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The effectiveness of local action of nano oxides on wound infection

O.Ya. Popadyuk[1], V.P. Polyovyy[2], S.M. Henyk[1], I.V. Shelefontiuk[2], A.S. Palyanytsia[2]

[1]*Ivano-Frankivsk National Medical University;*
[2]*Bukovinian State Medical University; e-mail: opopadyuk@ifnmu.edu.ua*

The treatment of wound-related combat injuries and antibiotic resistance require the search for new approaches to reduce the risk of infectious complications, this will increase the effectiveness of therapy and even save lives. The aim of our study was to establish the mechanism of action and effectiveness of nano oxides applied to wound infection. The antimicrobial activity of different concentrations of solutions of zinc (nZnO), magnesium (nMgO) and silicon (nSiO$_2$) nano oxides was studied by agar diffusion against clinical antibiotic-sensitive strains of opportunistic Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli), isolated from patients with purulent-septic diseases. It was found that nZnO exhibits extremely high antibacterial activity against S. aureus, E. coli. The ultra-low content of nZnO (1.56 mg/ml) completely destroys S. aureus, unlike nMgO, the effective concentration of which exceeds the minimum by 16 times. It is known that nZnO has the highest antimicrobial properties against S. aureus and E. coli, this effectiveness is achieved due to the features of the structure and the possibility of the formation of oxygen and a number of other radicals. These qualities of nZnO make it possible to integrate it into dressings for wound treatment.
Key words: nano oxide; antimicrobial activity; wound infection.

INTRODUCTION

The treatment of wound combat injuries requires the search for new approaches to reduce the risks of infectious complications, which will increase the effectiveness of therapy and even save lives [1-3]. Metal oxides nanoparticles (NPs), such as zinc oxide (ZnO), magnesium oxide (MgO), copper oxide (CuO), calcium oxide (CaO), silver oxide (AgO) and titanium oxide (TiO_2) are a new class of antimicrobial agents that are increasingly being studied and may be used in medicine [4]. As a nanoscale (<100 nm) of inorganic material, metal oxides NPs have special characteristics, including broad-spectrum of antibacterial properties, high activity, large surface area of interaction with cells and tissues, low ability for bacteria to resist their effects, long-term stability even in the field conditions. These features, as well as the ability to change the size, shape, surface properties and chemical compositions, provide great potential for the development and use of nanomaterials as effective antimicrobials [5].

It is known that metallic NPs of silver (Ag), gold (Au) and zinc (Zn) have effective bacteriostatic and bactericidal properties, low *in vivo* toxicity [6-9]. In particular, at a sufficient concentration zinc nano oxide (nZnO) exhibits antibacterial activity without affecting healthy cells. Thanks to ZnO chitosan-dextran hydrogel in dressings exhibits appropriate antimicrobial properties against *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus)*. In addition, ZnO is active against some antibiotic-resistant microorganisms [10]. ZnO NPs prevent the development of biofilms of *E. Coli, S. aureus* and other microorganisms due to their ability to generate free radicals [11, 12]. Magnesium nano oxide (nMgO) is a light white powder that easily absorbs water and is interesting due to its antibacterial activity, high thermal stability, and low cost. The toxicity of nMgO is similar to other nanomaterials with metal oxides and is usually associated with the release of active oxygen species [13].

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Thus, the limited use of antibiotics due to the insensitivity of pathogenic microorganisms to them, the negative effect on wound granulation tissue of known antiseptics for local treatment and prevention of wound infection, the not fully studied mechanism of action, the bioavailability of NPs of metals and their oxides, and the problem of known traditional dressings prompts the search for new effective methods and means of local treatment of wounds of various genesis, especially in war conditions.

The aim of the study: to establish the mechanism of action and effectiveness of nano oxides applying on wound infection, the possibility of their integration into wound dressings for wounds treatment.

METHODS

The microbiological study and quantum chemical substantiation of the antiseptic effect of modern nanoscale metal oxides were carried out. The antimicrobial activity of different concentrations of solutions of zinc (nZnO), silicon ($nSiO_2$) and nMgO nano oxides was studied by the method of diffusion into agar against the clinical antibiotic-sensitive strains of opportunistic *S. aureus* and *E. coli* isolated from patients with purulent-septic diseases. Petri dishes were poured with 30 ml of nutrient agar, in which wells with a diameter of 7.88 ± 0.55 mm were made. According to the optical turbidity standard (concentration $1 \cdot 10^7$ CFU/ml), test cultures suspensions were evenly sown on the agar surface. 100 μl of nano oxide solutions were added to the prepared wells. Pure solvent (saline) was added to the control wells. After cultivation for 24-48 h, the diameters of the growth retardation zones of the test cultures were determined. Digital images of the sowing on the plates were processed using the UTHSCSA ImageTool 2.0 computer program (The University of Texas Health Science Center at San Antonio). The antimicrobial activity of nano oxides was also studied by the method of serial dilutions in agar. At the next stage of the study, the mechanism of antimicrobial action of nZnO and nMgO was investigated on the basis of quantum chemical calculations [14]. The obtained results were analysed using the methods of variation statistics.

RESULTS AND DISCUSSION

As a result of the study, the antimicrobial activity of nZnO, nMgO, and $nSiO_2$ was determined (Table 1). The most pronounced antimicrobial effect of nZnO is noteworthy, while $nSiO_2$ does not affect the growth of *E. coli*.

During the study of the antimicrobial activity of nano oxides by the method of serial dilutions in agar, their minimum bactericidal

Table 1. Antimicrobial activity of nZnO, nMgO, and $nSiO_2$

Nano oxides	Concentrations, mg/ml	Diameters of growth inhibition zones, mm	
		S. aureus	*E. coli*
Control	0	7.88 ± 0.55	7.88 ± 0.55
nZnO	10	9.27 ± 0.46*	9.91 ± 0.87*
nZnO	50	10.24 ± 0.73*	11.83 ± 0.83*
nMgO	10	8.49 ± 0.56	– **
nMgO	50	[10.61 ± 0.59]***	7.85 ± 0.41
$nSiO_2$	10	8.35 ± 0.35	– **
$nSiO_2$	50	0.46	– **

Notes: *P < 0.05 reliable differences to the control; ** absence of growth inhibition zones; *** zones diameters of partial inhibition of culture growth (bacteriostatic effect) are given in square brackets.

concentrations (MBC) were determined. The concentrations of nZnO and nMnO were gradually reduced from 50 to 0.8 mg/ml and the growth of test cultures was monitored (Table 2). As a result of the study an extremely high antimicrobial activity of nZnO against *S. aureus*, especially at an ultra-low concentration of 1.56 mg/ml was revealed and against *E. coli* – 50 mg/ml. Thus, nZnO completely destroyed the pus-forming bacterium *S. aureus*, in contrast to nMnO, which efficiency exceeded the minimum concentration by 16 times. MBC nMgO against *S. aureus* and *E. coli* was 25 mg/ml.

The concentration of zinc ions in the test solution was determined at certain intervals using the Aquaquant® zinc colourimetric test system with a sensitivity of 0.1-5 mg/l ("Merck KGaA", Germany). The average weight of the samples was determined using an AXIS AD200 balance (Poland) with an accuracy of 0.001 g. The film samples were placed in chemical cups with 100.0 ml of distilled water and samples of the solution were taken after 5, 15, 30, 60 min, 24 and 48 h, respectively (the first sample – 5 ml, the second and third samples were 1 ml each, subsequently brought the volume to 5 ml with distilled water; the remaining samples were 0.5 ml each and also subsequently brought the volume to 5 ml with water). The obtained data were tabulated and the average values were calculated, with the corresponding graphs and analysis of the results. This technique allows detecting the presence and showing the concentration of zinc ions in the solution, which makes it possible to reliably and accurately study the excretory capacity of the experimentally developed polymer film.

The release of active substances from the developed polymeric films was studied using *in vitro* methods, as they are affordable, simple and require less material and instrumental costs to determine the bioavailability of active substances than *in vivo* methods.

The next stage of the study was the conduction of quantum chemical studies of the electronic properties of metal nano oxides. This study is important because it allows calculating the characteristics of NPs and predicting their mechanism of action. In general, such theoretical studies of physicochemical parameters usually coincide with the values obtained experimentally later.

The examination of homolytic or heterolytic dissociation processes on the surface of ZnO and MgO crystals can be represented by the following reactions:

$$Zn_nO_n \longrightarrow \cdot Zn_nO_{n-1}\!: + \cdot O \cdot \quad 1$$

$$Mg_nO_n \longrightarrow \cdot Mg_nO_{n-1}\!: + \cdot O \cdot \quad 3$$

$$Zn_nO_n \longrightarrow \cdot Zn_{n-1}O_n\!: + \cdot Zn \cdot \quad 5$$

$$Mg_nO_n \longrightarrow \cdot Mg_{n-1}O_n\!: + \cdot Mg \cdot \quad 7$$

$$Zn_nO_n \longrightarrow Zn_nO_{n-1}^{2+} + O^{2-} \quad 2$$

$$Mg_nO_n \longrightarrow Mg_nO_{n-1}^{2+} + O^{2-} \quad 4$$

$$Zn_nO_n \longrightarrow Zn_{n-1}O_n^{2-} + Zn^{2+} \quad 6$$

$$Mg_nO_n \longrightarrow Mg_{n-1}O_n^{2-} + Mg^{2+} \quad 8$$

Table 2. Growth pattern of test cultures in the presence of nano oxides

Cultures	Concentrations of nano oxides, mg/ml						
	50	25	12.5	6.25	3.13	1.56	0.8
				nMgO			
S. aureus	–	–	+	+++	+++	+++	+++
E. coli	–	–	++	+++	+++	+++	+++
				nZnO			
S. aureus	–	–	–	–	–	+++	+++
E. coli	–	–	++	+++	+++	+++	+++

Notes: "–" – absence of culture growth; "+"– intensity of culture growth

Solid particles have their own specific crystal structure: ZnO – hexagonal syngonium, MgO – cubic syngonium. Accordingly, the atoms located on the surface can be at the corners, on the edges or on the planes of the faces. The arrangement of atoms can affect their ability to dissociate, which can be determined by the decrease in the total energy of the system after dissociation ($\Delta E = E_{fin} - E_{in}$, where ΔE is the change in the energy of the system, E_{fin} and E_{in} – the energies in the final and initial states, respectively) [14].

The data of quantum chemical modelling of such systems are given in Table 3.

The decrease in the energy of the system is observed only with the formation of oxygen radicals on the surface of ZnO, which can provoke a number of free radical transformations, in particular, the simplest variant is described by equation:

$$\cdot O \cdot + H_2O \longrightarrow \cdot OH + \cdot OH$$

$$\cdot OH + \cdot OH \longrightarrow H_2O_2$$

Oxygen radicals (O_2^- – superoxide; $\cdot OH$ – hydroxyl; O_2H – peroxide) are highly reactive and interact with many substances in the body, including nucleic acids, proteins, lipids and other compounds, causing a violation of their functions [2, 12].

Modelling the structure of nano objects, which are usually tens or hundreds of nanometres in size and include several hundred or thousands of atoms, using precise quantum chemical Ab initio and DFT (Density Functional Theory) computational methods, and even more approximate semiempirical methods, is not possible on modern personal computers. For such objects, it is possible to use only methods of molecular mechanics, which are very inaccurate and do not take into account the interaction of molecular orbitals [15]. The model of crystal of ZnO hexagonal syngonium created by the method of molecular mechanics, when modelled by the semiempirical PM3 method, slightly changes the arrangement of atoms in the crystal, in which the surface oxygen atoms are slightly removed from the crystal, and vice versa, zinc atoms are shifted slightly to the middle. This results in a particle with mainly oxygen atoms on its surface. The more mobile surface oxygen atoms can be detached homolytic or heterolytic. In the case of heterolytic breaking, depending on the location of atom on the surface, the energy of the system increases by 2500-2700 kJ/mol, and vice versa, decreases in the case of homolytic breaking by 60-150 kJ/mol. When a radical or zinc ion is detached from the crystal, the energy of the system increases by 1000-1500 and 2700-2900 kJ/mol, respectively. Thus, the most energy-efficient process is the cleavage of oxygen radicals from the surface, which can subsequently form a number of other radicals,

Table 3. The change in energy of system (ΔE) of dissociation processes (1-8) calculated by the semiempirical method of PM3

Process number	The change in energy of system (kJ/mol) during the dissociation of an atom or ion, that is located in a crystal			
	in a corner	on the edge	on the planes	
1	-61	-141	-153	-103
2	2778	2674	2582	2683
3	731	485	677	706
4	2989	2684	2876	2950
5	968	1522	1523	1618
6	2475	2655	2659	2723
7	916	607	774	744
8	2843	2673	2659	2696

which leads to their antimicrobial effect. The smaller the particle size, the larger their surface area, and thus the greater the number of active oxygen atoms.

However, reducing the particles to molecular size will not always be effective. ZnO NPs exhibit semiconducting properties, and their energy gap (the energy difference between the highest occupied molecular orbital – HOMO and the lowest vacant molecular orbital – LVMO) is 3.3 eV (Figure) [14, 15]. As can be seen from the calculations by the PM3 method, a small crystal consisting of six ZnO molecules (a particle of approximately 0.4 nm in size) has an energy gap of 8.43 eV. As the crystal increases, more orbitals conjugate, which leads to interaction between the energy levels and their separation. Under such conditions, the energy gap decreases and approaches a certain steady-state value. When the ZnO crystal is enlarged to 38 molecules (a particle of about 1 nm in size), the energy gap is 4.85 eV, which is close to the experimental values.

During the modelling of the nMgO structure, surface oxygen atoms do not move away from the surface and dissociation in MgO (both radical and heterolytic) is not energetically favourable, and therefore is improbable. Since no radicals will be formed, nMgO will not have an antibacterial effect.

CONCLUSIONS

It has been proven that nZnO has the highest antimicrobial properties against *S. aureus, E. coli,* the effectiveness of which is achieved due to the features of the structure and the possibility of the formation of oxygen and a number of other radicals. These properties of nZnO make it possible to integrate it into dressings for wound treatment. The insufficient antibacterial ability of nMgO and $nSiO_2$ has been substantiated.

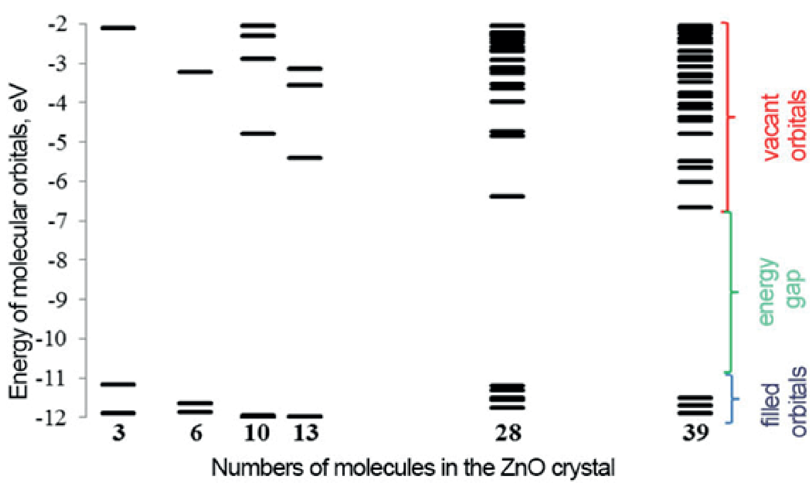
The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

О.Я. Попадюк[1], В.П. Польовий[2], С.М. Геник[1], І.В. Шелефонтюк[2], А.С. Паляниця[2]

ЕФЕКТИВНІСТЬ МІСЦЕВОЇ ДІЇ НАНОКСИДІВ НА РАНОВУ ІНФЕКЦІЮ

[1] Івано-Франківський національний медичний університет;
[2]Буковинський державний медичний університет;
e-mail: opopadyuk@ifnmu.edu.ua

Лікування ранових бойових травм, антибіотикорезистентність потребують пошуку нових принципів для зниження ризиків інфекційних ускладнень, що сприятиме



Reduction of the energy gap in the ZnO crystal calculated by the PM3 method

підвищенню ефективності терапії і навіть збереженню життя. Мета нашого дослідження – з’ясувати механізм дії та ефективність застосування нанооксидів на ранову інфекцію. Вивчали протимікробну активність різних концентрацій розчинів нанооксидів цинку (nZnO), магнію (nMgO) та кремнію (nSiO_2) методом дифузії в агар щодо клінічних антибіотикочутливих штамів умовно-патогенних Staphyloccus aureus (S. aureus) і Escherichia coli (E. coli), виділених від пацієнтів із гнійно-септичними захворюваннями. Протимікробну здатність нанооксидів визначали методом серійних розведень в агарі та обчислювали їхні мінімальні бактерицидні концентрації. Установлено, що nZnO виявляє надзвичайно високу антибактеріальну активність щодо S. aureus, E. coli. Дуже низький його вміст (1,56 мг/мл) повністю знищує S. aureus на відміну від nMgO, ефективна концентрація якого перевищує мінімальну у 16 разів. Відомо, що твердим частинкам властива певна кристалічна структура: оксид цинку – гексагональної сингонії, оксид магнію – кубічної сингонії. Відповідно, атоми можуть перебувати на кутах, ребрах чи площинах граней. Їхнє розташування впливає на здатність до дисоціації, яку можна визначити за зниженням загальної енергії системи після процесу. Доведено, що найвищі протимікробні властивості щодо S. aureus, E. coli має nZnO, ефективність якого досягається завдяки особливостям структури та можливістю утворення кисневих і низки інших радикалів. Такі якості nZnO дають змогу інтегрувати його у пов›язки для лікування ран.

Ключові слова: нанооксиди; протимікробна активність; ранова інфекція.

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