

Preclinical evaluation of the individualized approach for chronic non-healing wounds management

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Chronic non-healing wounds (CNHW) are very common and often incorrectly treated, the morbidity and associated costs of chronic wounds management highlight the need to implement wound prevention and treatment concepts.

OBJECTIVE — to evaluate the possibility of different metal nanooxide polymer nanofilms use for CNHW local treatment.

MATERIALS AND METHODS. The study design is based on evaluation of various types of dressing materials considering their option for use in CNHW local treatment. Samples of biodegradable polymer films (with an optimal composition of gelatin, polyvinyl alcohol, lactic acid, glycerin and distilled water) saturated with nanoparticles of several oxides with expected antibacterial and pro-regenerative feature — nZnO, nMgO in concentrations of 1 %, 5 %, and 10 % were used in the study of antimicrobial action and substance release profiling. Quarterly ammonium antiseptic decamethoxin 0.02 % was used for control.

RESULTS. Obtained data shows that polymer based biodegradable films incorporating optimal component composition (gelatin, polyvinyl alcohol, lactic acid and glycerin) enriched with 5 % and 10 % zinc nanooxide have potent antimicrobial activity against both gram-positive and gram-negative microorganisms, the most common causative agents of CNHW's. The ion release capacity analysis showed that the Zinc impregnated wound-healing biodegradable polymer film gradually releases the active substance in a time dependent manner, and the nano-sized particles of nanoZinc oxide are released from the polymer composition faster than ordinary zinc oxide.

CONCLUSIONS. Complex natural biodegradable polymer based nanofilms are composite materials impregnated with metal nanooxides showing high potential in local treatment of chronic non-healing wounds. Polymer film with 5 % nanoZnO showed up to the 58 % higher antimicrobial activity, comparable or exceeding the one of quarterly ammonium compound decamethoxin. Furthermore, nanoZnO impregnated polymer films compared to standard ZnO impregnated polymer films showed up to 63.2 % faster substance release profile with rapid and more unified curve.

KEYWORDS

chronic wound, ulcer, treatment, metal nanooxide, polymer nanofilm, antimicrobial activity, biofilms.

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Chronic non-healing wounds (CNHW) are those that do not progress through a normal, and timely sequence of repair, and one of the most common chronic conditions today impacting lifestyle and health status of millions worldwide [1]. They are very common and often incorrectly treated, the morbidity and associated costs of chronic wounds management highlight the need to implement wound prevention and treatment

concepts [3]. The mainstay of CNHW treatment is the TIME concept, which includes Tissue debridement, Infection control, Moisture balance, and Edges of the chronic wound [2, 9]. Following these common approaches are addressed, treatment may be focused on specific to the ulcer type features [7, 19].

More than 70 % of patients with diabetes mellitus (DM) suffer from various complications of

diabetes, among which the most common is diabetic foot syndrome or diabetic foot syndrome (DFS), which is a complex of morpho-functional and pathogenetic changes in the tissues of the lower extremities against the background of diabetic micro- and macroangiopathy, neuropathy, osteoarthropathy [11]. In the majority of patients with DFS, CNHW of the foot and lower leg are registered, which in almost half of the cases ends in forced amputations [4, 10].

CNHW are commonly associated with metabolic, regulatory and vascular conditions. In addition to DFS, another important types of CNHW are venous, arterial and pressure ulcers, which are slightly less common but similarly poses a great risk for the respective patients in terms of morbidity, mortality and various complications. High levels of disability and mortality determine the medical and socio-economic significance of the disease [12, 19].

According to the existing recommendations, the general principles of treatment of patients with CNHW [1, 3] include compensation and normalization of metabolism, including carbohydrate and lipid violations, correction and compensation of organ dysfunctions, detoxification; rational systemic and local etiotropic antibacterial therapy; elimination/correction of angio- and neuropathy, unloading (immobilization) of a limb; reduction of oedema and ischemia; timely necessary and adequate surgical intervention; local treatment of a chronic wound (ulcers or other defects of covering and soft tissues), vascular resuscitation. The role of local CNHW's treatment is preserved throughout decades as majority of these patients have multiple comorbidities and high risk for development of complications, making high-scale surgical interventions dangerous and ineffective [2].

Hitherto, there are significant differences in the interpretation of many aspects of the treatment of patients with CNHW, especially regarding the choice and phasing of surgical interventions, selection of local therapies, debridement techniques and selection of remedies. At the current stage, opposing views on surgical activity in CNHW have been formed. On the one hand, the implementation of radical interventions already at the beginning of the development of purulent-necrotic processes, which is justified by the expected inevitability of further pathological changes progression, on the other hand, the maximum limitation of interventions, and selection of merely conservative approach, which is justified by the high risk of their complications [1, 2, 7]. As a result, there is a high risk of unjustified choice of methods and stages of surgical treatment in patients with CNHW,

especially in terms of selecting the local procedures and measures [18].

The above determines the need to develop an individualized approach to local surgical treatment of patients with CNHW.

OBJECTIVE — to evaluate the possibility of different metal nanooxide polymer nanofilms use for CNHW local treatment.

Materials and methods

The study design is based on evaluation of various types of dressing materials considering their option for use in CNHW local treatment and was fully compliant with acting national and international legislation in bioethics. Following the study, it may act as a background for selecting the proper dressing material for individualized treatment of CNHWs.

Samples of biodegradable polymer films (with an optimal composition of gelatin, polyvinyl alcohol, lactic acid, glycerin and distilled water) saturated with nanoparticles of several oxides with expected antibacterial and pro-regenerative feature — nZnO, nMgO in concentrations of 1 %, 5 %, and 10 % were used in the study of antimicrobial action. Quarterly ammonium antiseptic decamethoxin 0.02 % samples (n = 5) were used for control. The study of antimicrobial activity was carried out by standard solid media disk diffuse method (by inoculating the surface of an agar plate with bacteria isolated from clinical material). The inhibition zone in mm was calculated after 24 hrs. of cultivation in thermostat at temperature 36 °C. Ninety clinical strains of microorganisms that are most often isolated in 98 patients' wounds content: *S. aureus* MSSA (n = 10), *S. aureus* MRSA (n = 10), *S. epidermidis* MSSE (n = 10), *S. haemolyticus* MRSH (n = 10), *S. pyogenes* (n = 10), *E. coli* (n = 10), *P. aeruginosa* (n = 10), *C. freundii* (n = 10), *Candida* spp. (n = 10). Five samples of each tested polymer films were used for obtaining more statistically valid results.

Study of the substance release capacity of biodegradable polymer films (n = 5, each type, respectively) was performed spectrophotometrically or using the colorimetric test system with a sensitivity of 0.1–5 mg/l [17].

The raw digital data obtained in the study was processed by the variation statistics method in MS Excel software. Student's t-test was used to test whether the difference between two groups is statistically significant or not. The frequency characteristics of the investigated indicators (n, %), the average values (mean — M) of the variability estimates of the quantitative indicators (mean square deviation) and the average error (m) were calculated.

Results and discussion

Testing the activity of polymer films saturated with antibacterial agents against clinical strains of microorganisms showed that the highest antimicrobial effect was observed in films impregnated with decamethoxin, as expected, taking into account its topical antimicrobial activity. The results of the study (Fig. 1) indicate the size of microorganism's suppression using the 5% concentration of zinc nanooxide in a biodegradable film base. This type of film has the widest spectrum of antimicrobial activity and effectively suppressed the growth of all tested strains of microorganisms, especially emphasizing *S. pyogenes*, coagulase-negative cocci (including the methicillin-resistant strain of *S. haemolyticus*), as well as *Candidae* spp. The nano-sized zinc oxide films showed their acceptable antimicrobial potential in relation to all tested taxonomic group of opportunistic microorganisms. However, tested films with magnesium nanooxide introduced into the polymer base showed no significant antimicrobial activity (not shown in the diagram).

The overview of biofilm's antimicrobial efficacy is presented in the table. Zones of microorganisms' growth inhibition (mm) under the influence of films with zinc nanooxide to some extent increased in ratio to its concentration.

The best antimicrobial effect against resistant MSSE and MRSH was observed in 5% ZnO films. Whereas, β -hemolytic *S. pyogenes* was found to be the most sensitive of all ZnO film samples, especially at concentrations of 1% and 5%. Regarding polyresistant *P. aeruginosa*, all studied nanofilms showed minor but only a bacteriostatic influence. The antimicrobial

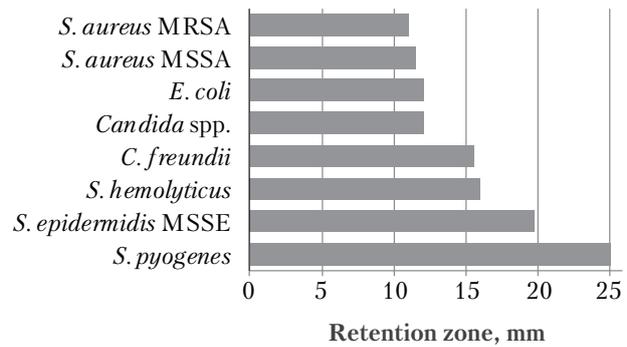


Figure 1. Efficacy of 5% ZnO antimicrobial action against most common CNHW pathogens.

Data shows respective growth retention zone's sizes

activity of the 5% and 10% ZnO nanofilms was comparable to decamethoxin. A 5% ZnO impregnated nanofilm showed top activity against *E. coli*, whilst two times weaker antimicrobial effect was observed in films impregnated with 10% magnesium nanooxide.

Obtained data shows that polymer based biodegradable films incorporating selected component composition (gelatin, polyvinyl alcohol, lactic acid and glycerin) enriched with 5% and 10% zinc nanooxide have potent antimicrobial activity against both gram-positive and gram-negative microorganisms, the most common causative agents of CNHW's.

Expectedly, based on the literature data [4] the speed of Zn²⁺ ions release from the films significantly increased when using its nanoform. Only during first 5–15 minutes of test standard ZnO film showed comparable release of ions. The most remarkable difference between samples was observed during 30–1140 minutes of exposure (Fig. 2).

Table. Comparison of antimicrobial action of tested biofilms

Pathogen	DKT	1% ZnO	5% ZnO	10% ZnO	1% MgO	5% MgO	10% MgO
<i>S. aureus</i> MSSA	14.29 ± 0.21	9.78 ± 0.17*	11.49 ± 0.35**	11.98 ± 0.33#	6.45 ± 0.63#&	6.32 ± 0.17#&	6.01 ± 0.31#
<i>S. aureus</i> MRSA	13.65 ± 0.25	11.47 ± 0.84*	10.90 ± 0.46*	10.53 ± 0.18	–	–	–
<i>S. epidermidis</i> MSSE	13.87 ± 1.10	15.39 ± 0.46*	17.90 ± 0.77**	15.78 ± 0.60&	–	–	–
<i>S. haemolyticus</i> MRSH	15.81 ± 0.77	6.17 ± 0.37*	16.23 ± 0.32**	15.43 ± 0.55#	–	–	–
<i>Str. pyogenes</i>	20.96 ± 0.68	23.08 ± 0.42*	24.60 ± 0.11**	18.21 ± 0.63#&	–	–	–
<i>E. coli</i>	8.92 ± 0.47	8.88 ± 0.27	11.64 ± 0.16**	9.40 ± 1.06	6.63 ± 0.64#&	6.83 ± 0.15#&	7.01 ± 0.42#&
<i>P. aeruginosa</i>	[10.20 ± 0.36]	–	[9.46 ± 0.18]	[9.83 ± 0.20]	–	–	–
<i>Citrobacter freundii</i>	4.82 ± 0.67	14.33 ± 0.79*	15.48 ± 0.23*	11.00 ± 0.43#&	–	–	–
<i>Candida</i>	11.69 ± 0.36	9.77 ± 0.34	12.13 ± 0.89**	13.20 ± 0.67#	–	–	–

Note. DKT — decamethoxin (control). Values in square brackets mean presence of bacteriostatic effect only.

* The difference from the control (decamethoxin) is statistically significant (p < 0.05).

The difference compared to 1% ZnO film is statistically significant (p < 0.05).

& The difference compared to 5% ZnO film is statistically significant (p < 0.05).

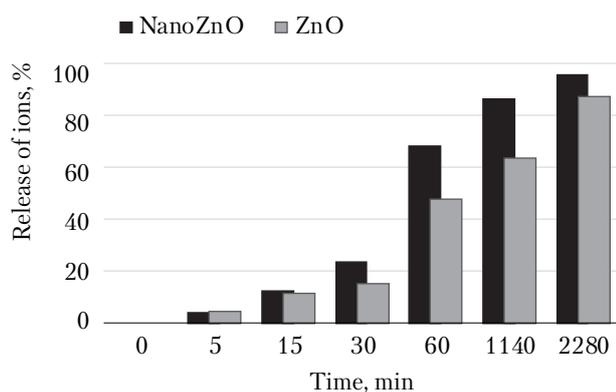


Figure 2. **Time-dependent comparison of 5% ZnO and 5% nanoZnO ions release from polymer films**

The ion-release capacity analysis showed that the Zinc impregnated wound-healing biodegradable polymer film gradually releases the active substance in a time dependent manner, and the nano-sized particles of nanoZinc oxide are released from the polymer composition faster than ordinary zinc oxide. This phenomenon may have both positive and negative interpretations as longer release may decrease the number and frequency of dressings, alleviating iatrogenic wound trauma, however, faster release of ions let achieving of higher drug concentration and better, and more reliable antimicrobial action.

Adequate management of chronic non-healing wounds remains challenging both in terms of prevalence and complexity [10]. In recent decades, considerable progress has been achieved in understanding the background of wound healing, and creating novel approaches for their treatment [1, 6].

Whereas explosive volumes of studies and related publications may be found, and the developments of hundreds of dressing and therapy options became available, the issue is far from being solved. Moreover, existing database sometimes cause confusions due to different research approaches used in different studies, making extrapolation and use of data problematic [5].

The efficacy of the complex gelatin-containing polymer films use as carriers of therapeutic agents has been previously mentioned in literature [2, 4, 15]. Available sources [16] indicate that on the basis of natural biodegradable polymers combined with active substances the composite materials with specified substance release properties can be created [12, 21], and metal nanooxides are well released from the polymer carrier, which significantly increases the effectiveness of CNHWs' local treatment applying a smaller dose of drug [17, 18].

In this study we have confirmed well established data concerning zinc containing [2, 13, 14, 20]

polymer film efficacy. However, it remained unclear whether different zinc concentrations could have demonstrated significant variations in antimicrobial activity. Moreover, this is one of the first studies to introduce and explore nanoforms of polymer films dedicated to CNHW treatment. Therefore, it was shown that 5% zinc as a nanoZnO is preferable emphasizing its higher antimicrobial effect and ion-releasing potential.

The limitations of the study include comparatively small dataset in terms of its size and *in vitro* character of the methodical approach. Further clinical studies of nanoZnO polymer films use may accurately accomplish this study.

Conclusions

Complex natural biodegradable polymer based nanofilms are composite materials impregnated with metal nanooxides showing high potential in local treatment of chronic non-healing wounds. Polymer film with 5% nanoZnO showed up to the 58% higher antimicrobial activity, comparable or exceeding the one of quarterly ammonium compound decamethoxin. Furthermore, nanoZnO impregnated polymer films compared to standard ZnO impregnated polymer films showed up to 63.2% faster substance release profile with rapid and more unified curve.

DECLARATION OF INTERESTS

The authors declare no conflicts of interest.

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AUTHORS CONTRIBUTIONS

The authors have contributed equally to concept and design, acquisition and interpretation of data, drafting the article.

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Доклінічна оцінка індивідуального підходу до лікування хронічних тривало незагоєваних ран

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Хронічні незагоєвані рани (ХНР) дуже поширені та часто неправильно лікуються, захворюваність і пов'язані з цим витрати на лікування хронічних ран підкреслюють необхідність впровадження концепцій профілактики та лікування ран.

Мета — оцінити можливості використання різних місцевих методик і засобів у лікуванні ХНР.

Матеріали та методи. Дизайн дослідження ґрунтується на оцінці різних типів перев'язувальних матеріалів з урахуванням їх можливості використання при місцевому лікуванні ХНР. Зразки біорозкладних полімерних плівок (з оптимальним складом желатину, полівінілового спирту, молочної кислоти, гліцерину та дистильованої води), насичених наночастинками кількох оксидів з очікуваною антибактеріальною та прорегенеративною властивістю — nZnO, nMgO у концентраціях 1 %, 5 % та 10 %, були використані для вивчення антимікробної дії та профілю вивільнення діючої речовини. Для контролю використовували четвертинно амонієвий антисептик декаметоксин 0,02 %.

Результати. Отримані дані свідчать, що біорозкладні плівки на полімерній основі з оптимальним компонентним складом (желатин, полівініловий спирт, молочна кислота та гліцерин), збагачені 5 % та 10 % наноксидом цинку, демонструють потужну антимікробну дію як щодо грамположитивних, так і грамнегативних мікроорганізмів, найбільш поширених збудників ХНР. Аналіз здатності до вивільнення іонів показав, що просочена цинком ранозагоєвальна біорозкладна полімерна плівка поступово вивільняє активну речовину залежно від часу, а нанорозмірні частинки наноксиду цинку вивільнюються з полімерної композиції швидше, ніж звичайний оксид цинку.

Висновки. Комплексні природні біорозкладні наноплівки на основі полімерів — це композиційні матеріали, просочені наноксидами металів, які мають високий потенціал для місцевого лікування хронічних ран, що не загоєються. Полімерна плівка з 5 % наноZnO показала найвищу антимікробну активність, порівнянню або таку, що перевищує четвертинно амонієву сполуку — декаметоксин. Крім того, полімерні плівки, просочені наноZnO, порівняно зі стандартними полімерними плівками, просоченими ZnO, показали кращий профіль вивільнення речовини з швидшою та більш уніфікованою кривою.

Ключові слова: хронічна рана, виразка, лікування, наноксид металу, полімерна наноплівка, антимікробна активність, біоплівка.

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