Application of aqueous dispersions of silver nanostructures for treatment of pyoinflammatory diseases with a chronic component

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Abstract.
Bactericidal properties of aqueous dispersions of oxide silver nanostructures (ADSN) produced by means of pulsed electric discharges (PED) in water can use in surgery for treatment of upper purulent wounds with a chronic component. The patients with such wounds are of large number and differ on etiology of diseases but their mutual feature is long treatment without marked positive changes. Thus long application of antibiotics leads to abnormality of immune processes and antibacterial resistance of microbial flora. Moreover, local antiseptics are frequently toxic and one can oppress processes of reparation in a wound. The investigation is addressed to finding out the opportunity of usage of an ADSN for treatment of purulent wounds with a chronic component and comparison of its efficiency with the sodium hypochlorite. At investigation, the ADSN formed at PED of 5 - 10 µs duration, with highest share of “small” (hydrodynamic diameter \(\leq 100\) nm) nanostructures and greatest surface electric charge we used. It was found that the usage of ADSN during the first 5 days characterized by high active reparative processes with their maximum at 3rd – 4th days and subsequent moderate further healing. At local use of ADSN, there were no cellular atypia and preternatural representations about inflammatory reactions. It is possible to assume that usage of ADSN will allow in prospect to correct the practice of out-patient therapy of chronic and slow pyoinflammatory diseases.

1. Introduction
Surgical infection is one of the priorities in clinical medicine due to high number of morbidity and significant difficulties in treatment. Surgical infection reaches 35% in total surgical morbidity and it takes place in the form of acute and chronic purulent processes.

ADSN produced by means of PED in water have high biological activity and bactericidity. However, to date, many issues of action ADSN on purulent wounds are poorly understood, so they are vital so far.

The study is addressed to determination the possibility of ADSN for development of a method for purulent wounds treatment.

2. Materials and procedure
One of effective ways of oxide metal nanostructures manufacture is processing of water by PED. In this case, ADSN formed due to electrodes erosion by PED contains both nanostructures of electrode metal or its oxides and metal’s ions [4, 7]. For determination of ADSN parameters, the techniques of dynamic quasi-elastic scattering and high effective ionic capillary electrophoresis were used.

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Distribution of a share of “small” nanostructures subfractions (≤ 100 nm) and a surface electric charge of nanostructures depending on pulse duration by laser correlation spectrometer “LCS-03” and the device capillary electrophoresis “NANOPHORE 01” had been determined (Figure 1).

For the clinical researches, the ADSN with maximal share of “small” nanostructures and maximal surface electric charge has been chosen. Such nanostructures at pulse duration of 5 - 10 µs are formed. The parameters of electrodischarge unit were next: voltage - 60 kV, pulsed current - 40 A, pulse duration 5 - 20 µs, and pulse frequency - 50 Hz.

Clinical research on 133 rats of both sexes of line “VISTAR” of weight 170 ± 30 g was carried out. The animals were divided in three groups: one control group of 31 animals and two test groups of 51 animals each.

For treatment of wounds, the ADSN of alloy (~ 70 % of silver and ~ 30 % of copper) in 2 months after its preparation and solution of sodium hypochlorite of 600 mg/l were used.

Purulent wounds of initial area of 150 ± 50 mm² by means of sewing in inter shoulder-blade areas of a gauze napkin impregnated with a solution of low viral strain of Staphylococcus aureus were made. Lancing and surgical sanitation of an abscess on 7th day after infecting were carried out. Treatment procedures at using of ADSN of 4.5 mg/l and solution of sodium hypochlorite of 600 mg/l in test groups of animals in one time per day during 14 days were performed.

For a comprehensive assessment of wound healing process, the methods of planimetric, bacteriological, histological examination of the wounds, which were carried out on the 3rd, 5th, 7th, 10th, 14th days were used.

3. Results and discussion
Bacteriological investigation of purulent wounds included a study of the qualitative composition of microbial pathogens and quantitative count of microorganisms.

Determination of quantitative composition of microflora in wounds per 1 g of tissue is shown in table 1.
Table 1. Number of microorganisms on 1g of tissue in experimental animals.

| Groups of animals | 1st day (CFU) | 3rd day (CFU) | 5th day (CFU) | 7th day (CFU) |
|-------------------|---------------|---------------|---------------|---------------|
| Control           | (3.5±1.6)×10⁷ | (3.9±1.9)×10⁷ | (3.8±1.4)×10⁷ | (3.7±1.4)×10⁷ |
| Sodium hypochlorite | (3.9±1.7)×10⁷ | (5.3±1.6)×10⁵ | (3.8±0.5)×10⁴ | No growth     |
| ADSN              | (3.7±1.5)×10⁷ | (6.5±1.1)×10⁵ | (3.9±0.6)×10⁴ | No growth     |

Thus, the use of sodium hypochlorite solution and ADSN make the effective elimination of the pathogens from the wounds, which was not observed in control group of animals.

Morphodynamic changes of wound healing process at the application of ADSN and sodium hypochlorite compared with the control are presented in table 2.

Table 2. Morphodynamics of wound healing process.

| Clinical signs                             | Control (days) | Sodium hypochlorite (days) | ADSN (days) |
|--------------------------------------------|----------------|---------------------------|-------------|
| Period of wound cleansing                  | 9.1±0.1        | 4.5±0.2                   | 4.3±0.2     |
| Period of appearance of granulations       | 8.3±0.2        | 4.3±0.2                   | 4.2±0.2     |
| Period of wound granulations filling       | 13.0±0.3       | 6.0±0.1                   | 5.9±0.2     |
| Beginning of edge epithelialization        | 10.6±0.2       | 6.3±0.2                   | 6.3±0.1     |

The data analysis revealed reducing wound healing time for experimental series using sodium hypochlorite and ADSN for the purulent wound treatment.

Application of sodium hypochlorite and ADSN for the purulent wound treatment led to changes in the area of wounds in experimental animals. The data presented in table 3.

Table 3. The area of wounds in experimental animals during treatment.

| Days | Control (mm²) | Sodium hypochlorite (mm²) | ADSN (mm²) |
|------|---------------|---------------------------|------------|
| 1    | 40.2±5.1      | 400.1±3.1                 | 400.2±6.2  |
| 3    | 390.1±1.9     | 355.2±2.5                 | 333.5±5.1  |
| 5    | 366.4±0.7     | 238.5±6.3                 | 219.4±4.7  |
| 7    | 355.7±8       | 182.3±13.5                | 165.7±9.1  |
| 10   | 334.2±1.1     | 154.4±10.4                | 138.9±11.3 |
| 14   | 275.8±0.55    | 70.0±5.3                  | 52.3±2.3   |

It should be noted that there is diurnal decrease of wound areas in experimental animals due to daily application of sodium hypochlorite and ADSN. The data presented in table 4.
Table 4. Daily decrease of wound areas in experimental animals.

| Days | Control (%) | Sodium hypochlorite (%) | ADSN (%) |
|------|-------------|-------------------------|----------|
| 3    | 2.5±0.1     | 11.2±0.2                | 16.6±0.2 |
| 5    | 6.0±0.1     | 32.9±0.1                | 34.2±0.1 |
| 7    | 2.9±0.1     | 23.4±0.1                | 24.4±0.1 |
| 10   | 6.0±0.2     | 15.3±0.1                | 16.1±0.1 |
| 14   | 17.4±0.3    | 54.6±0.1                | 62.3±0.1 |

Analysis of the data on the daily decrease in wound areas shows a positive impact of sodium hypochlorite and ADSN on the purulent wound. It should be noted that more effective impact on wound healing has ADSN. In the control group of animals the dynamics of decreasing the surface area of wounds was significantly smaller. Total reduction in the area of wounds for 14th day is presented in table 5.

Table 5. Total reduction in the area of wounds for 14th day.

| Groups of animals |
|-------------------|
| Control (%)       |
| Sodium hypochlorite (%) |
| ADSN (%)          |
| 31.08±0.2         | 82.5±0.1             | 86.9±0.1 |

Data of table 5 shows that the greatest common factor of decreasing the wound areas for 14 days stated in the test group with the use of ADSN.

Changes of wound areas in mm²/day during treatment are presented in table 6.

Table 6. Terms of wound healing in experimental animals.

| Days | Control (mm²/day) | Sodium hypochlorite (mm²/day) | ADSN (mm²/day) |
|------|------------------|-------------------------------|----------------|
| 3    | 10.1±1.2         | 44.9±3.5                      | 66.7±2.5       |
| 5    | 23.7±2.7         | 117.0±5.6                     | 114.1±5.2      |
| 7    | 10.7±1.1         | 55.9±6.7                      | 53.7±5.4       |
| 10   | 21.5±1.2         | 27.9±4.5                      | 26.8±3.7       |
| 14   | 58.4±5.7         | 84.4±6.5                      | 86.6±4.9       |

The data of table 6 shows that the shortest time of healing was observed with the use of ADSN. This period was slightly shorter than that in case of sodium hypochlorite solution application.

The analysis of planimetric data and the healing time shows high activity of wound healing at using of ADSN, which exceed that of sodium hypochlorite solution.

Thus, the findings of our investigation of wound healing in animals with purulent wounds indicated a reasonably effectiveness of ADSN, which not lower than sodium hypochlorite solution.

Our study has shown that ADSN with local application for the treatment of purulent wounds can be recommended as a means of comprehensive prevention and healing of wound infections because of its antiseptic properties.

Current practice of treatment of chronic purulent wounds on the background of the damaged tissue metabolism and local pathomorphism shows that there is no reason to rely on their quick healing, and therefore, the aim of treatment is to prevent complications.

In addition, the reactivation of an existing microflora when adapting it to antibiotics and the accession of additional associations of pathogens in the nutrient medium of wounds result to
considerable adverse effects. Antibiotic resistance is becoming every year more and more pressing problem. Traditionally used antiseptics can inhibit regeneration in the wound and sometimes they lead to total intoxication patient with their long-term use in large-affected areas.

The investigation showed that the ADSN have not of systemic toxicity, which may open new possibilities to cure a broad category of patients. Another significant positive quality of ADSN is the relative ease and cheapness of their manufacturing, as well as the fact that their storage does not require special conditions and they retain their antibacterial properties for a long time.

4. Conclusion
For realization of investigation was used ADSN, which has maximal share of “small” nanostructures (<100 nm) with the greatest surface electric charge. They formed at pulse duration of 5 - 10 µs.

It was founded that bacterial contamination of purulent wounds disappears for the 7th day in the local use of ADSN. The acceleration of wound cleansing and a positive healing morphodynamics with the earlier boundary epithelization was noted. As a result of local application of ADSN enhances the appearance of early signs of purulent wounds healing in comparison with sodium hypochlorite solution.

The attributes of cellular atypia and preternatural representations about inflammatory reactions are not revealed at local use of ADSN.

The positive results of local use of ADSN in the study allow us to recommend this method for use in clinical practice.

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