобувачам опанувати, поглибити а також вдосконалити рівень засвоєння начального матеріалу, розвинути індивідуальний підхід до вирішення поставлених завдань відповідно до цілей та програмних результатів. У роботі вказано на важливість формування у здобувачів цілісної уяви про паразитизм, як форму взаємовідносин між організмами в природі. Разом із тим, розкрито роль форм навчання, які застосовуються під час викладання вибіркової дисципліни «Сучасні аспекти медичної паразитології» у закладі вищої освіти, а також аналізується ефективність використання засобів навчання для організації навчальної діяльності студентів під час вивчення основ медичної паразитології, зокрема ролі музейних експонатів у освітньому процесі і кафедри медичної біології. Використання засобів із високим ступенем наочності, інформаційно-комунікаційних технологій, добре продумане навчально-методичне забезпечення, правильно організована аудиторна діяльність студентів дозволить удосконалити якість, зміст і принципи надання освітніх послуг за освітньою програмою «Сестринська справа» спеціальності 223 Медсестринство

**Ключові слова:** освітній процес; медична біологія; медична паразитологія; спеціальність 223 Медсестринство; сестринська справа



## Modern methods of researching autonomic functions in children with syncope: A literature review

**Tetiana Kovalchuk\***

PhD in Medical Sciences, Associate Professor

I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine

46001, 1 Maidan Voli, Ternopil, Ukraine

<https://orcid.org/0000-0003-2455-3278>

**Abstract.** The research of autonomic functions in children with non-cardiogenic syncope allows improving differential diagnosis and treatment tactics in children depending on the pathogenetic mechanisms of syncope development. The purpose of the research was to analyse modern scientific achievements in the field of autonomic functions in children with different types of syncope. The review used the full texts of English-language studies published between January 2018 and December 2022 and published in the PubMed Medline and Scopus databases. It has been established that the active orthostasis test, tilt test, circadian blood pressure rhythms and heart rate variability are the most commonly used functional autonomic tests in paediatric practice. They allow evaluating the spectrum of pathological cardiovascular reactions in a standing position; identifying types of orthostatic hypotension and causes of orthostatic intolerance; diagnosing orthostatic hypertension, postural orthostatic tachycardia syndrome, presyncope or syncope; to differentiating between sympathetic and parasympathetic autonomic dysfunctions and psychogenic transient syncope and epilepsy; to recommend orthostatic training as a method of treatment of syncope with an orthostatic mechanism of development. Despite this, there is no consensus on the definition of autonomic disorders and the methodology for conducting functional autonomic tests in children of different ages, considering their gender, body mass index and time of assessment during the day. Thus, functional autonomic tests are additional methods of physical and instrumental examination of the patient that allow effective assessment of the autonomic nervous system and possible mechanisms of syncope development, differentiation of transient loss of consciousness, stratification of future risks and optimisation of treatment and preventive tactics of the child's management based on an individual patient-centred approach

**Keywords:** nervous system; autonomic dysfunction; functional tests; vasovagal syncope; syncope due to orthostatic hypotension; children

### ✦ INTRODUCTION

Since the 2000s, significant progress has been made in understanding the various aspects of syncope diagnosis and management. A significant part of this progress has been in the development of clear criteria for the diagnosis of syncope, according to the European Society of Cardiology (ESC) 2018 revision [1]. These guidelines call attention to the importance of conducting functional autonomic tests in patients with syncope as a manifestation of dysautonomia, as they provide an objective and quantitative assessment of the integrity and reactivity of autonomic nerves, ganglia, and the central nervous system in general. Since the autonomic nervous system is not available for direct physiological testing, clinical autonomic tests usually allow evaluating

of the response of an end organ to a specific physiological provocation. The search for new effective methods of assessing autonomic functions in children with syncope is one of the priority areas of modern medicine, which will allow expanding the understanding of the pathogenetic mechanisms of syncope development and evaluating the effectiveness of treatment measures in the long term.

It is well-known that the phenomenon of syncope is the result of transient global cerebral hypoperfusion, which, regardless of the initiating mechanism, occurs due to a decrease in the activity of vasoconstrictor neurons and an increase in the parasympathetic activity of cardio-motor neurons [2]. The complexity of these mechanisms

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\*Corresponding author



determines the significance of assessing the autonomic nervous system in patients with syncope, which involves studying the reactivity of the heart and/or blood pressure (BP) in response to tests such as active orthostasis, tilt test, Valsalva test, hyperventilation test, analysis of circadian BP rhythms and heart rate variability, etc [3]. Functional autonomic tests can help determine autonomic dysfunction as the main cause of classical orthostatic hypotension, characterise cardiovascular sympathetic and parasympathetic autonomic function, and assess the severity of autonomic dysfunction [4].

A.P. Wang *et al.* [5], Y. Cui *et al.* [6], M.R. Khalilian *et al.* [7] substantiate the significance of the research, quantification and topical diagnosis of autonomic dysfunction in the verification of vasovagal syncope and syncope due to orthostatic hypotension in childhood. The research of B. Gu *et al.* [8], T. Kovalchuk *et al.* [9] demonstrated that sympathetic function is dominant in children with vasovagal syncope under non-provocative conditions. Changes in such parameters as heart rate variability, QT interval dispersion, P-wave dispersion, late ventricular potentials, and the ability to slow the heart rate (HR) may indicate autonomic nervous system dysfunction and provide a new therapeutic target in the treatment of noncardiogenic syncope [10]. The results of observations by M. Kisanuki *et al.* [11], F. Aghajani *et al.* [12] indicate that training in autonomic functions is an effective method of treating patients with syncope, provided that autonomic dysfunction is involved in the pathogenesis of transient loss of consciousness. Thus, the research on autonomic nervous function in patients with non-cardiogenic syncope is useful both from the standpoint of a better understanding of syncope mechanisms and their diagnosis and treatment.

The purpose of this work was to analyse the current scientific achievements in the research of autonomic functions in children with different pathogenetic mechanisms of syncope development – vasovagal syncope, syncope due to orthostatic hypotension and cardiogenic syncope. The review focuses on autonomic functional tests that are considered by experts to be scientifically valid, reliable and clinically useful.

To analyse the data, the PubMed Medline and Scopus databases were used, using the following search terms: “vasovagal syncope” and “autonomic functions”; “syncope due to orthostatic hypotension” and “autonomic functions”; “cardiogenic syncope” and “autonomic functions”. The research includes full texts of studies published in English between January 2018 and December 2022. A total of 294 scientific publications were identified – 187 in PubMed Medline and 107 in Scopus. Due to duplicates, 155 sources were excluded from the research. After screening 139 publications, 104 scientific works were excluded from the search due to irrelevant research subjects, lack of full-text versions of articles and the results of own research. Thus, 35 scientific publications were checked for relevance to the subject of the search.

#### ✦ USING ACTIVE AND PASSIVE ORTHOSTASIS TESTS IN THE ASSESSMENT OF AUTONOMIC FUNCTIONS IN CHILDREN

The active orthostasis test is used to evaluate the spectrum of pathological cardiovascular reactions in the standing

position, to establish the types of orthostatic hypotension and causes of orthostatic intolerance, to diagnose orthostatic hypertension, postural orthostatic tachycardia syndrome, presyncope or syncope, and to determine autonomic function and dysfunction [13]. The initial decrease in BP during the first 10 seconds of active orthostasis reflects the discrepancy between the increase in cardiac output due to the outflow of blood from the lower extremity muscles and its deposition in the abdomen and the instantaneous vasodilation of the leg vessels. Short-term mechanisms of the baroreflex are accompanied by a rapid increase in HR (within 1-3 s), cardiac contractility (within 3-8 s), peripheral arterial and venous vasoconstriction (within 10-30 s), which lead to the recovery of BP. In most healthy people, these compensatory mechanisms are characterised by the restoration of baseline BP values within 20-30 s [14].

The spectrum of pathological haemodynamic reactions that are most often identified during an active orthostasis test with continuous BP monitoring is as follows [14]: initial orthostatic hypotension – a decrease in systolic blood pressure (SBP)  $> 40$  mm Hg and/or diastolic blood pressure (DBP)  $> 20$  mm Hg within 15 s of standing in the absence of persistent orthostatic hypotension; late recovery – delayed or inability to restore BP to  $\leq 20$  mm Hg from baseline values after 30-40 s of active orthostasis in the absence of criteria for classical orthostatic hypotension; classical orthostatic hypotension – a persistent decrease in SBP  $\geq 20$  mm Hg or DBP  $\geq 10$  mm Hg, that occurs between 60 and 180 seconds of orthostasis; orthostatic hypertension – sustained increase in SBP  $\geq 20$  mm Hg or DBP  $\geq 10$  mm Hg after 60-180 seconds of orthostasis; postural orthostatic tachycardia syndrome – sustained tachycardia with a HR  $> 40$  beats/min from baseline in children or  $> 120$  beats/min in general without concomitant orthostatic hypotension.

It is generally accepted that the active orthostasis test with continuous BP monitoring is a more accurate method of diagnosing syncope compared with single BP measurements at the 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup>, and 10<sup>th</sup> minute in a standing position [15]. Therewith, H. Cai *et al.* [16] substantiated the effectiveness of the active orthostasis test in patients with postural orthostatic tachycardia syndrome, which was equivalent to the Tilt test. In any case, the active orthostasis test is the only tool for diagnosing syncope due to orthostatic hypotension, which is confirmed by a fall in SBP  $\geq 20$  mm Hg or DBP  $\geq 10$  mm Hg from baseline, or a fall in SBP  $< 90$  mm Hg, accompanied by spontaneous reproduction of symptoms in orthostasis [1].

The tilt test (tilt table test, passive orthostasis test) is a useful diagnostic test in the diagnosis of syncope with an orthostatic component. The main purpose of the tilt test is to provoke an event with recognition of the complaint and demonstrate a pathophysiological correlate [17]. Both of these aspects are key: recognition can refer to subjective sensations reported by patients and visible aspects such as changes in facial colour or movements that the examiner judges to be spontaneous. Along with the demonstration of pathophysiological measurements, a clinical and pathophysiological correlate is obtained to confirm the cause of the transient loss of consciousness.

The Tilt test is a useful tool for the diagnosis of those forms of syncope that are accompanied by an orthostatic

component, such as reflex syncope, syncope due to orthostatic hypotension, and postural orthostatic tachycardia syndrome [17]. In addition, the Tilt test has an important diagnostic value for provoking psychogenic transient syncope by excluding epilepsy, which is not usually provoked by orthostasis [18]. The Tilt test can help to differentiate between sympathetic or parasympathetic autonomic dysfunction and distinguish between neurogenic and non-neurogenic causes of classical orthostatic hypotension [19]. Thus, L. Norcliffe-Kaufmann *et al.* [20] demonstrated that the ratio of  $\Delta\text{HF}/\Delta\text{BP}$  from the supine position to the standing position within 3 minutes  $< 0.5$  beats/min/mm Hg indicates neurogenic orthostatic hypotension (sensitivity 91%; specificity 88%; area under the curve (AUC) = 0.96).

G. Barón-Esquivias *et al.* [21] proved that the combined use of the European Society of Cardiology guidelines and the tilt test in 1 058 adult patients with syncope was accompanied by the diagnosis of syncope of unknown etiology in 19.5% of cases. To confirm vasovagal syncope in patients, the tilt test with drug provocation – intravenous administration of isoproterenol or sublingual administration of nitroglycerine is increasingly used [22]. Drug provocation increases the sensitivity of the tilt test, but slightly reduces its specificity [23]. Due to a large number of side effects, drug provocation in paediatric practice is limited and not standardised [24].

Although the tilt test has been used in clinical practice for more than 30 years, it lacks standardisation and therefore different protocols are used in different centres [25]. The sensitivity of the tilt test in the diagnosis of vasovagal syncope varies and ranges from 21 to 85%, depending on the specific protocol used [26]. The prevalence of asystole during tilt-induced vasovagal syncope may depend on the test methodology [27]. In addition, the European Society of Cardiology guidelines note that a positive tilt test occurs in 92% of patients with vasovagal syncope and 47% of patients with cardiogenic syncope, and the presence of a negative result does not refute the diagnosis of vasovagal syncope [1]. N. Kulkarni *et al.* [28] drew attention to the high frequency of false positive and false negative results of the body test, and suggested that the tilt table test should no longer be used to diagnose syncope.

Thus, active and passive orthostasis tests are important methods for assessing the functional state of the autonomic nervous system and diagnosing syncope with an orthostatic component. Therewith, there is no clear standardised protocol for conducting a tilt test, and therefore, the results of the test interpretation may differ significantly depending on the methodology.

#### ◆ THE VALUE OF THE VALSALVA TEST, HYPERVENTILATION TEST AND CAROTID SINUS MASSAGE IN THE RESEARCH OF AUTONOMIC FUNCTIONS

The Valsalva test – an exercise test characterised by changes in intrathoracic pressure and accompanied by a specific haemodynamic response. The latter can be divided into four separate phases: Phase I – an increase in mean arterial pressure at the beginning of exercise due to an increase in intrathoracic pressure; Phase IIa – a decrease in stroke volume due to a decrease in atrial filling pressure; Phase IIb – an increase in HR due to the activation of the arterial

baroreflex to compensate for the reduced stroke volume; Phase III – a rapid decrease in mean arterial pressure due to a decrease in exercise; Phase IV – a rapid recovery and increase in mean arterial pressure as the restored cardiac output is released into the constricted arterial vessels [28]. Thus, the Valsalva test provides an autonomous analysis of BP and HR modifications during respiratory activity.

The parasympathetic activity of the autonomic nervous system is often assessed by calculating the Valsalva coefficient. According to the results of the study by U. Zafar *et al.* [29] there was no significant difference between the functioning of the parasympathetic and sympathetic systems in young men and women. An effective response of peripheral vascular resistance of end organs is crucial for controlling BP in the upright position and preventing syncope. The Valsalva test causes a decrease in BP, which causes baroreflex-mediated vasoconstriction. B.C.D. Hockin *et al.* [30] characterised the forearm vascular resistance responses to the Valsalva test in healthy subjects and explored their connection with orthostatic intolerance. The authors suggested that people with a reduced forearm vascular resistance response are more susceptible to syncope, regardless of gender.

Pathological changes in BP and HR during the Valsalva test are a marker of orthostatic intolerance. Thus, neurogenic orthostatic hypotension is characterised by a progressive decrease in BP in phase II and its slow recovery in phase IV [31]. J. Baker *et al.* [32] found that the Valsalva test, like other baroreflex-mediated tests, affects the function of the cerebellum, which plays an essential role in the vestibulo-sympathetic reflexes of BP changes in orthostasis. Nevertheless, the distinct haemodynamic patterns and associated baroreflex hypersensitivity in orthostatic intolerance are still understudied and controversial, and there are no clear protocols for interpreting Valsalva test results in children with transient syncope.

Whether hyperventilation is caused by rapid or deep breathing, it is common in stressful situations and may contribute to symptoms of presyncope or syncope. The effects of some previously tested interventions to prevent syncope, such as applied tension, may be associated with a reduction in hyperventilation. More targeted respiratory control techniques may be useful and promising additional methods to reduce vasovagal symptoms [33].

Hyperventilation in the upright position is observed in 25% of patients with postural orthostatic tachycardia syndrome as a manifestation of orthostatic intolerance with a high risk of syncope [33]. Hyperventilation in postural orthostatic tachycardia syndrome is characterised by hyperpnoea, in contrast to a panic attack, which causes tachypnoea. In orthostatic failure, hyperventilation decreases cardiac output, and peripheral resistance and BP increase. Such changes distinguish hyperventilation from arbitrary hyperventilation, when cardiac output increases, while resistance and BP decrease, and from a panic attack when all these parameters increase [34].

The hyperventilation test is one of the methods for assessing autonomic function in patients with syncope. Usually, the HR increases during inhalation and decreases during exhalation. The variability of HR during deep breathing (expiratory-inspiratory index) is  $\geq 15$  beats/min in healthy individuals over the age of 50 [1]. There is convincing

evidence that a decrease in this index is an indication of parasympathetic dysfunction [35]. The hyperventilation test can be used in the diagnosis of autonomic dysfunction in children, but there is no clear algorithm for performing this test and its interpretation in paediatric practice.

Carotid sinus massage is another diagnostic test used in patients over 40 years of age. A cardiac pause of > 3 s and/or a drop in SBP > 50 mm Hg is the result of carotid sinus hypersensitivity and may be the cause of syncope in older age [36]. Carotid sinus massage is an effective test of vagal stimulation in children with paroxysmal supraventricular tachycardia but has not been proven effective in assessing autonomic dysfunction in paediatrics [1].

The above is evidence of the effectiveness of using the Valsalva test and hyperventilation test in children with syncope to determine sympathicotonia or vagotonia in the functioning of the CVS. Further research is needed to explore the reference values and features of the interpretation of these tests in children of different ages.

#### ★ EVALUATION OF CIRCADIAN BLOOD PRESSURE RHYTHMS AND HEART RATE VARIABILITY

Due to physiological circadian rhythms, human BP undergoes significant daily fluctuations. The research of circadian rhythms of BP using daily BP monitoring is a modern, effective and easily accessible method of evaluating the functioning of the autonomic nervous system in children [5]. Thus, the daytime period is characterised by sympathicotonia and increased plasma catecholamine concentrations, while the nighttime period is characterised by vagotonia and decreased blood catecholamine concentrations. Staying in the supine position during sleep is accompanied by a redistribution of systemic blood flow with blood accumulation in the lower extremities and causes increased blood flow to the trunk, increased central venous pressure and afferent impulses in baroreceptors, and increased inhibitory effects of afferent vasomotor centres in orthostasis. After that, the sympathetic arousal reflex is weakened, cardiac output decreases, peripheral vascular resistance decreases, general hypotension of the body muscles develops, and, as a result, BP levels decrease during the night [37].

Notably, there are no clear diagnostic criteria for daily BP monitoring in patients with suspected syncope. Several studies indicate specific patterns of circadian BP rhythms in this group of patients. G. Rivasi *et al.* [38] proved that patients with reflex syncope have significantly more frequent episodes of low SBP according to the results of daily BP monitoring compared with healthy subjects. The group of authors R. Zou *et al.* [39] identified a significantly higher percentage of insufficient (non-dipper) degree of nocturnal BP reduction among children with vasovagal syncope, which was associated with vitamin D deficiency. T. Kovalchuk & O. Boyarchuk [40] proved that the prevalence of the pathological profile of “non-dippers” of nocturnal SBP reduction is 47.7% and is the justification for the dysfunction of the autonomic nervous system in children with vasovagal syncope.

In patients with vasovagal syncope, a comparison of daily BP monitoring with an activity diary can establish correlations between hypotension episodes and their potential triggers [41]. In patients with neurogenic orthostatic

hypotension, daily BP monitoring can detect nocturnal hypertension and hypotension associated with food, medication, or exercise, which, in turn, offers a window of opportunity for individualised treatment [42].

To evaluate the functioning of the autonomic nervous system, several cardiac electrophysiological parameters have been developed and implemented in clinical practice, such as HR variability, Q-T interval dispersion, P wave dispersion, late ventricular potentials, HR deceleration, etc. The implementation of these electrophysiological parameters of the cardiovascular system allows for the selection of individual treatment plans for children with different haemodynamic types of syncope [43]. However, there is still a lack of systematic multicentre long-term studies that would confirm or refute the diagnostic and prognostic value of these indicators in the group of patients with syncope.

H. Akizuki *et al.* [44] proved that in adults with vasovagal syncope, there is a depression of the sympathetic and parasympathetic autonomic nervous system, and the increase in HR after a syncope episode is due to parasympathetic inhibition rather than sympathetic reactivation. In addition, the authors proposed to use the coefficient of variation of the R-R interval as an effective clinical biomarker of syncope in the emergency department. However, S. Akinci *et al.* [45] found no difference in HR variability parameters between tilt-positive and tilt-negative patients and concluded that basal autonomic functions do not affect the pathogenesis of vasovagal syncope.

The results of the research by Q. Zhang *et al.* [46] demonstrated that children with vasovagal syncope have a decrease in the parasympathetic tone of the cardiovascular system in the setting of vitamin D deficiency. While Y. Wang *et al.* [47] established that ULF (average spectral power value at a frequency of  $\leq 0.003$  Hz) and VLF (average spectral power value at a frequency of  $< 0.05$  Hz) are higher in children with vasovagal syncope compared to postural orthostatic tachycardia syndrome and recommended using these indicators of HR variability for the differential diagnosis of these groups of patients.

Thus, the active orthostasis test, tilt test, and research of circadian BP rhythms and HR variability in children are the functional autonomic tests most often used in paediatric clinical practice and research. There is no doubt that most methods for assessing autonomic functions are restricted by limited experience in children and the lack of clear criteria for diagnosing autonomic disorders depending on age, gender, body mass index, and time of assessment during the day.

#### ★ CONCLUSIONS

Assessment of the autonomic nervous system functioning can be performed non-invasively using autonomic tests based on determining the response of the cardiovascular system to applied stimuli or provocations. These include the active orthostasis test, tilt test, Valsalva test, hyperventilation test, assessment of daily BP rhythms and HR variability. Functional autonomic tests are additional methods of physical and instrumental examination of the patient, which allow effective assessment of the autonomic nervous system with an assessment of the dominance of sympathicotonia or vagotonia, establish the possible mechanisms of syncope development, including orthostatic intolerance,

and differentiate between types of transient loss of consciousness. Orthostatic training improves the functioning of the autonomic nervous system and is an essential method of treating syncope with an orthostatic mechanism, although its effectiveness is still controversial. The results of studies of the autonomic nervous system will allow stratifying the risk of developing syncope in the future and making decisions about the specifics of treatment of a child in each case of syncope. Considering the significance of evaluating the autonomic nervous system in patients with syncope, notably, a significant number of functional autonomic tests are non-standardised and have limited use in paediatric patients due to the lack of criteria for autonomic disorders depending on age, gender, body mass index and time of assessment during the

day. Given the wide variety of available methods and their variations, there is a need to develop a single consensus methodology for the assessment of autonomic nervous system functioning in children. Establishing commonly accepted standards for the assessment and classification of autonomic disorders will ensure homogeneity of results between studies and allow for more effective comparisons of data obtained in different clinical settings and patient groups.

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#### ✦ CONFLICT OF INTEREST

The author declares no conflict of interest.

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## Сучасні методи дослідження вегетативних функцій у дітей із синкопе: огляд літератури

**Тетяна Анатоліївна Ковальчук**

Кандидат медичних наук, доцент

Тернопільський національний медичний університет імені І. Я. Горбачевського МОЗ України

46001, майдан Волі, 1, м. Тернопіль, Україна

<https://orcid.org/0000-0003-2455-3278>

**Анотація.** Вивчення вегетативних функцій у дітей із некардіогенними синкопе дозволяє покращити диференційну діагностику та лікувальну тактику у дітей залежно від патогенетичних механізмів розвитку непритомності. Метою дослідження був аналіз сучасних наукових досягнень у вивченні вегетативних функцій у дітей із різними видами синкопе. В огляді використані повні тексти англійських статей, опубліковані у період з січня 2018 року по грудень 2022 року та розміщені у базах даних PubMed Medline і Scopus. Встановлено, що тест активного ортостаза, тілт-тест, дослідження циркадних ритмів артеріального тиску та варіабельності серцевого ритму є найбільш застосованими функціональними вегетативними тестами у педіатричній практиці. Вони дозволяють оцінити спектр патологічних серцево-судинних реакцій у положенні стоячи; ідентифікувати типи ортостатичної гіпотензії та причини ортостатичної непереносимості; діагностувати ортостатичну гіпертензію, синдром постуральної ортостатичної тахікардії, пресинкопе або синкопе; диференціювати симпатичну і парасимпатичну вегетативні дисфункції та психогенні транзиторні втрати свідомості і епілепсію; рекомендувати ортостатичні тренування, як метод лікування синкопе з ортостатичним механізмом розвитку. Попри це, не існує консенсусного підходу щодо визначення вегетативних розладів і методології проведення функціональних вегетативних тестів у дітей різного віку із врахуванням їх статі, індексу маси тіла та часу оцінки впродовж доби. Таким чином, функціональні вегетативні тести є додатковими методами фізикального та інструментального обстеження пацієнта, які дозволяють ефективно оцінити стан вегетативної нервової системи та ймовірні механізми розвитку синкопе, диференціювати транзиторні втрати свідомості, стратифікувати майбутні ризики та оптимізувати лікувально-профілактичну тактику ведення дитини, базуючись на індивідуальному пацієнт-центрованому підході

**Ключові слова:** нервова система; вегетативна дисфункція; функціональні тести; вазовагальне синкопе; синкопе внаслідок ортостатичної гіпотензії; діти



## The role of microbial biofilms in the development of respiratory system complications in patients with COVID-19: A literature review

**Olena Kochnieva\***

PhD in Medical Sciences, Senior Lecturer  
Kharkiv National Medical University  
61022, 4 Nauky Ave., Kharkiv, Ukraine  
<https://orcid.org/0000-0002-1039-9313>

**Olena Kotsar**

PhD in Medical Sciences, Associate Professor  
Kharkiv National Medical University  
61022, 4 Nauky Ave., Kharkiv, Ukraine  
<https://orcid.org/0000-0002-3797-1068>

**Abstract.** One of the complications of COVID-19 is the development of acute respiratory failure, which may require artificial ventilation using an endotracheal tube to correct hypoxaemia. However, the establishment of biofilms during intubation of patients can pose a risk of microbial growth that can cause severe complications. Therefore, the research on the microbial composition of biofilms that causes such diseases becomes an urgent issue. The purpose of the research was to analyse and summarise the data from current studies on the role of microbial biofilms and their impact on the development of respiratory system complications in patients with COVID-19. After reviewing the literature, it was determined that *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Candida albicans* accounted for the majority of biofilms isolated from endotracheal tubes in patients with COVID-19. The level of antimicrobial resistance among the isolated strains was almost 70%. The examination of samples from endotracheal tubes identified representatives of the lung microbiome, *Prevotella* spp. and some species of *Streptococcus*, *Veillonella*. However, in the research on the microbial composition of biofilms isolated from endotracheal tubes, pathogenic representatives dominated, such as *Pseudomonas* spp., *Staphylococcus* spp., *Streptococcus* spp., *Stenotrophomonas* spp., *Enterobacterales*, *Haemophilus* spp. and *Actinomyces* spp. Changes in the composition of the lung microbiome in patients with COVID-19 can lead to the development of severe complications accompanied by the establishment of biofilms. Microorganisms in biofilms can be a reservoir for secondary pulmonary infections, which affects the duration of mechanical ventilation and the admission of patients with COVID-19 to intensive care units. The development and implementation of effective measures for the prevention and treatment of biofilm-related infections is an important task for modern medical practice

**Keywords:** microbial biofilms; respiratory failure; pneumonia; secondary infection

### INTRODUCTION

Secondary bacterial infections play a crucial role in the morbidity and mortality of patients with COVID-19. One of the complications of coronavirus infection is the development of acute respiratory failure, which requires artificial lung ventilation (ALV) using an endotracheal tube to correct hypoxaemia. Most microorganisms in natural and artificially designed environments exist as structured forms attached to biotic or abiotic surfaces, forming com-

plex microbial communities surrounded by an exopolysaccharide matrix called biofilms [1]. The establishment of biofilms during the intubation of patients poses a risk of the growth of microorganisms that can cause pneumonia and other complications of the respiratory system. Such undesirable consequences can affect the treatment of patients and prolong their hospital stay. Despite ongoing research in this area, the impact of biofilms on the

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\*Corresponding author



mortality rate of patients with coronavirus infection remains unexplored.

Lung damage in COVID-19 and impaired immune response can promote the growth and persistence of microorganisms in hospitalised patients and increase the risk of biofilm development. Studies by F.M. Carvalho *et al.* [2] demonstrate that secondary bacterial pneumonia is a potential risk factor for severity and complications in patients with COVID-19. L. Meng *et al.* [3] and F. Zhou *et al.* [4] believe that mechanical ventilation is necessary to treat patients with acute respiratory symptoms and hypoxia, can increase the risk of pneumonia and promote the establishment of biofilms. Changes in oxygen levels, alveolar ventilation, and the density of affected cells affect microbial growth conditions in the lungs.

According to a study by T.M. Rawson *et al.* [5], the overuse of broad-spectrum antibiotics creates an ideal environment for opportunistic bacterial colonisation, secondary bacterial infections and increased levels of multidrug resistance, which leads to microflora disruption, the spread of resistant strains among COVID-19 patients and contributes to the ability of pathogens to form biofilms. Studies demonstrate that bacteria spend most of their life cycle in the biofilm matrix. J. Yan & B.L. Bassler [6] found that the planktonic stage can only be considered as a way of moving a microbial cell from one surface to another, a short-term state in the life of bacteria that are free in the environment. Microorganisms in biofilms increase their resistance to disinfectants, antibacterial drugs, bacteriophages, antibodies and phagocytes by 50-500 times [7, 8].

The extracellular matrix accounts for 85% of the biofilm mass, consisting of exopolysaccharides, proteins and nucleic acids. It is synthesised by the extracellular components of attached microorganisms and has important functions in the biofilm's life. The extracellular matrix is a powerful biological glue that allows the biofilm to be firmly fixed to any surface [9, 10]. The synthesis of virulence factors and the development of biofilms occurs only when there is a sufficient population density (Quorum sensing (QS)) [11]. QS is implemented by several means of perception and transmission of information: physical contact between cells; generation of physical fields; and synthesis of chemicals diffusing into the environment, which are called autoinducers. The concentration of extracellular autoinducers correlates with the density of cells in the population, and when certain thresholds are reached, autoinducers enter the cell interior or activate receptors on their surface, which leads to changes in the expression of various genes [12, 13]. Thus, bacteria can "sense" the density of the cell population, and this mechanism allows bacteria to function as a multicellular organism. Studies have demonstrated that the establishment of biofilms in coronavirus infection can affect the development of respiratory system complications and determine the severity and outcome of the disease.

The purpose of the research was to explore the microbial composition of biofilms and their role in the development of respiratory system complications in patients with COVID-19. The research reviewed modern works of scientists from different countries, including scientific researches, clinical data, using Internet resources, publications of professional journals, and the Medscape/PubMed medical database. A comparative analysis was conducted between

the composition of the lung microbiome and isolates isolated from endotracheal tubes. The main complications of the respiratory system in patients with COVID-19 and their connection with the development of biofilms were explored. The issues of treatment and prevention of complications in patients with COVID-19 associated with the development of biofilms are considered.

#### ★ FEATURES OF THE MICROBIAL COMPOSITION OF BIOFILMS ISOLATED FROM PATIENTS WITH COVID-19

The composition of the lung microbiome can vary significantly depending on the influence of many factors. Among the representatives of normal biocenosis are microorganisms of the genus *Prevotella*, *Streptococcus*, and *Veillonella*. In addition, pathogenic representatives, such as *Haemophilus* spp., *Neisseria* spp. and *Pseudomonas* spp. can be isolated from the respiratory tract, but they account for a smaller proportion of the microbiome [14, 15].

S. Alhumaid *et al.* [16] found that against the background of COVID-19, the lung microbiome is usually disturbed and is characterised by an increase in commensals such as *Prevotella* spp. Thus, when examining samples from endotracheal tubes, representatives of the lung microbiome, *Prevotella* spp. and some species of *Streptococcus*, *Veillonella* were identified. However, in the examination of the microbial composition of biofilms isolated from endotracheal tubes, pathogenic representatives, such as *Pseudomonas* spp., *Staphylococcus* spp., *Streptococcus* spp., *Stenotrophomonas* spp., *Enterobacterales*, *Haemophilus* spp. and *Actinomyces* spp. dominated [17, 18].

Notably, out-of-hospital bacterial infections associated with COVID-19 are quite rare, while hospital-acquired infections are more common and account for about 47% of cases [19]. Research conducted by G. Giacomo *et al.* [20] demonstrated that the percentage of clinically significant bacterial infections in hospitalised patients ranged from 4 to 14%, with these diseases often being recorded in patients in intensive care units. In COVID-19 infection, hospital-acquired pathogens such as *Mycoplasma pneumoniae*, *Pseudomonas aeruginosa* and *Haemophilus influenzae* are spreading. In addition, other pathogens have been identified, including *Enterobacter cloacae*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* [21].

The literature review identified that isolated microorganisms from biofilms established on endotracheal tubes included both commensal and pathogenic agents. *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Candida albicans* accounted for the majority of biofilms. Pathogens such as *Paracoccus yeei* were unusual, but they were detected in several patients. In addition, the isolated strains were tested for antibiotic susceptibility using the disc diffusion method. Thus, the level of antimicrobial resistance among the isolated strains was almost 70%. Among the tested strains were isolates with resistance to meropenem and gentamicin. In addition, differences in antibiotic susceptibility between various isolates of the same species isolated from the same endotracheal biofilm have been reported [22].

Studies have demonstrated a significant role of *Acinetobacter baumannii* in the development of ventilator-associated pneumonia in patients with COVID-19 undergoing

mechanical ventilation. The pathogen is characterised by a significant level of resistance to many clinically relevant antibiotics, including meropenem, imipenem, gentamicin, tobramycin and levofloxacin. Antibiotic resistance has increased through using empirical broad-spectrum antibiotic therapy to treat COVID-19 bacterial superinfections. In addition, these pathogens have been identified as having a high ability to form biofilms [23].

The development of tracheobronchitis in patients with COVID-19 on mechanical ventilation is not uncommon, with an incidence of almost 15%. Microbiological studies have demonstrated that potentially resistant gram-negative bacteria such as *P. aeruginosa* and carbapenem-resistant strains of *Klebsiella pneumoniae* are isolated from the pathological material of such patients [24]. Other enterobacteria are isolated: *Alcaligenes xylosoxidans*, *Acinetobacter* spp. and *Stenotrophomonas*. Depending on the type and virulence of the bacteria and their interaction with the immune system, colonisation and biofilm development subsequently occur.

Thus, normal biocinosis of the respiratory system plays an important role in maintaining homeostasis. Changes in the microbiome and the development of biofilms during using endotracheal tubes during coronavirus infection can affect the course of the disease and lead to the development of severe complications.

#### ◆ MAIN COMPLICATIONS OF THE RESPIRATORY SYSTEM IN PATIENTS WITH COVID-19

Both innate and acquired immunity are involved in the response to the infectious process. The development of complications in coronavirus infection is associated with significant dysfunction of the immune system. I. Sulaiman *et al.* [25] established that patients with COVID-19 had a decrease in the number of CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes, an increase in neutrophils, and a decrease in the concentration of gamma interferon in the serum. Further studies confirmed these findings and identified the presence of such processes as a cytokine storm, characterised by an excess of proinflammatory molecules, inhibition of natural killer cells and cytotoxic lymphocytes, and morphological and phenotypic changes in monocytes [26]. Such immune response disorders can contribute to increased adhesion, growth, and spread of bacteria that can form biofilms. In addition, bacterial infection leads to an increased probability of virus survival and replication, and tissue damage promotes the further spread of pathogens, which increases the risk of bloodstream infections. Thus, the presence of secondary infection in COVID-19 leads to further complications, including the development of septic shock.

Acute respiratory distress syndrome (ARDS), which often develops in patients with COVID-19, is a life-threatening form of respiratory failure. According to statistics, ARDS develops in about 1/3 (33%) of hospitalised patients and almost 3/4 (75%) of patients in intensive care units [27]. The known mechanisms of ARDS are associated with the development of severe pulmonary infiltration, oedema and inflammation, which leads to a violation of alveolar homeostasis, changes in lung physiology, pulmonary fibrosis, endothelial inflammation and thrombosis. ARDS can occur both as a result of direct exposure to the

virus and due to the action of substances synthesised by host cells. Activated cells of the immune system secrete specific enzymes and pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), which leads to the induction of a cytokine storm. Patients with ARDS require ALV and have a high mortality rate due to shock, septicaemia and multiple organ dysfunction syndrome. Invasive ventilation in patients with ARDS can directly lead to lung damage, and prolonged use of mechanical ventilation causes the development of biofilms by microorganisms, which significantly worsens the prognosis for recovery [27].

The most common bacterial complication of COVID-19 is ventilator-associated lower respiratory tract infection, which includes ventilator-associated pneumonia and ventilator-associated tracheobronchitis. The mechanism underlying bacterial secondary infection in viral pneumonia is damage to ciliated cells, which leads to impaired mucociliary clearance and increased bacterial adhesion and colonisation of the airways [28].

During complications of coronavirus infection, mechanical ventilation using an endotracheal tube can support pulmonary gas exchange disorders in critically ill patients. The development of biofilms inside the endotracheal tube and their subsequent movement to the distal airways during mechanical ventilation cycles is considered a possible pathogenic pathway for the development of ventilator-associated pneumonia. Previous studies have demonstrated that biofilm formation on endotracheal tubes affects the incidence of bacterial infections in intubated patients and is a factor in the development of ventilator-associated pneumonia, which occurs in 20-40% of cases [29]. T. Sakano *et al.* [30] established that a biofilm can be established on the surface of the tube within 24 hours after intubation and can be a reservoir for microorganisms that subsequently cause infection in the lungs. In addition, biofilm cells are inherently more resistant to antibiotics for a variety of reasons, including the lower metabolic rate of bacteria in the biofilm and poor penetration of antimicrobial drugs. The hypothesis that biofilms on endotracheal tubes play a role in the development of ventilator-associated pneumonia is supported by the fact that in many cases the same bacteria are identified in tube biofilms and other airway specimens [31]. Such pathogenetic features can complicate the treatment of biofilm-related infections.

Ventilator-associated tracheobronchitis is a frequent and clinically significant infectious complication in patients on mechanical ventilation for more than 48 hours, with a frequency similar to ventilator-associated pneumonia. Studies conducted by D. Koulenti *et al.* [32] demonstrated that tracheobronchitis can be considered as an intermediate process leading to ventilator-associated pneumonia. In addition, ventilator-associated tracheobronchitis has a limited impact on overall mortality but demonstrates a significant association with increased patient costs, length of hospital stay, antibiotic use, and duration of mechanical ventilation.

Thus, patients affected by the coronavirus can develop various complications, the most common of which are pneumonia and ARDS. One of the important factors leading to such complications is using endotracheal tubes and the development of biofilms, which worsen the patient's

condition and increase mortality. The impact on microbial biofilms and the treatment of associated infections is a complex and unresolved problem.

#### ★ STRATEGIES FOR THE TREATMENT AND PREVENTION OF MICROBIAL BIOFILM FORMATION IN PATIENTS WITH COVID-19

Since antibiotic therapy is ineffective in the treatment of biofilm infections, maximum efforts should be devoted to preventing the formation of biofilms. The main measures are designed to prevent the adhesion of microorganisms when using endotracheal tubes in patients with severe coronavirus infection. For this purpose, modified endotracheal tubes coated with antimicrobial compounds can be used, which has a high clinical effect. Using antimicrobial-coated endotracheal intubation tubes for prolonged mechanical ventilation allows delaying contamination of the respiratory tract tissues with microflora and reduces the microbial load on the lung parenchyma. Animal studies have demonstrated that endotracheal tubes coated with silver ions lead to reduced adhesion of *P. aeruginosa* and a lower prevalence of ventilator-associated pneumonia compared to uncoated tubes [33]. Other modifications are used, including silicone or noble metal coating. Such modified endotracheal tubes can be used in combination with devices for drainage of bronchial secretions, which prevents the development of respiratory system complications.

In addition, it is necessary to constantly monitor endotracheal aspirate in patients with COVID-19 on mechanical ventilation. Such monitoring allows identifying pathogens that colonise the lower respiratory tract and determining their quantitative content. M.L. Blasco *et al.* [34] note that timely detection of pathogens will allow for early targeted antibiotic therapy and prevent the development of ventilator-associated pneumonia.

Prevention of biofilm development in patients with COVID-19 on mechanical ventilation consists of measures designed to reduce mucus viscosity and activate its evacuation. Mucus viscosity can be reduced with mucolytic agents. Special attention should be devoted to using N-acetylcysteine, which can prevent the establishment and destruction of bacterial biofilms, which is becoming especially important in the era of antibiotic resistance. There is a growing body of evidence confirming the antimicrobial and antibiofilm activity of N-acetylcysteine against many respiratory pathogens, including pathogens of the following genera and species: *Escherichia*, *Pseudomonas*, *Staphylococcus*, *Acinetobacter*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis* and *Klebsiella*. F.L. Poe & J. Corn [35] proved that this mucolytic inhibits the establishment of biofilms by bacteria and fungi, and destroys mature biofilms. The destruction of the biofilm matrix facilitates the penetration of antibacterial drugs into the deeper layers of biofilms and significantly increases the effectiveness of treatment of bacterial infections.

Clear criteria for prescribing antimicrobial therapy should be followed to prevent the development of antibiotic resistance. Antibiotic therapy in patients with a diagnosed coronavirus infection is justified if there are convincing signs of bacterial infection. These signs include: a change from a dry cough to a productive cough (especially with purulent sputum) in a patient with confirmed SARS-

CoV-2 infection, a significant increase in blood procalcitonin levels, an increase in white blood cell count > 10-12-109/L and/or a stain shift > 10%, signs of consolidation (alveolar infiltration) of the lung parenchyma according to computed tomography [36].

In addition, inappropriate treatment with broad-spectrum antibiotics may increase the level of mycobacterial resistance and mortality of patients with COVID-19. C. Rhee *et al.* [37] believe that early antibiotic treatment should be avoided in more than 75% of cases if the aetiology of superinfection is not proven using standard microbiological diagnostic methods.

Recently, using monoclonal antibodies that can affect the proteins of the SARS-CoV-2 spike-like envelope proteins has become widely used to prevent and treat complications in patients with COVID-19. Their mechanism of action is to block the attachment of the virus to the cell membrane, which prevents SARS-CoV-2 from entering human cells, neutralises the virus's effect and helps prevent the development of the disease, reducing the nature and duration of its clinical manifestations. M.P. O'Brien *et al.* [38] found that using a combination of drugs containing monoclonal antibodies in outpatients with coronavirus reduces the incidence of hospitalisation or death by 70% due to a rapid reduction in viral load. Such measures to prevent complications can help reduce the frequency of ventilator use and reduce the risk of biofilm development.

An important task of modern medicine is to develop new approaches to the identification and research of biofilms, including the immune response to these infections, changing the tactics of antibiotics, and searching for and introducing new antimicrobial agents. Scientists from different countries are researching the design of preventive measures and treatment of infections associated with the development of biofilms, but no general recommendations have been established, which requires further research on this issue.

#### ★ CONCLUSIONS

The literature review demonstrated that patients with COVID-19 may develop severe respiratory complications associated with the development of biofilms. The composition of biofilms is diverse and contains many microorganisms that are clinically important and highly resistant to antibiotics. Among the microorganisms that are part of biofilms, the most common are representatives of the genera *Pseudomonas* spp., *Staphylococcus* spp., *Streptococcus* spp., *Stenotrophomonas* spp., *Enterobacterales*, *Haemophilus* spp. and *Actinomyces* spp. These pathogens can cause severe complications associated with the development of ventilator-associated pneumonia, ventilator-associated tracheobronchitis, ARDS and septic shock. The main measures for the treatment and prevention of complications associated with biofilm formation in patients with COVID-19 should include the prevention of microbial adhesion and the principles of rational antibiotic therapy.

The correlation between the microbial composition of biofilms isolated from patients with respiratory system complications and clinical outcomes remains unexplored. However, microorganisms in biofilms can probably be a reservoir for secondary pulmonary infections. These processes can affect the duration of mechanical ventilation

and the admission of patients with COVID-19 to intensive care units. The prospect of this research is to establish the impact of the microbial composition of biofilms on the development of complications, which will reduce the number of bed days spent in hospital and the cost of treatment.

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#### ✦ CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## Роль мікробних біоплівки при розвитку ускладнень дихальної системи у пацієнтів з COVID-19: огляд літератури

### Олена Володимирівна Кочнева

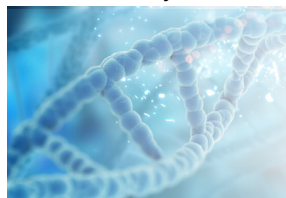
Кандидат медичних наук, старший викладач  
Харківський національний медичний університет  
61022, просп. Науки, 4, м. Харків, Україна  
<https://orcid.org/0000-0002-1039-9313>

### Олена Василівна Коцар

Кандидат медичних наук, доцент  
Харківський національний медичний університет  
61022, просп. Науки, 4, м. Харків, Україна  
<https://orcid.org/0000-0002-3797-1068>

**Анотація.** Одним із ускладнень COVID-19 є розвиток гострої дихальної недостатності, що може потребувати штучної вентиляції легень із використанням ендотрахеальної трубки для корекції стану гіпоксемії. Однак утворення біоплівки в процесі інтубації хворих може стати ризиком розмноження мікроорганізмів, здатних викликати важкі ускладнення. Тому, актуальним питанням стає дослідження мікробного складу біоплівки, який викликає такі захворювання. Мета дослідження полягала в аналізі та узагальненні даних сучасних досліджень, які стосуються вивчення ролі мікробних біоплівки та їх впливу на розвиток ускладнень дихальної системи у пацієнтів з COVID-19. Після проведеного аналізу літератури встановлено, що в структурі біоплівки, виділеної з ендотрахеальних трубок у пацієнтів із COVID-19 більшу частку склали *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* і *Candida albicans*. При цьому рівень резистентності до антимікробних препаратів серед виділених штамів складав майже 70 %. При дослідженні зразків з ендотрахеальних трубок були виявлені представники мікробіому легень, *Prevotella* spp., деякі види *Streptococcus*, *Veillonella*. Однак при дослідженні мікробного складу біоплівки, виділеної з ендотрахеальних трубок, домінували саме патогенні представники, такі як, *Pseudomonas* spp., *Staphylococcus* spp., *Streptococcus* spp., *Stenotrophomonas* spp., *Enterobacterales*, *Haemophilus* spp. та *Actinomyces* spp. Зміни складу мікробіому легень у хворих з COVID-19 можуть призводити до розвитку важких ускладнень, які супроводжуються утворенням біоплівки. Мікроорганізми у біоплівках можуть бути резервуаром для вторинних легеневоїх інфекцій, що впливає на тривалість використання штучної вентиляції легень та перебування пацієнтів з COVID-19 у відділеннях інтенсивної терапії. Розробка та впровадження ефективних заходів для профілактики та лікування інфекцій, пов'язаних з утворенням біоплівки є важливим завданням для сучасної медичної практики

**Ключові слова:** мікробні біоплівки; дихальна недостатність; пневмонія; вторинна інфекція



## Application of gaseous ozone and its aqueous solution for inactivation of pathogenic microorganisms: A literature review

**Taras Pyatkovskyy**

PhD in Medical Sciences, Associate Professor

I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine

46001, 1 Maidan Voli, Ternopil, Ukraine

<https://orcid.org/0000-0003-1240-1680>

**Abstract.** Due to its potent oxidising properties, ozone presents itself as a promising disinfectant and may serve as a substitute for conventional techniques. The purpose of this study was to analyse the literature on the effectiveness of gaseous ozone and its aqueous solution against pathogenic and opportunistic microorganisms or their surrogates. More than 150 English-language sources have been studied, of which 72 have been selected for this review. To illustrate the effectiveness of ozone against bacteria, bacterial biofilms and viruses, data from selected publications are summarised in tables for the respective groups of disinfectant targets. In cases where data were presented in the form of kinetic curves in the reviewed publications, the maximum inactivation values were chosen. It was shown that both gaseous ozone and its aqueous solution are widely used due to their powerful bactericidal properties. The study noted that ozone is an effective oxidant that neutralises gram-negative, gram-positive, and spore-forming microorganisms, the biofilms they form, as well as fungi, protozoa, viruses, and prions. Among the industries that have implemented or may implement ozone applications are medicine (including dentistry and pharmacy), as well as the food industry and recreation. One of the notable advantages of using ozone is its relatively short half-life, with conversion to non-toxic oxygen. It was also noted that ozone treatment is non-thermal, which allows it to be used for processing fresh fruit and vegetables. The main disadvantages and challenges for safe implementation are ozone toxicity at exceeding the maximum permissible concentration in the air, corrosiveness, and explosiveness at high concentrations

**Keywords:** ozone; bactericidal properties; disinfection; non-thermal treatment; safety

### ✦ INTRODUCTION

Disinfectants play a crucial role in preventing the spread of infectious diseases and controlling microbial contamination. Scientific research helps determine the effectiveness of disinfectants in killing or inhibiting the growth of target microorganisms. Furthermore, the research helps assess the safety of disinfectants for human and animal health and the environment. Since the creation of the first commercial ozone generator, ozone has been used as an alternative to traditional disinfectants using chlorine-containing compounds [1]. The ability of ozone to inactivate a wide range of microorganisms has attracted interest among researchers. M. Murakami *et al.* [2] investigated the use of ozone in dentistry. Its effectiveness against various oral pathogens, including planktonic bacteria and biofilms formed by them, was emphasised. M. Marino *et al.* [3] inves-

tigated the effect of ozone on bacterial biofilms and found that ozone treatment can effectively disrupt the biofilm structure and reduce the viability of bacteria in it. M. Ziyaina & B. Rasco [4] demonstrated the wide possibilities of using ozone for the inactivation of microorganisms in the food industry. They have shown antibacterial effects of both gaseous and aqueous ozone against a wide range of microorganisms on dairy, meat products, and vegetables. M. Alimohammadi & M. Naderi [5] investigated the use of ozone and showed its effectiveness for inactivating viruses in indoor air. Researchers C. Wolf *et al.* [6] used ozone to inactivate enteroviruses in water.

As a strong oxidising agent, ozone effectively inactivates bacteria, damaging their cellular structure and disrupting vital metabolic processes. Among the means by

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\*Corresponding author



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which ozone can inactivate microorganisms are the following: oxidation of fatty acids and the breakdown of cell membranes, which results in increased permeability [7]; oxidation of amino acids and sulfhydryl groups in proteins and enzymes, which in turn leads to their denaturation and inactivation [8]; damage to nucleic acids [9]; and the generation of reactive oxygen species, which creates oxidative stress for microbial cells and viruses [10].

Until recently, Ukrainian science considered ozone mainly as a means of water purification. This is covered in the study by T.V. Hernaya [11], who among the methods of water disinfection described the use of ozone both independently and in combination with ultraviolet irradiation. O. Shmychkova *et al.* [12] demonstrated the use of ozone for the treatment of wastewater from pharmaceuticals. The complete destruction of antibiotic molecules by oxidation was shown. The purpose of this study was to review the English-language literature on the effectiveness of ozone against various groups of microorganisms and individual pathogens. This paper brings together a variety of scientific studies that both highlight the potential of ozone as a powerful disinfectant and reveal limitations and warnings regarding its use.

The Google Scholar web resource was used to search for literature. The literature was searched using the keywords “ozone generation”, “gaseous ozone”, “aqueous ozone”, “ozonated water”, “inactivation”, “log reduction” in combination “+” with groups of pathogens (“gram-negative bacteria”, “gram-positive bacteria”, “spore-forming bacteria”, “bacterial biofilms”, “fungi”, “protozoa”, “virus”, “prion”) and their individual species. English-language literature sources from the last 30 years were reviewed. Preference was given to papers describing ozone treatment alone, without the use of combined treatment with other non-thermal methods. Data on ozone-only treatment were selected

from publications with several types of treatment or combined treatment. Book chapters, dissertations, and reports or abstracts from scientific conferences were excluded from the search. Publications with the results of using ozonated oil were also excluded. The data from the selected publications were critically analysed, systematised, and presented in the form of a literature review. Both open-access articles and articles from paid resources were investigated.

#### ★ THE USE OF GASEOUS OZONE AND ITS AQUEOUS SOLUTION TO INACTIVATE BACTERIA

Due to the mechanisms described above, both gaseous and aqueous ozone have been effectively used to inactivate many Gram-negative microorganisms, including pathogens such as *Escherichia coli* [13, 14], *Salmonella* spp. [15], *Pseudomonas aeruginosa* [14, 16], *Legionella pneumophila* [17, 18], *Vibrio cholerae* [19, 20] and *Shigella* spp. [4, 19]. The effectiveness of ozone neutralisation of gram-positive flora, including *Staphylococcus aureus* [16, 21], *Streptococcus* spp., *Enterococcus* spp. [22, 23], and *Listeria* spp. [24]. Inactivation of gram-positive spore-forming microorganisms, both vegetative cells of *Bacillus* spp. [25], *Clostridium* spp. [26] and their spores [27, 28], was also reported. As various methods of treating varied contaminated substrates were employed, comparing the sensitivity of microorganisms to ozone based on diverse sources' outcomes is impractical. To illustrate the effectiveness of ozone against various microorganisms, data from selected publications are summarised in Table 1. In most of the selected cases, ozone-saturated water was used. In cases where data were presented in the form of kinetic curves in the reviewed publications, the maximum inactivation values expressed in logarithms of colony forming units (log CFU) per gram or millilitre of substrate were selected.

Table 1. Ozone inactivation of microorganisms

Pathogen	Inactivation (log CFU)	Processing time (min)	Ozone concentration (mg/L)	Temperature (°C)	Substrate	Processing type	Reference
<i>Bacillus cereus</i> (vegetative cells)	3.62	420	0.4	20±3	Cooked rice inoculated with bacterial suspension	Gaseous ozone	[26]
<i>Bacillus subtilis</i> (spores)	5.2	3	140	Not specified	Suspension of spores in a buffer solution	Ozone dissolved in a buffer solution	[28]
<i>Clostridium perfringens</i> – vegetative cells – spores	1.5 0.87	5	5	4 → 45 4 → 55	Microbial suspension applied to pieces of beef	Ozonated water (ozonation method not specified)*	[27]
<i>Enterococcus faecalis</i>	98%**	10	5 mg/min***	13	Naturally contaminated water from a private source	Water ozonated by bubbling	[22]
<i>Escherichia coli</i> O157:H7	2.9	30	1.5	10	Bacterial suspension applied to spinach leaves	Pressurised gaseous ozone 69 kPa*	[9]
<i>Legionella pneumophila</i>	>3	15	0.033****	22	Suspension of bacteria in 0.01 M phosphate buffer solution	Ozone dissolved in a buffer solution	[18]

Continued Table 1.

Pathogen	Inactivation (log CFU)	Processing time (min)	Ozone concentration (mg/L)	Temperature (°C)	Substrate	Processing type	Reference
<i>Listeria monocytogenes</i>	0.79	1	2-3	25	Pasteurised apple juice inoculated with a bacterial suspension	Passing gaseous ozone through juice samples	[24]
<i>Listeria monocytogenes</i>	3.7	10	50	Indoor (without specified values)	Bacterial suspension applied to TSA medium	Gaseous ozone	[25]
<i>Pseudomonas aeruginosa</i>	4.8	4	0.095****	Indoor (without specified values)	Suspension of bacteria in distilled water	Distilled water ozonated by bubbling	[16]
<i>Salmonella enterica</i> serovar <i>Enteritidis</i>	2.9	30	160	Not specified	Bacterial suspension applied to almonds	Pressurised gaseous ozone 12.5 psig***	[15]
<i>Shigella flexneri</i>	3.19 4.46	15 30	20.83 mg-min/l***	Indoor (without specified values)	Bacterial suspension in a wastewater model	Wastewater ozonated by bubbling	[19]
<i>Shigella sonnei</i>	5.6	1	2.2	Not specified	Suspension of bacteria in distilled water	Distilled water ozonated by bubbling	[4]
<i>Staphylococcus aureus</i>	5.4	2	0.095****	Indoor (without specified values)	Suspension of bacteria in distilled water	Distilled water ozonated by bubbling	[16]
<i>Staphylococcus aureus</i>	>2	4	2	23-24	Bacterial suspension applied to fabric (65% polyester, 35% cotton)	Gaseous ozone	[21]
<i>Streptococcus mutans</i>	2.91	10 sec*	Not measured, manufacturer's data are given: 0.052% (v/v), flow rate 13.33 ml/sec***	23	Bacterial suspension applied to glass beads	Gaseous ozone	[23]
<i>Vibrio cholerae</i>	3.26 4.52	15 30	20.83 mg-min/l***	Indoor (without specified values)	Bacterial suspension in a wastewater model	Wastewater ozonated by bubbling	[19]

**Notes:** \* – combined treatment with ozonated water for 5 min and subsequent heating of samples for 30 min was used; \*\* – units of measurement according to the original publication, corresponds to inactivation up to 2 log; \*\*\* – units of measurement according to the original publication; \*\*\*\* – initial ozone concentration

**Source:** developed by the author based on the results of a literary search

Thus, the effectiveness of both gaseous and aqueous ozone against a wide range of both Gram-negative and Gram-positive pathogens, including bacillus and clostridium spores, has been demonstrated.

#### ★ USE OF OZONE TO INACTIVATE FUNGI AND PROTOZOA

Ozone was used to inactivate fungal spores. Scientists have demonstrated the effectiveness of water ozonated by bubbling with a concentration of 2 mg/L at 20°C against *Aspergillus niger*, *Penicillium polonicum*, and *Trichoderma harzianum* [29]. Ozone gas at a concentration of 10 mg/L at 20°C for 15 minutes was used to inactivate *Candida albicans* and *Aspergillus fumigatus* on the surface of dense and porous materials. E.I. Epelle *et al.* [21] compared the effectiveness of ozonation with irradiation with ultraviolet light. The

authors attributed the higher efficiency of ozonation to the better permeability of gaseous ozone to porous materials, regardless of the orientation of the substrate relative to the source of the disinfectant. The same authors showed that under the same conditions, they could achieve better inactivation of bacterial strains of *Escherichia coli* and *Staphylococcus aureus*, suggesting that the thicker and stronger cell wall of fungi, compared to bacteria, makes it difficult for ozone to penetrate (and subsequently oxidise cell components). Even if fungi are not completely destroyed, ozonation slows their growth. Thus, O.J. Sujayasree *et al.* [30] proved that when citrus fruits artificially inoculated with *Penicillium digitatum* and *Penicillium italicum* cultures were treated with gaseous ozone at a concentration of 0.3-1 mg/L at 20°C for 4-9 weeks, the development of mould on fruits occurred a week later compared to the control. The same

treatment slowed or stopped the sporulation of fungi without any noticeable phytotoxicity to the fruit. Ozonation can neutralise not only fungal spores or vegetative cells, but also mycotoxins. Scientists have investigated the use of gaseous and aqueous ozone for the degradation of several mycotoxins (aflatoxins, ochratoxin A, fumonisins, deoxynivalenol, zearalenone, patulin) in various food products [31].

There is scarce data on the successful use of ozone against protozoa, as the cyst membranes make them resistant to disinfectants. For instance, K.E. Wainwright *et al.* [32] and A. Dumètre *et al.* [33] demonstrated that ozonation failed to inactivate *Toxoplasma gondii* oocysts at an ozone concentration of 6 mg/L for 12 minutes and 9.4 mg×min/L. However, ozone can still be used to inactivate some protozoa of medical importance. The results of studies by Z. Ran *et al.* [34] showed that ozonated water with an ozone concentration of 0.5 to 5 mg/L neutralised *Cryptosporidium* oocysts within 5 minutes. A more recent study by A.P. Ogura *et al.* [35] showed the killing of 3.44 log *Cryptosporidium parvum* by ozonated water with an ozone concentration of 10 mg/L for 5 minutes at 25°C. Ozone at a concentration of 5 mg/L was used in an attempt to inactivate *Giardia duodenalis* cysts in drinking water. The results of L.Y. Kondo Nakada *et al.* [36] showed that although this ozone concentration was not sufficient to inactivate all cysts, ozonation reduced the ability of cysts to cause disease in laboratory

animals and also reduced the reactivity of natural organic matter in treated water samples.

In general, the reviewed literature on the inactivation of fungi and protozoa showed moderate effectiveness of ozone against these microorganisms. This may be explained by the fact that eukaryotic cells have a more complex structure and are more resistant to ozone due to their thicker cell wall and the ability to form cysts that are quite resistant in the environment.

#### ★ AN ANALYSIS OF PAPERS ON THE USE OF OZONE TO INACTIVATE VIRUSES

Ozone has been used to inactivate viruses for several decades. Since the first studies of the antiviral properties of ozone, its ability to inactivate a wide range of human pathogenic viruses that cause both intestinal [6] and respiratory infections [5], as well as their surrogates [37], have been investigated and used. With the onset of the pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) virus, interest in the antiviral effect of ozone has increased considerably [5]. The data on virus inactivation by ozone are presented in Table 2. The inactivation values were expressed as logarithms of plaque forming units per millilitre of substrate (log PFU/ml) and/or the presence of the virus after treatment was confirmed by polymerase chain reaction.

**Table 2.** Inactivation of viruses with ozone

Pathogen	Inactivation (log PFU/ml)	Processing time (min)	Ozone concentration (mg/L)	Temperature (°C)	Substrate	Processing type	Reference
Adenovirus type 40	3.55	10	0.3	5	Phosphate buffer solution	Distilled water ozonated by bubbling	[38]
Hepatitis A virus	2.9 1.5	10	6.25	20	Virus applied to the surface of green onions Virus internalised in green onions	Distilled water ozonated by bubbling	[39]
Influenza virus type A subtype H1N1	99.999%*	150	20	23-29	Virus suspension dried on the surface of glass and plastic petri dishes	Gaseous ozone	[40]
SARS-CoV-2 coronavirus	3.3	55	6	25	Virus suspension dried on the surface of a stainless steel plate	Gaseous ozone	[41]
Mouse coronavirus (SARS virus surrogate)	3.0	60	20-25**	20	Virus suspension dried on the surface of a glass slide	Gaseous ozone	[42]
Mouse norovirus 1 (human norovirus surrogate)	4.1	10	6%***	25	Phosphate buffer solution	Gaseous ozone	[37]
Human norovirus	2.5	30	6.25	22	Alfalfa seeds inoculated with viral filtrate	Ozonated water (ozonation method not specified)	[43]
Poliovirus type 1	2.76	0.7 sec****	0.25	5	Phosphate buffer solution	Phosphate buffer solution ozonated by bubbling	[44]

Continued Table 2.

Pathogen	Inactivation (log PFU/ml)	Processing time (min)	Ozone concentration (mg/L)	Temperature (°C)	Substrate	Processing type	Reference
Respiratory syncytial virus A2	4.0	40	20	24	Virus on the surface of the N95 respirator	Gaseous ozone	[45]
Rhinovirus 1A and 14	2.0	60	28**	20	The virus is applied to a sterile glass surface	Gaseous ozone	[42]
Rotavirus strain Wa	8.0	1	25	25	Virus suspension	Distilled water ozonated by bubbling	[46]

**Notes:** \* – units according to the original publication, corresponds to inactivation of 5 log; \*\* – gradual increase of the concentration to the specified within 15 minutes; \*\*\* – units according to the original publication, corresponds to 60,000 mg/L; \*\*\*\* – units according to the original publication

**Source:** developed by the author based on the results of a literary search

The publications from the late 1980s and early 1990s reported data on effective inactivation (2.7-5 log) of hepatitis A virus by ozone (not included in this review). For the experiments, an aqueous ozone solution with a concentration of 0.25-1 mg/L was used, and the substrate was a virus suspension in phosphate buffer solution [47]. However, more recent publications have demonstrated both the effectiveness of aqueous ozone against hepatitis A virus applied to green onions (Table 2) and the inability of its gas state at a concentration of 5 mg/L to achieve a significant reduction in the concentration (<0.6 log) of hepatitis A virus on raspberry fruit during treatment for 3 minutes [48]. In this context, continuing research into the inactivation of viruses by ozone is important for optimising and improving the effectiveness of disinfection methods, ensuring the purity of water and food, and possibly preventing viral disease outbreaks.

#### ★ THE USE OF GASEOUS OZONE AND ITS AQUEOUS SOLUTION TO INACTIVATE PRIONS

While ozone has been widely investigated for its disinfectant properties against a wide range of microorganisms, including bacteria, viruses, and fungi, there are fewer publications on its effectiveness against prions. This is quite natural, given that these pathogens cause neurodegenerative disorders that are quite rare and that the research to inactivate these pathogenic proteins is difficult and expensive. The number of altered proteins was determined by Western blot, and qualitative changes were observed during the development of the disease in laboratory animals [49]. Prions are persistent in the environment and resistant to conventional disinfection methods such as heat treatment, chemical disinfectants, and ultraviolet radiation, which makes them difficult to inactivate [49]. Effective decontamination of prions is possible by hydrolysis of these proteins or destruction of the tertiary structure of the protein [50]. The study by N. Ding *et al.* [51] demonstrated the use of ozone as a disinfectant against prions. It has been shown that ozone dissolved in water by bubbling at a concentration of 13.7 mg/L can be effectively used to disinfect PrP<sup>Sc</sup>-contaminated clean water and wastewater at a treatment time of 5 min. In another publication, the

same researchers continued to demonstrate the effectiveness of ozone for wastewater treatment. They showed that inactivation of 2.8 log was achieved by ozonation with an initial concentration of 43.4 mg/L for 5 minutes, while preliminary settling of wastewater with the removal of some organic matter allowed to increase the inactivation of prion protein scrapie (PrP<sup>Sc</sup>) to more than 4 log [49]. The effectiveness of gaseous ozone has also been investigated for the inactivation of prions, specifically on the surface of stainless steel. It was found that prion disease developed later in mice intracerebrally implanted with prion-contaminated stainless-steel wires treated with gaseous ozone (25,000 mg/L, for 3 minutes) than in the control group of mice, indicating that ozone treatment reduces the infectivity of prions on stainless steel [50].

Although ozone treatment of prions has shown promise in laboratory studies, its practical application to neutralise these pathogenic proteins is still being explored. Research into the inactivation of prions by ozone can be challenging due to the unique properties of prions, but the potential benefits to public health safety make this area of research valuable.

#### ★ EFFICIENCY OF OZONE APPLICATION AGAINST BACTERIAL BIOFILMS

Bacteria can form biofilms on various surfaces of the human body, including teeth, mucous membranes, respiratory and urinary tracts, as well as on medical devices such as catheters, contact lenses and medical implants [2, 52]. Biofilms can be used to cover various artificial surfaces, including pipes of water supply and sewage systems, ventilation systems, and various equipment in such industries as the medical (including dentistry), pharmaceutical, food, and recreational sectors [2, 53]. Biofilms create a protective barrier for bacteria that can prevent the penetration of various disinfectants [2, 54]. Although ozone has demonstrated some effectiveness against biofilms, its ability to penetrate and inactivate bacteria within biofilms is generally lower than its effectiveness against planktonic bacteria [2, 25]. However, ozone can still have a certain effect on biofilms. Ozone treatment can disrupt biofilm structure, damage the matrix of extracellular

polymers, and reduce the total biomass of the biofilm. This can make the biofilm more sensitive to subsequent antimicrobial treatments and facilitate the removal of

bacteria from surfaces [2, 55]. To illustrate the effectiveness of ozone against biofilms, data from selected publications are summarised in Table 3.

**Table 3.** Effect of ozone on biofilms formed by bacteria

Pathogen	Surface type	Processing type	Ozone concentration (mg/L)	Processing time (min)	Effect	Reference
<i>Bacillus cereus</i>	Polypropylene plate	Gaseous ozone	45 ± 2	30	Inactivation 2.16 log CFU/cm <sup>2</sup>	[53]
<i>Bacillus subtilis</i>	Stainless steel plate	Gaseous ozone	1.4	4.5	Inactivation > 6 log CFU/ml	[54]
<i>Listeria monocytogenes</i>	Stainless steel cylinder	Tap water ozonated by electrolytic treatment	0.5	20	Inactivation 1.65 log CFU/cm <sup>2</sup> at static treatment (soaking) Inactivation 3.29 log CFU/cm <sup>2</sup> when treated with water flow (1 l/min)	[3]
<i>Listeria monocytogenes</i>	Stainless steel cylinder	Gaseous ozone	0.2 2	60 2	Inactivation 2.12 log CFU/cm <sup>2</sup> Inactivation > 5.33 log CFU/cm <sup>2</sup> (no growth)	[3]
<i>Pseudomonas aeruginosa</i>	Contact lens container (material not specified)	Distilled water ozonated by bubbling	4	20	Inactivation 7.42 log CFU/cm <sup>2</sup>	[52]
<i>Pseudomonas fluorescens</i>	Stainless steel cylinder	Tap water ozonated by electrolytic treatment	0.5	20	Inactivation 1.56 log CFU/cm <sup>2</sup> at static treatment (soaking) Inactivation 3.52 log CFU/cm <sup>2</sup> when treated with water flow (1 l/min)	[3]
<i>Pseudomonas fluorescens</i>	Stainless steel cylinder	Gaseous ozone	20	60	Inactivation 5.51 log CFU/cm <sup>2</sup>	[3]
<i>Salmonella</i> spp.*	Stainless steel plate	Ozonated water (ozonation method not specified)	16	20	Inactivation < 0.8 log CFU/cm <sup>2</sup> but potentiation (up to 2 log CFU/cm <sup>2</sup> ) was observed in combination with ultrasound (40 kHz)	[55]
<i>Staphylococcus aureus</i>	Stainless steel cylinder	Tap water ozonated by electrolytic treatment	0.5	20	Inactivation 2.15 log CFU/cm <sup>2</sup> at static treatment (soaking) Inactivation 4.47 log CFU/cm <sup>2</sup> when treated with water flow (1 l/min)	[3]
<i>Staphylococcus aureus</i>	Stainless steel cylinder	Gaseous ozone	20	20	Inactivation > 5.47 log CFU/cm <sup>2</sup> (no growth)	[53]

**Notes:** \* – a mixture of strains of *Salmonella enterica* serovar *Agona*, *Salmonella enterica* serovar *Enteritidis*, *Salmonella enterica* serovar *Infantis*, *Salmonella enterica* serovar *Typhimurium*

**Source:** developed by the author based on the results of a literary search

In general, further research in this area is promising and may lead to the discovery of new approaches or combinations of treatment methods that will increase the efficiency of destroying biofilms or preventing their formation on surfaces.

#### ★ OZONE PROPERTIES AND CHALLENGES ASSOCIATED WITH ITS USE

Ozone is a powerful oxidant and therefore a promising disinfectant due to its high reactivity and ability to penetrate deeper than liquid disinfectants [56]. This gas is unstable in the environment, it spontaneously decomposes to non-toxic oxygen with a half-life of about 30

minutes in aqueous solution and from 40 minutes to 25 hours in air, depending on the conditions (temperature, humidity, air movement) [57]. One of the substantial advantages of ozonation is its ability to operate without the need for thermal energy, which leads to energy savings. In contrast to conventional thermal treatment processes that rely on heating or boiling, ozonation uses the powerful oxidising properties of ozone to achieve the effect [58]. However, while ozonation itself saves energy, the energy requirement for ozone generation can vary depending on the method used [59]. Several methods can be used to produce gaseous ozone, among which the corona discharge method is most commonly

used for industrial ozonators [60]. In laboratories, the photochemical method can also be used to produce gaseous ozone [4]. Most often, an aqueous solution of ozone was obtained by bubbling gaseous ozone through water, which was saturated with it. Moreover, it can be produced by electrolytic method [61].

Ozone is widely used in various industries, but there are certain limitations to processing with this agent. Due to its strong oxidising properties, ozone not only destroys microorganisms but also damages everything it comes into contact with. Thus, there are limitations in the choice of an effective ozone dose and ozone treatment time. In medicine and dentistry, dose limitation is used to prevent tissue damage to patients. P.S. Subiksha [62], in the study on the use of ozone in dentistry, points out that even low concentrations of ozone can cause headaches, irritation or dryness of the nose, throat and eyes. There are also restrictions on ozone exposure for workers in other areas where it is used. In the commercial application of ozone, screening people who may come into contact with it in industry is mandatory because it affects the human respiratory tract. According to P. Wolkoff *et al.* [63], staying in a room with ozone in the air can cause eye irritation, while inhaling ozone can cause irritation and damage to the respiratory tract. People with pre-existing respiratory conditions, such as asthma or chronic obstructive pulmonary disease, may be more susceptible to the respiratory effects of ozone [64]. Its toxicity is primarily due to its strong oxidative properties. Exposure to high levels of ozone can lead to oxidative stress, inflammation, and damage to cells and tissues. According to C. Michaudel *et al.* [65], a single exposure to ozone caused rapid destruction of the epithelial barrier with increased neutrophil recruitment, reactive oxygen species production, airway hypersensitivity, and IL-33 expression in epithelial and myeloid cells in mice. In another *in vivo* experiment, G. Valacchi *et al.* [66] demonstrated that at concentrations of 0.8 mg/L for 6 days (exposure for 6 hours per day), ozone can affect both lungs and skin, causing depletion of  $\alpha$ -tocopherol and inducing stress-related reactions in both the skin epidermis and airway epithelium. Furthermore, M.I. Gilmour *et al.* [67] showed that ozone exposure increases the susceptibility of mice to pulmonary bacterial infection caused by *Streptococcus zooepidemicus*, reduces the function of alveolar macrophages, and increases the mortality of animals from infection.

In the food industry, the restriction applies to food processing, especially to fresh leafy vegetables. M.V. Shynkaryk *et al.* [56] demonstrated a change in the colour of spinach leaves after prolonged treatment with gaseous ozone at a concentration of 0.106 mg/L or short-term treatment with a higher (1.5 mg/L) concentration. Ozone is suitable for the treatment of rooms, surfaces, and equipment, but it should be borne in mind that the elements treated with ozone must be resistant to corrosion [68]. R. Pandiselvam *et al.* [69] showed that the use of ozone at a concentration of 47-106 mg/L for two months can lead to damage to rubber parts. Ozone decomposes to non-toxic oxygen, but when it reacts with a substrate, it can form by-products and modify the biomolecules in it. P. Li *et al.* [70] proved that ozone reacts with bromide

dissolved in water to form bromate, which is a carcinogen. T. Manasfi *et al.* [71] have demonstrated that ozonation by oxidation of polyunsaturated fatty acids produces aldehydes, ketones, and keto acids.

Another disadvantage is that ozone cannot be stored and decomposes quickly, and therefore on-site ozone production is mandatory. When producing ozone, it should be kept in mind that at elevated concentrations in the air, it may become explosive. The experiments conducted by K. Koike *et al.* [72] have shown that ozone mixed with oxygen at a concentration of more than 10-11% at room temperature and atmospheric pressure can lead to an explosive chain reaction until the complete decomposition of ozone. The above disadvantages and warnings do not detract from the benefits of ozone as an antibacterial agent. By overcoming these obstacles, it is possible to realise the potential benefits of ozonation in these industries, which would contribute to an improvement in hygiene, safety, and product and service quality. To mitigate the potential risks associated with ozone exposure, it is important to follow the recommended safety instructions and practices. Appropriate technical controls such as ventilation systems and personal protective equipment are recommended to minimise exposure and protect people from excessive ozone concentrations.

## ★ CONCLUSIONS

The literature review demonstrates the extensive application and efficacy of gaseous ozone and its aqueous solution in deactivating pathogenic microorganisms. The collected data from scientific publications demonstrate the bactericidal properties of ozone against various groups of microorganisms, including gram-negative and gram-positive bacteria, spore-forming microorganisms, biofilms formed by them, fungi, protozoa, viruses, and prions. This broad spectrum of action makes ozone a versatile option for microbial control in medicine and other industries. In most of the sources reviewed, ozone was used without combination with other treatments, but there are many publications demonstrating potentiation of inactivation when ozone and other thermal and non-thermal treatments are combined.

The introduction of ozone technology in sectors such as healthcare, food processing, and recreation is showing a growing recognition of its potential benefits. The relatively short half-life of ozone, which results in its conversion to non-toxic oxygen, is an advantage from a safety perspective. Furthermore, the non-thermal nature of ozone treatment allows it to be used in the processing of fresh fruit and vegetables, thereby improving product safety and quality. However, it is important to recognise the potential disadvantages and safety concerns associated with ozone. High concentrations of ozone are necessary to deactivate bacterial spores and protozoan oocysts, rendering the use of this treatment method challenging to deactivate these microorganisms on food or living organisms. Moreover, ozone toxicity at concentrations above the maximum permissible level in the air must be carefully regulated and monitored to ensure the well-being of workers in ozone-using industries. Additionally, the potential explosive nature of ozone in high concentrations requires proper handling and adherence to safety protocols.

Further research and development are needed to optimise the use of ozone in various industries and to address the challenges associated with its implementation. With proper precautions and new research, ozone technology can contribute to enhanced microbial control and improved safety standards in various sectors, ultimately benefiting public health and quality of life.

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#### ★ CONFLICT OF INTEREST

The author declares no conflict of interest.

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## Застосування газоподібного озону та його водного розчину для інактивації патогенних мікроорганізмів: огляд літератури

**Тарас Іванович П'ятковський**

Кандидат медичних наук, доцент  
Тернопільський національний медичний університет ім. І. Я. Горбачевського МОЗ України  
46001, майдан Воли, 1, м. Тернопіль, Україна  
<https://orcid.org/0000-0003-1240-1680>

**Анотація.** Завдяки своїм потужним окислювальним властивостям озон є перспективним засобом дезінфекції і може використовуватися, як альтернатива традиційним засобам. Метою даної роботи було проведення аналізу літературних даних стосовно ефективності газоподібного озону та його водного розчину проти патогенних та умовно патогенних мікроорганізмів чи їх сурогатів. Опрацьовано більше 150 англомовних джерел, з яких 72 вибрано для даного огляду. Для ілюстрації ефективності озону проти бактерій, бактеріальних біоплівки та вірусів дані з вибраних публікацій зібрано у таблицях по відповідних групах мішеней дії дезінфектанта. У випадках представлення даних у оглянутих публікаціях у вигляді кінетичних кривих обиралися максимальні значення інактивації. Показано, що як газоподібний озон, так і його водний розчин знайшли широке застосування завдяки їх потужним бактерицидним властивостям. Зазначається, що озон є ефективним окисником, який знешкоджує грам-негативні, грам-позитивні та спороутворюючі мікроорганізми, утворені ними біоплівки, а також грибки, найпростіші, віруси та пріони. Серед галузей, які впровадили чи можуть впровадити застосування озону є медицина (включаючи стоматологію та фармацію), а також харчова промисловість та рекреаційна галузь. Однією з помітних переваг застосування озону є його відносно короткий період напіврозпаду з перетворенням у нетоксичний кисень. Також зазначається, що обробка озоном є нетермічною і дозволяє його застосовувати для обробки свіжих фруктів та овочів. Основними недоліками та проблемою для безпечного впровадження є токсичність озону при перевищенні гранично допустимої концентрації у повітрі, корозійна активність та вибухонебезпечність при високих концентраціях

**Ключові слова:** озон; бактерицидні властивості; дезінфекція; нетермічна обробка; безпека

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