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Antimicrobial effect of wound healing nano-containing polymer materials

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Abstract

Background: Treatment of infected wounds presents the biggest difficulty for the surgeon. The treatment of skin lesions has recently increased due to the effect of developed local multi-application forms saturated with nano-sized antiseptic preparations, namely with metals nano-oxides: zinc oxide, magnesium oxide, iron oxide, silver oxide, etc.

Material and methods: We have developed a biodegradable polymer film that is flexible and has the ability to biodegrade and deliver the remedy to the area of injury. Saturated with active components, polymer films have been studied for their antimicrobial effect against clinical strains of opportunistic microorganisms commonly present in wounds, which were isolated from patients with purulent-septic diseases: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pyogenes*, *Escherichia coli*, *Citrobacter freundii*, *Pseudomonas aeruginosa* and *Candida tropicalis yeasts*.

Results: The films saturated with zinc nano-oxide have been characterized by high antimicrobial effect against all used microbial cultures. Films containing 5 and 10% of ZnO appeared to produce larger areas of growth inhibition against the most strains in comparison to the films containing 1% of ZnO. In contrast to zinc nano-oxide, the presence of magnesium in films hasn't proved to have any antimicrobial effect.

Conclusions: Biodegradable films with 5% of nano-oxide content have optimal antimicrobial effect in vitro against relatively opportunistic microorganisms and require further experimental and clinical studies.

Key words: wound healing polymer materials, zinc nano-oxide.

Introduction

The biggest difficulty for the surgeon is the treatment of infected wounds. The incidence of surgical infection in surgical diseases is not reducing and makes up 24-36% [1].

Recurring infectious diseases, as well as the continuous development of antibiotic resistance among different bacteria is a serious problem and danger to public health in the world [2].

Enterococci, Staphylococci, Streptococci and other pathogens cause a wide range of infectious diseases and result in surgical wounds infection and abscess that are very difficult to treat. Despite antimicrobial therapy, morbidity and mortality associated with the bacterial infection still remain high, partly due to the ability of these organisms to develop resistance to almost all antibiotics [3,4,5].

Nowadays, there are two main approaches to the purulent wounds treatment in the first phase of wound healing. The first one deals with finding the most effective ways of necrotic masses quick removing from the purulent wound (developing of experimental wound healing coatings to remove damaged tissue caused by different mechanical, thermal and other purulent-inflammatory or degenerative processes). The second one is based on the medications and tools development and their application that can limit and eliminate wound infections [6,7,8].

Improving local wound treatment is aimed primarily at the application of modern highly efficient drugs depending on the particular phase of wound healing [9,10]. Therefore, to solve these problems a new generation of drugs or agents should be used to combat bacterial infections in the wound. So, local multi-application forms with prolonged osmotic effect for the treatment of damaged skin have been developed and applied increasingly, to prevent drying of the wound, stimulate the growth of granulation and saturated antiseptic nano-sized drugs, namely metals nano-oxides: zinc oxide, magnesium oxide, iron oxide, silver oxide, etc. [11,12,13,14,15,16,17].

One of such promising antimicrobial agents in polymer materials that currently is intensively investigated, and has both anti-inflammatory and antiseptic effect is zinc nano-oxide (ZnO) [18,19,20].

The aim of the study was to investigate the antimicrobial properties of biodegradable wound healing polymer materials in the form of films with different concentrations of metals nano-oxides during the experiment in vitro.

Material and methods

We have developed a biodegradable polymer film [21] based on gelatin, polyvinyl alcohol (PVA), lactic acid, distilled water and glycerin, which are blended under the influence of microwave radiation, and which is flexible and has the ability to biodegrade and deliver the remedy to the area of injury. There was synthesized a polymer base by the method optimized previously (sample №8) and there was also synthesized a base saturated with nano-oxides of zinc (samples №1, 2, 3), of magnesium (samples №4, 5, 6) at concentrations (1%, 5% and 10%) respectively, and also saturated with common antiseptic decamethoxinum (sample №7) (tab.1)

Saturated with active components polymer films have been studied for their antimicrobial effect against clinical strains of opportunistic microorganisms commonly present in wounds, which were isolated from patients with purulent-septic diseases: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus pyogenes*, *Escherichia coli*, *Citrobacter freundii*, *Pseudomonas aeruginosa* and *Candida tropicalis yeasts*.

Identification of microorganisms clinical strains was based on morphological and cultural properties in accordance with the recommendations of the 9th edition of 'Bergey's Manual of Determinative Bacteriology'[22] and biochemical microtests sets 'STAPHYtest 16', 'STREPTOtest 16', 'ENTEROtest 24', 'NEFERMtest 24' (Lachema, Czech Republic).

Staphylococcus test strains differed with their antibiotic

Table 1

Polymer films compositional analysis

| Sample № | Gelatin, g | PVA, g | Water, ml | Lactic acid, ml | Glycerin, ml | Decame-thoxi-num, ml | Nano-oxides | |
|----------|------------|--------|-----------|-----------------|--------------|----------------------|-------------|---------|
| | | | | | | | mg | % |
| 1 | 2 | 1 | 15 | 0.05 | 0.02 | 0 | 0.025 | 1% ZnO |
| 2 | 2 | 1 | 15 | 0.05 | 0.02 | 0 | 0.125 | 5% ZnO |
| 3 | 2 | 1 | 15 | 0.05 | 0.02 | 0 | 0.25 | 10% ZnO |
| 4 | 2 | 1 | 15 | 0.05 | 0.02 | 0 | 0.25 | 1% MgO |
| 5 | 2 | 1 | 15 | 0.05 | 0.02 | 0 | 0.125 | 5% MgO |
| 6 | 2 | 1 | 15 | 0.05 | 0.02 | 0 | 0.25 | 10% MgO |
| 7 | 2 | 1 | 0 | 0.05 | 0.02 | 15 | 0 | 0 |
| 8 | 2 | 1 | 15 | 0.05 | 0.02 | 0 | 0 | 0 |

resistance: there were used methicillin-resistant and methicillin-susceptible strains in research. Methicillin-resistance of *S. aureus* and *S. haemolyticus* strains was proved with positive latex agglutination reaction to penicillin-binding protein PBP2 (Slidex® MRSA Detection, bioMerieux, France). Both methicillin-resistant strains of staphylococci are also characterized by the associated resistance to macrolides, tetracyclines, aminoglycosides and fluoroquinolones. Culture of *E. coli* proved to be sensitive to antibiotics, including aminopenitsyline, cephalosporins and carbapenems.

Other used in research Gram-negative bacteria are β -lactamase producers of extended range (ES β L). β -lactamase activity was detected on the comparative basis of strains sensitivity to cefoperazone and to combinations of tsefoperezon / sulbactam. The strain of *Candida tropicalis* showed weak sensitivity to polyenes (nystatin, amphotericin B) and alimines (terbinafine) in a dose-dependent sensitivity to imidazole (especially ketoconazole) and triazoles (fluconazole, itraconazole, voriconazole).

Microbial cultures were grown in liquid nutrient medium for 24 hours. Then 1 ml of daily microbial culture was diluted with isotonic sodium chloride solution at the ratio of 1: 1000. The obtained suspension was planted into the elective nutrient media prepared by 'a spread bacterial lawn' approach.

Determination of films antibacterial properties was conducted by disk-diffusion method. 6 mm diameter discs made from films samples were applied on the surface of agar which was planted smoothly with standardized test cultures suspensions. Experiments results were calculated after plating incubation in the thermoregulator for 24 hours. The obtained digital images on the plates were processed with the help of a computer program UTHSCSA ImageTool 2.0 [23].

Dimeters of microorganisms inhibition zones were determined around the investigated disks. Experiments with each microbial strain were performed three times. The results were processed with the help of variation statistics methods.

Results

We have previously investigated the antimicrobial properties of medicinal films containing various common antiseptic agents in combination with weak acids (lactic, salicylic, succinic, orthophosphoric) [24]. The results proved films

benefits after introducing decamethoxinum and chlorhexidine into biodegradable polymer-basis. At the same time we have proved that decasan in combination with lactic or salicylic acid increases antimicrobial films effect against putrefactive cocci flora (staphylococci, enterococci, β -hemolytic streptococci). This work is a logical continuation of research initiated and attempted to evaluate the biodegradable films antimicrobial effect made by our previously introduced method with adding zinc, magnesium nano-oxides particles, sized of 30 nm (Yurui (Shanghai) Chemical Co., Ltd., China) and decamethoxinum.

Made by us film containing zinc nano-oxide, is characterized by high antimicrobial effect against all microbial cultures used, except *Pseudomonas aeruginosa* (tab. 2). No significant differences in diameter of microorganisms inhibition zones have been determined around zinc nano-oxide films from methicillin-resistant and methicillin-susceptible staphylococci. Coagulase-negative staphylococci (*S. epidermidis* and *S. haemolyticus*) and β -hemolytic streptococcus *S. pyogenes* revealed higher sensitivity to zinc nano-oxide than strains of *Staphylococcus aureus* *S. aureus*. Films containing 5 and 10% of ZnO appeared to produce larger areas of growth delay against most strains in comparison to the films containing 1% of ZnO. In contrast to zinc nano-oxide, the presence of magnesium in films hasn't proved to have any antimicrobial effect.

For a more detailed analysis of the zinc concentration impact in nano-oxide polymer films on the growth of different types of microorganisms, the obtained results are presented in diagrams.

Regarding the strains of *S. aureus*, the greatest antimicrobial effect was observed in the films containing decamethoxinum. Zones of MSSA growth inhibition, being under the influence of zinc nano-oxide films, were increasing in proportion to the increase of zinc concentration. Optimal antimicrobial effect was produced by the film containing 5 and 10% of zinc nano-oxide. Zones of MSSA growth inhibition under the influence of magnesium nano-oxide film were two times smaller in comparison to those under the influence of ZnO containing film of a similar concentration. Zinc nano-oxide films retained high activity against MRSA. MgO film appeared to be absolutely ineffective against MRSA.

Similar patterns were found in the study of films samples against coagulase-negative staphylococci. The best effect against both *MSSE* and *MRSH* was produced by 5% ZnO containing film. MgO film did not appear to be effective against coagulase-negative staphylococci either *MSSE* or *MRSH*.

β-hemolytic S. pyogenes appeared to be the most sensitive of all ZnO films samples, particularly in concentrations of 1 and 5%. However, under the influence of MgO film, growth inhibition of *β-hemolytic streptococcus* was not observed.

Regarding antibiotic sensitive *E. coli*, the most active film appeared to be 5% ZnO. 10% MgO film produced almost half weaker antimicrobial effect. Diameters of inhibition zones of antibiotic-resistant citrobacter culture under the influence of ZnO film at concentrations of 1 and 5%, were 1,6 and 1,3 times bigger in comparison to antibiotic sensitive *E. coli*. MgO films and films containing decasan did not produce any antimicrobial effect against *ESβL + C. freundii*.

Regarding polyantibiotic resistant *Pseudomonas aeruginosa*, all investigated films proved to have only bacteriostatic effect. The effect produced by 5 and 10% ZnO films was equal to the one produced by the films containing decasan. ZnO films effectively inhibited the growth of *Candida tropi-*

calis which is resistant to classical culture antimycotics. This antifungal effect of 5 and 10% ZnO films was even greater than the effect produced by the films containing decasan.

The results of the experiments indicate that the optimum is to apply 5 % ZnO into biodegradable film basis. This type of films has the widest range of antimicrobial effect. They effectively inhibited the growth of all test strains, especially *S. pyogenes*, coagulase-negative staphylococci (including *MRSH* and *S. haemolyticus*), and the yeasts *Candida tropicalis* (fig.1).

Discussion

Antimicrobial properties of zinc oxide ions and its salts are well known and have been used in medicine for a long time [25]. In recent years, practical application of zinc oxide nano-particles has been intensively investigated [17, 20]. Into surgical practice there have been introduced and incorporated antimicrobial biomaterials as well as metal substrates [26]. There were applied fabrics impregnated with zinc nano-oxide [27]. Nano-particles of zinc oxide with gentamicin adsorbed have been reported to provide a synergistic effect of both components regarding *S. aureus*, *E. faecalis*, *E. coli*, *Salmonella sp.*, *L. monocytogenes*, *P. aeruginosa* [28,29,30]. We have applied ZnO biodegradable polymer films for the treatment of septic and infected wounds.

The ZnO biodegradable polymer films suggested by the author are characterized by high antimicrobial effects against all used cultures of opportunistic microorganisms.

It should be noted that similar MgO films did not produce any antimicrobial effect. This result is consistent with literature data that point to a distinct correlation of antimicrobial effect of magnesium nano-oxide film to the size of its particles. Thus, 24-hour contact with the MgO nano-particles which are smaller than 10 nm, leads to intensive cell death of *S. aureus* spores of *B. subtilis*. However, MgO nano-particles of 50 nm have the ability to inhibit the growth of *E. coli* and *B. subtilis* partially [31].

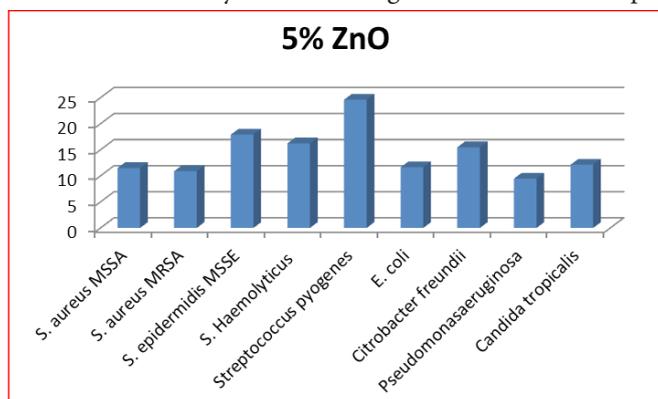


Fig. 1. Effect of 5 % ZnO films on the growth of microorganisms test strains.

Table 2

Antimicrobial effect of the investigated polymer films (diameters of microorganisms inhibition zones, mm)

| | | <i>S. aureus</i> MSSA | <i>S. aureus</i> MRSA | <i>S. epidermi-</i> <i>dis MSSE</i> | <i>S. haemo-</i> <i>ly-ticus</i> MRSH | <i>Strepto-</i> <i>coccus</i> <i>pyogenes</i> | <i>E. coli</i> | <i>Pseudomona</i> <i>saeruginosa</i> | <i>Citrobacter</i> <i>freundii</i> | <i>Candida</i> <i>tropicalis</i> |
|---|---------------------|--------------------------|--------------------------|--|---|---|----------------|---|---------------------------------------|-------------------------------------|
| 1 | 1% ZnO | 9.78±0.17 | 11.47±0.84 | 15.39±0.46 | 6.17±0.37 | 23.08±0.42 | 8.88±0.27 | 0 | 14.33±0.79 | 9.77±0.34 |
| 2 | 5% ZnO | 11.49±0.35 | 10.90±0.46 | 17.90±0.77 | 16.23±0.32 | 24.60±0.11 | 11.64±0.16 | [9.46±0.18] | 15.48±0.23 | 12.13±0.89 |
| 3 | 10%ZnO | 11.98±0.33 | 10.53±0.18 | 15.78±0.60 | 15.43±0.55 | 18.21±0.63 | 9.40±1.06 | [9.83±0.20] | 11.00±0.43 | 13.20±0.67 |
| 4 | 1% MgO | 6.45±0.63 | 0 | 0 | 0 | 0 | 6.63±0.64 | 0 | 0 | 0 |
| 5 | 5% MgO | 6.32±0.17 | 0 | 0 | 0 | 0 | 6.83±0.15 | 0 | 0 | 0 |
| 6 | 10% MgO | 6.01±0.31 | 0 | 0 | 0 | 0 | 7.01±0.42 | 0 | 0 | 0 |
| 5 | Decame- thoxinum | 14.29±0.21 | 13.65±0.25 | 13.87±1.10 | 15.81±0.77 | 20.96±0.68 | 8.92±0.47 | [10.20±0.36] | 4.82±0.67 | 11.69±0.36 |
| K | Control (basis) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Note: in brackets, zones of partial growth inhibition are presented (bacteriostatic effect).

Thus, *in vitro* experiments showed that biodegradable polymer-based films made of gelatin, polyvinyl alcohol, lactic acid and glycerin also containing 5% and 10% of zinc nano-oxide proved to have high antimicrobial effect against Gram-positive and Gram-negative opportunistic microorganisms that are most common causative agents of surgical wound infections. It is important from a practical point of view as this effect spreads to polyantibiotic resistant strains. So, developed by us nano-containing biodegradable polymers can be used as a means of drug delivery in the treatment of septic and infected wounds of various origins.

Conclusions

1. Biodegradable films containing zinc nano-oxide have high antimicrobial effect against opportunistic microorganisms – pathogens of wound infections, including their polyantibiotic resistant strains.
2. *In vitro* experiments showed that biodegradable polymer-based films containing 5% zinc nano-oxide proved to have high antimicrobial effect.
3. New polymer biodegradable films with nano-oxide content have optimal antimicrobial effect *in vitro* against relatively opportunistic microorganisms and require further experimental and clinical studies.

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