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The studies on the pharmaceutical development of dosage forms with silver and gold nanoparticles for use in dentistry and surgery

In dentistry and surgery a special role belongs to medications of the local action that possess the antimicrobial and anti-inflammatory properties and can stimulate the tissue regeneration.

Aim. To study the antimicrobial activity of colloidal solutions of silver and gold nanoparticles *in vitro*, estimate their pharmacological activity *in vivo* and substantiate the composition and technology of 3 dosage forms based on them – a hydrophilic ointment, gel and solution for wound cleansing.

Materials and methods. Colloidal solutions of spherical silver and gold nanoparticles with the particle size of 30 nm and the concentration of 8.0 mg/ml and 77.2 μg/ml nanoparticles, respectively, were used to develop dosage forms. The antimicrobial activity of colloidal solutions of silver and gold nanoparticles was determined using the test strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Enterococcus faecalis*, *Candida albicans*, *Proteus vulgaris*, as well as clinical isolates of pathogenic strains of microorganisms isolated from patients with purulent inflammatory diseases of the maxillofacial area. The therapeutic efficacy of colloidal solutions of silver and gold nanoparticles *in vivo* was studied on the model of the jaw abscess of the experimental animals (*Wistar* rats). Technological methods were used to make dosage forms in accordance with the general rules for the manufacture of liquid and semi-solid medicines.

Results and discussion. It has been found that silver nanoparticles in the concentration of 0.16 mg/ml and their combination with gold nanoparticles in the concentration of 0.08 mg/ml for Ag and 1.93 μg/ml for Au exhibit a high level of the antimicrobial activity against all test microorganisms. Gold nanoparticles as independent antimicrobial agents within the concentration range of 1.93-38.6 μg/ml exhibit a weak antimicrobial activity. The combination of silver nanoparticles with gold nanoparticles exhibit the marked anti-inflammatory action and contribute to decontamination and healing of the wound in the studies *in vivo* compared to the effect of silver nanoparticles only. The studies conducted made it possible to substantiate the choice of the concentration of colloidal solutions in dosage forms.

Conclusions. According to the results obtained the marked complex action (antimicrobial, anti-inflammatory, regenerative) of the combination of colloidal solutions of silver and gold nanoparticles in the treatment of purulent-inflammatory diseases of the maxillofacial area has been determined. The composition and technology of 3 dosage forms based on them – an ointment, gel, solution for wound cleansing, which can be promising for application in dentistry and surgery, have been substantiated.

Key words: *silver nanoparticles; gold nanoparticles; solution for wound cleansing; ointment; gel*

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Дослідження з фармацевтичної розробки лікарських форм з наночастинками срібла і золота для застосування у стоматології і хірургії

У стоматології і хірургії особлива роль належить засобам місцевої дії, які володіють антимікробними та протизапальними властивостями, а також здатні стимулювати процеси репарації.

Мета роботи. Вивчити антимікробну дію колоїдних розчинів наночастинок срібла і золота в дослідженнях *in vitro*, їх фармакологічну активність *in vivo* та обґрунтувати склад і технологію 3-х лікарських форм на їх основі – гідрофільної мазі, гелю та розчину для промивання ран.

Матеріали та методи. Для розробки лікарських засобів використані колоїдні розчини наночастинок срібла і золота сферичної форми із середнім розміром частинок 30 нм та концентрацією наночастинок 8,0 мг/мл і 77,2 мкг/мл відповідно. Антимікробну активність колоїдних розчинів наночастинок срібла і золота визначали щодо музейних штамів мікроорганізмів *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis, Enterococcus faecalis*, *Candida albicans*, *Proteus vulgaris*, а також клінічних ізолятів патогенних штамів мікроорганізмів, виділених від хворих на гнійно-запальні захворювання щелепно-лицевої ділянки. Ефективність фармакологічної дії і*n vivo* колоїдних розчинів наночастинок срібла і золота досліджували на моделі абсцесу щелепної ділянки дослідних тварин. Для виготовлення лікарських форм застосовували технологічні методи відповідно до загальних правил виготовлення рідких і м'яких лікарських засобів.

Результати та їх обговорення. Встановлено, що наночастинки срібла у концентрації 0,16 мг/мл та їх комбінація з наночастинками золота у концентрації 0,08 мг/мл за Ag та 1,93 мкг/мл за Au виявляють високий рівень антимікробної активності відносно усіх досліджуваних тест-культур мікроорганізмів. Наночастинки золота як самостійні антимікробні агенти в концентраційному діапазоні 1,93-38,6 мкг/мл за металом виявляють слабку антимікробну активність. У дослідженнях *in vivo* встановлено, що комбінація колоїдного розчину наночастинок срібла і золота виявляє виражену протизапальну дію і сприяє знезараженню та загоєнню рани порівняно із характером впливу монодисперсії наночастинок срібла. Проведені дослідження дали можливість обґрунтувати вибір концентрації колоїдних розчинів наночастинок у лікарських формах.

Висновки. За результатами проведених досліджень встановлена виражена комплексна дія (антимікробна, протизапальна, регенеративна) комбінації колоїдних розчинів наночастинок срібла і золота при лікуванні гнійно-запальних захворювань щелепно-лицевої ділянки. Обґрунтовано склад і технологію 3-х лікарських форм на їх основі – мазі, гелю, розчину для промивання ран, що можуть бути перспективними для застосування у стоматології і хірургії.

Ключові слова: *наночастинки срібла; наночастинки золота; розчин для промивання ран; мазь; гель*

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Исследования по фармацевтической разработке лекарственных форм с наночастицами металлов для применения в стоматологии и хирургии

В стоматологии и хирургии особая роль отведена средствам местного действия, которые обладают антимикробными и противовоспалительными свойствами, а также способны влиять на процессы репарации.

Цель работы. Изучить антимикробное действие коллоидных растворов наночастиц серебра и золота в исследованиях *in vitro*, их фармакологическую активность *in vivo* и обосновать состав и технологию 3-х лекарственных форм на их основании – гидрофильной мази, геля и раствора для промывания ран.

Материалы и методы. Для разработки лекарственных средств использованы коллоидные растворы наночастиц серебра и золота сферической формы со средним размером частиц 30 нм и концентрацией наночастиц 8,0 мг/мл и 77,2 мкг/мл соответственно. Антимикробную активность коллоидных растворов наночастиц серебра и золота определяли на музейных штаммах микроорганизмов *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis, Enterococcus faecalis*, *Candida albicans*, *Proteus vulgaris*, а также клинических изолятах патогенных штаммов микроорганизмов, выделенных от больных гнойно-воспалительными заболеваниями челюстно-лицевой области. Эффективность фармакологического действия *in vivo* коллоидных растворов наночастиц серебра и золота исследовали на модели абсцесса челюстного участка подопытных животных. Для изготовления лекарственных форм применяли технологические методы в соответствии с общими правилами изготовления жидких и мягких лекарственных средств.

Результаты и их обсуждение. Установлено, что наночастицы серебра в концентрации 0,16 мг/мл и их комбинация с наночастицами золота в концентрации 0,08 мг/мл по Ag и 1,93 мкг/мл по Au проявляют высокий уровень антимикробной активности в отношении всех исследуемых тест-культур микроорганизмов. Наночастицы золота как самостоятельные антимикробные агенты в диапазоне концентраций наночастиц 1,93-38,6 мкг/мл по металлу проявляют слабую антимикробную активность. В исследованиях *in vivo* установлено, что комбинация наночастиц серебра и золота оказывает выраженное противовоспалительное действие и способствует обеззараживанию и заживлению раны по сравнению с характером влияния монодисперсии наночастиц серебра. Проведенные исследования позволили обосновать выбор концентрации коллоидных растворов наночастиц в лекарственных формах.

Выводы. По результатам проведенных исследований установлено выраженное комплексное воздействие (антимикробное, противовоспалительное, регенеративное) комбинации коллоидных растворов наночастиц серебра и золота при лечении гнойно-воспалительных заболеваний челюстно-лицевой области. Обоснованы состав и технология 3-х лекарственных форм на их основе – мази, геля, раствора для промывания ран, которые могут быть перспективными для применения в стоматологии и хирургии.

Ключевые слова: *наночастицы серебра; наночастицы золота; раствор для промывания ран; мазь; гель*

In dentistry and surgery, an alternative to the antimicrobial medicines that are available at the pharmaceutical market for the treatment of infectious diseases is creation of new drugs based on silver and gold nanoparticles considering their high biological activity: antimicrobial [1, 2], anti-inflammatory [3] and the ability to stimulate the tissue regeneration [4, 5, 6].

Among dosage forms in dentistry today it is important to use semi-solid preparations, in particular in the practice of maxillofacial surgery and for the treatment of gingivitis and periodontitis since purulent-inflammatory diseases constitute a significant percentage among such patients. Annually, this percentage increases due to the expansion of multi-resistant strains of microorganisms to the traditional antibiotics and weakening of the immune status of patients [7, 8].

In the complex treatment of periodontal diseases it is important to use local medicines having the ability to inhibit the pathological process to avoid complications due to antimicrobial and anti-inflammatory properties, as well as to be able to stimulate the regeneration processes [9, 10].

To achieve the desired effect of local therapy of the mucous membrane of the oral cavity it is necessary to take into account not only the pharmacological properties of the active substances, but also the properties of excipi-

ents, namely the base of the medicinal product. When developing such medicines it should be considered that hydrophobic ointments have an occlusion effect, they are difficult to wash off with water and not mixable with the exudate; when using emulsion products in dentistry the concentration of active substances rapidly reduces due to their dilution with saliva, medicinal substances are washed into the lower gastrointestinal tract, and this is not acceptable in the case of wound processes in phases II and III and inflammation of the oral mucosa [11]. Therefore, development of solutions for wounds cleansing and semi-solid preparations on hydrophilic bases, especially in the form of gels intended to be applied to the wound surface of the mucous membrane of the oral cavity is relevant.

The aim of the work was to study the antimicrobial action of colloidal solutions of silver and gold nanoparticles in *in vitro*, their pharmacological activity *in vivo* and substantiate the composition and technology of 3 dosage forms on their basis for the use in dentistry and surgery – a hydrophilic ointment, gel and solution for wound cleansing.

Materials and methods

To develop medicines for dentistry and surgery colloidal solutions of silver (AgNP) and gold (AuNP) nanoparticles with an average particle size of 30 nm and the concentration of 8.0 mg/ml and 77.2 μg/ml nanoparticles, respectively, were synthesized by the method of chemical condensation in the aqueous medium according to the original protocols.

Colloidal solutions of silver and gold nanoparticles were characterized as biosafe by indexes of cytotoxicity, genotoxicity, mutagenicity and biochemical markers in accordance with the criteria of the Guidelines "Safety assessment of medical nanopreparations" approved by the Scientific Expert Council of the State Expert Center of the Ministry of Health of Ukraine (Recoed No. 8, September 26, 2013).

The antimicrobial activity of the colloidal solutions of silver and gold nanoparticles was determined in the studies *in vitro* according to the standard protocol for the method of serial dilutions in agar according to the guidelines for susceptibility testing of microorganisms to antibacterial agents [12]. The test-strains of microorganisms of *Staphylococcus aureus* MRSA ATCC 43300, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 2592, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* 209P, *Enterococcus faecalis* G35 No 4-410, *Escherichia coli* No 25, *Pseudomonas aeruginosa* ATCC 27853 (F-51), *Candida albicans*, *Proteus vulgaris* HX 19 No. 222 from the ATCC Collection and from the Collection of the State Scientific Control Institute of Biotechnology and Strains of Microorganisms (Kyiv, Ukraine), as well as pathogenic clinical isolates of microorganisms isolated from the patients with purulent-inflammatory diseases of the maxillofacial area were used to estimate the antimicrobial activity of the metal nanoparticles synthesized *in vitro*.

The therapeutic efficacy of silver and gold colloidal solutions *in vivo* was studied on the model of the jaw abscess of Wistar rats. The studies *in vivo* were conducted in the vivarium of the Institute of Veterinary Medicine NAS of Ukraine (Kyiv). All experiments with animals were carried out according to the accepted ethical norms and standards.

The jaw abscess was modeled according to [13] by a subcutaneous injection of 0.5 ml of the suspension containing a daily culture of *Staphylococcus aureus* clinical isolate (5×10^7 CFU/ml) and the activated carbon powder with a mass fraction of 9 % in the suspension; it was injected in the submandibular area of laboratory animals. The opening of the abscess was carried out by the skin incision over the center of infiltration on the 4th day after injection; the length of the cut was not less than the length of the infiltrate. Irrigation of the opened abscess by colloidal solutions was done once a day for 3 days, at the rate of 4 ml of each solution per animal. There were the following animal groups in the experiment (10 animals in each group): group 1 – control group – healthy animals, group 2 – control group of animals with opened untreated abscess, group 3 – irrigation of the abscess with a colloidal solution of silver nanoparticles (AgNP) in the concentration of 0.8 mg/ml, group 4 – irrigation of the opened abscess with a colloidal solution containing the combination of silver and gold nanoparticles (Ag/AuNP) in the concentration of 0.8 mg (Ag)/19.3 μg (Au) per 1 ml.

The therapeutic effectiveness in vivo was assessed by the results of the histological studies. For the histological study the affected areas of the mandibles were isolated. Fixation and preparation of histological sections were performed according to the generally accepted protocols [14]. The specimens of histological sections were stained with hematoxylin-eosin. The specimens for histological studies were taken on day 5 after the last irrigation of the opened abscesses under the general anesthesia.

Technological methods were used for preparing of dosage forms according to the general rules for the manufacture of liquid and semi-solid preparations.

Results and discussion

To select the optimal concentration of silver and gold nanoparticles in the preparations developed for dentistry and surgery their antimicrobial activity *in vitro* was studied at the first stage of the research. Test strains of microorganisms – representatives of the main types of pathogens isolated in patients with purulent inflammatory diseases of the maxillofacial area were used to assess the antimicrobial activity of nanoparticles.

Colloidal solutions of silver nanoparticles with the concentration of 0.08 mg/ml and 0.16 mg/ml (dilution of the initial dispersion 1 : 100 and 1 : 50, respectively) were used for the study based on the literature data, the minimum inhibitory concentration of silver nanoparticles for *S. aureus* was in the range of 8-80 mg/L, and for *P. aeruginosa* was in the range of 8-70 mg/l [15].

When assessing the antimicrobial activity of gold nanoparticles *in vitro*, due to the existing controversial literature data in this area of research, the proven safe concentration of such nanoparticles for eukaryotic cells was chosen [16]. Gold nanoparticles in the concentra-

tion range of 1.93-38.6 μg/ml (dilution of the initial solution 1 : 40, 1 : 25, 1 : 10, 1 : 4 and 1 : 2, respectively) were used.

The results obtained showed the complete inhibition of growth for all pathogenic test strains examined under the effect of silver nanoparticles in the concentration of 0.16 mg/ml in the culture medium. A single colony growth was observed only for *C. albicans* and *B. subtilis* test strains with the seed doses of 10^5 CFU/cm^3 and silver nanoparticles with the concentration of 0.08 mg/ml by metal in the detection medium (Tab. 1).

Gold nanoparticles as an independent antimicrobial agent showed a weak antimicrobial activity in the similar study. There was no pronounced growth inhibition of all test strains in the nanoparticles concentration range of 1.93-38.6 μg/ml in the detection medium. The study of the antimicrobial action effectiveness of gold nanoparticles in the higher concentration range was considered to be inappropriate because of the significant increase in the potential final cost of the drug based on these nanoparticles.

Compared to the antimicrobial activity of the monodispersion of silver nanoparticles the combination of silver and gold nanoparticles (Ag/AuNP) in the concentration of 0.08 mg/ml (Ag) and 1.93 μg/ml (Au) revealed a pronounced bactericidal effect against all test strains and for all seed doses studied (Tab. 1). Thus, it is possible that gold nanoparticles without their own expressed antimicrobial activity have the ability to increase the antimicrobial activity of silver nanoparticles in the mixture.

Table 1

The antimicrobial activity of the colloidal solution of silver nanoparticles (AgNP) against pathogenic test strains of microorganisms

Test-strain	Seed dose of a test-strain, CFU/cm^3	Concentration of AgNP in the detection medium, mg/ml by metal		Control of the test strain growth
		0.08	0.16	
Staphylococcus aureus MRSA ATCC 43300	10^3	I	I	++++
	10^4	I	I	++++
	10^5	I	I	++++
Pseudomonas aeruginosa ATCC 27853	10^3	I	I	++++
	10^4	I	I	++++
	10^5	I	I	++++
Escherichia coli ATCC 2592	10^3	I	I	++++
	10^4	I	I	++++
	10^5	I	I	++++
Bacillus subtilis ATCC6633	10^3	I	I	++++
	10^4	I	I	++++
	10^5	+	I	++++
Staphylococcus aureus 209P	10^3	I	I	++++
	10^4	I	I	++++
	10^5	I	I	++++
Enterococcus faecalis G35 No. 4-410	10^3	I	I	++++
	10^4	I	I	++++
	10^5	I	I	++++
Escherichia coli No. 25	10^3	I	I	++++
	10^4	I	I	++++
	10^5	I	I	++++
Pseudomonas aeruginosa ATCC27853 (F-51)	10^3	I	I	++++
	10^4	I	I	++++
	10^5	I	I	++++
Candida albicans	10^3	I	I	++++
	10^4	I	I	++++
	10^5	+	I	++++
Proteus vulgaris HX 19 No. 222	10^3	I	I	++++
	10^4	I	I	++++
	10^5	I	I	++++

Note: "I" – total inhibition of the test strain growth; "++++"– intensive growth of the test strain; "+" – a single colony growth on the Petri dish; CFU – colony-forming unit.

Table 2

The antimicrobial activity of silver nanoparticles and the combination of silver and gold nanoparticles against pathogen clinical isolates of microorganisms

Clinical isolates	Growth of strains in the presence of AgNP in the concentration of 0.08 mg / ml by metal in the detection medium	Growth of strains in the presence of Ag/AuNP in the concentration of 0.08 mg/ml (Ag) and 1.93 μg/ml (Au) in the detection medium	Control of the strain growth
Staphylococcus aureus	I	I	++++
Micrococcus	I	I	++++
Escherichia coli	I	I	++++
Pseudomonas aeruginosa	I	I	++++
Candida albicans	I	I	++++
Yeastlike microorganisms	I	I	++++
Staphylococcus epidermidis	I	I	++++
Enterobacter aerogenes	I	I	++++
Staphylococcus haemolyticus	I	I	++++
Haemophilus influenzae	I	I	++++
Klebsiella spp.	I	I	++++
Enterococcus faecalis	I	I	++++

Note: "I" – total inhibition of the test strain growth; "++++" – intensive growth of the test strain.

However, with an increase in the gold concentration from 1.93 μg/ml to 19.3 μg/ml there were no significant differences in the antimicrobial activity of the nanoparticles in the mixture.

Thus, silver nanoparticles in the concentration of 0.16 mg/ml and their combination with gold nanoparticles in the concentration of 0.08 mg/ml by Ag and 1.93 μg/ml by Au exhibited a high level of the antimicrobial activity against all test strains studied.

At the next stage of research the level of the antimicrobial activity of nanoparticles against clinical isolates of microorganisms isolated from the patients with purulent-inflammatory diseases of the maxillofacial area was determined. The results obtained are shown in Tab. 2.

The total inhibition of growth for all pathogen clinical isolates studied was observed in the presence of silver nanoparticles in the concentration of 0.08 mg/ml in the medium, as well their combination with gold nanoparticles in the concentration of 1.93 μg/ml.

The therapeutic efficacy of the colloidal solutions of silver nanoparticles and their combination with gold nanoparticles *in vivo* was studied taking into account the possibility of the solution dilution with saliva, as well as washing out of the medicinal substances in the lower gastrointestinal tract. Therefore, the concentrations of nanoparticles that were in 10 times higher than the effective concentrations determined in the studies *in vitro* were used. Thus, for irrigation of model abscesses after their opening the colloidal solution of silver nanoparticles in the concentration of 0.8 mg/ml by metal and gold nanoparticles with the concentration of 19.3 μg/ml by metal was used.

It was found that in animals with the experimental abscess treated by colloidal solutions of silver and gold a significant stimulation of wound healing with fast transition of the inflammatory process to the stages of proliferation and organization of affected areas was observed.

Thus, when irrigating the model abscess with the colloidal solution of silver nanoparticles (AgNP) in the area of the infectious agent administration the suppression of the inflammatory process was observed. A large mass of foreign bodies was found in macrophages. Among histiocytes around the abscess there were fibroblasts and tissue basophils. Collagen fibers were well visible in the capsule of the abscess.

When the experimental abscesses were rinsed with the mixture of silver and gold nanoparticles (Ag/AuNP) during the histological studies the marker particles of the activated carbon powder were found in a short distance from the surface of the skin where they occupied a small area (Fig. 1).

The edema of adjacent tissues was insignificant, in the microenvironment of the abscess macrophages and fibroblasts dominated among the cells; there were only

Fig. 1. Photomicrography of tissues around the abscess on day 12 of formation after 3-time irrigation with the combination of silver and gold nanoparticles. Hematoxylin-eosin, × 200

a few leukocytes. Blood vessels sprouted to the central parts of the infectious lesion where fibroblasts were also found, indicating the organization of the damaged areas.

Significant acceleration of the inflammatory process transition to the stages of proliferation and organization of the damaged areas in animals with the experimental abscess rinsed with colloidal solutions of metal nanoparticles may indicate destruction of the infectious agent. It should be particularly noted when using the colloidal solution with the combination of silver and gold nanoparticles (Ag/AuNP) the healing process was accelerated (Fig. 2).

The results of the marked complex therapeutic efficacy of the mixture of silver and gold nanoparticles obtained *in vivo* may indicate the synergism of the antimicrobial and anti-inflammatory action of silver and gold nanoparticles.

Conclusions concerning the possibility of the therapeutic efficacy intensification due to the action of gold nanoparticles are confirmed by the literature data, in particular about the presence of the antimicrobial activity, the expressed anti-inflammatory effect and the ability to stimulate the tissue regeneration in gold nanoparticles [3, 5, 17-20].

For the first time in official medicine aurotherapy (gold treatment) was applied in 1929 for the treatment of rheumatoid arthritis and polyarthritis [19]. Aurotherapy is still one of the effective treatments for rheumatoid arthritis, along with the use of non-steroidal anti-inflammatory drugs [21].

Since in the treatment of infectious and purulent-inflammatory diseases in dentistry and surgery not only the antimicrobial effect of drugs was important, but also their anti-inflammatory action, the combination of silver and gold nanoparticles based on the given active concentrations was used for development of drugs for dentistry and surgery.

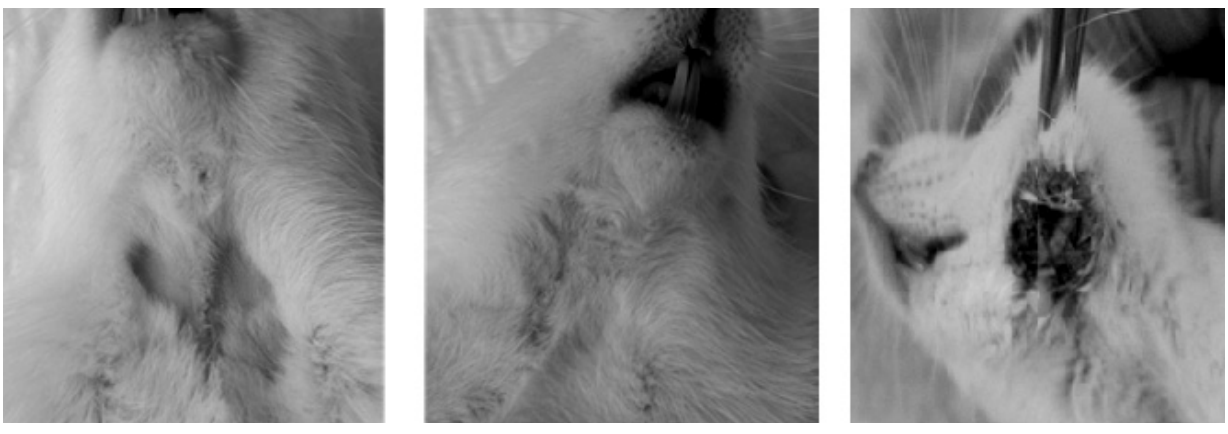
To develop a solution for wound cleansing in dentistry and surgery the combination of silver and gold nanoparticles was used in the concentrations, which therapeutic efficacy was confirmed by the results of the histological studies *in vivo* on the model of the jaw abscess of laboratory animals taking into account the results of the studies *in vitro*. Thus, it was experimentally confirmed that the concentration of silver nanoparticles in the solution for wound cleansing was 0.8 mg/ml, and of gold nanoparticles it was 19.3 μg/ml.

The neutral pH was therapeutically acceptable for the use of the drug developed; therefore, the concentration of a buffer agent (disodium phosphate) was selected experimentally. As a solvent purified water was used. The solutions were packed in 10 ml containers and closed hermetically. According to the State Pharmacopeia of Ukraine preparations used for wound treatment should be sterile; therefore, the solution was sterilized at 121 °C for 8 min.

The technological process of manufacturing the solution consists of the preparatory stage, dissolution of the active components and excipients, packing of the solution in the containers, sterilization, packaging and labeling of the solution.

Taking into account that in the semi-solid dosage forms the base affected the release of an active substance, and therefore, required the use of higher concentrations of the active pharmaceutical ingredient (API) than the given minimum antimicrobial concentration in solutions we used the combination of colloidal solutions of silver and gold nanoparticles in the concentrations that were 2.5; 5; 10 and 20 times more than the active concentrations established for preparing semi-solid preparations for dentistry. Since adding significant amounts of colloidal solutions into compositions of semi-solid preparations created certain technological complications and required the use of a significant amount of excipients, colloidal solutions with the initial concentration of silver nanoparticles of 8.0 mg/ml and gold nanoparticles of 77.2 μg/ml were concentrated. For concentration the initial aqueous dispersions of metal nanoparticles were lyophilized using an ALPHA 1-4 LD-2 lyophilic dryer with the preliminary freezing of the samples for 12 hours at -55 °C. To prepare semi-solid preparations the colloidal solution of silver and gold nanoparticles with the concentration of silver 4 mg/ml and gold of 0.15 mg/ml was used.

To develop a hydrophilic ointment with the composition of silver and gold nanoparticles a traditional macrogol base providing the osmotic and dehydration action



a b c

Fig. 2. The effectiveness of the model abscess treatment after 3-day irrigation with: (a) – colloidal solution of silver nanoparticles; (b) – colloidal solution of the combination of silver and gold nanoparticles compared to (c) – the initial state of the opened abscess

Table 3

The composition of semi-solid preparations with silver and gold nanoparticles

Name of the component	Quantity			
	Ointment			
	1	2	3	4
Solution of silver and gold nanoparticles (C_{Ag} =4.0 mg/ml, C_{Au} = 0.15 mg/ml)	5 ml	10 ml	20 ml	40 ml
Macrogol 400	60.0	55.0	45.0	25.0
Macrogol 1500	30.0	30.0	30.0	30.0
Propylene glycol	5.0	5.0	5.0	5.0
	Gel			
	1	2	3	4
Solution of silver and gold nanoparticles (C_{Ag} =4.0 mg/ml, C_{Au} = 0.15 mg/ml)	5 ml	10 ml	20 ml	40 ml
Carbopol	0.5	0.5	0.5	0.5
Ammonium hydroxide solution 25 %	to pH 7.0	to pH 7.0	to pH 7.0	to pH 7.0
Macrogol 1500	5.0	5.0	5.0	5.0
Glycerol	5.0	5.0	5.0	5.0
Purified water	to 100.0	to 100.0	to 100.0	to 100.0
Concentration of active substances calculated with reference to metals	C_{Ag} 0.2 mg/ml C_{Au} 7.5 μg/ml	C_{Ag} 0.4 mg/m C_{Au} 15 μg/ml	C_{Ag} 0.8 mg/ml C_{Au} 30 μg/ml	C_{Ag} 1.6 mg/ml C_{Au} 60 μg/ml

and accelerating the time of wound healing was used. To reduce the drying effect of the ointment propylene glycol in the concentration of 5 % was added into the composition.

When developing the gel as a gelling agent carbopol allowing to obtain transparent non-sticky gels was used in the concentration of 0.5 % [22]. In the composition of the gel developed this component formed a protective coating and promoted prolonged release of active ingredients. Macrogol 1500 was introduced into the composition in order to increase viscosity and the osmotic activity of the gel. The compositions of the semi-solid preparations studied are given in Tab. 3.

The technological study showed that the introduction of 40 ml of colloidal solution of nanoparticles into the composition of semi-solid preparations (ointment **4** and gel **4**) was problematic since a stable ointment and gel could be obtained with a maximum 20 % colloidal solution; therefore, for further studies the ointment and the gel composition **3** with the concentration of silver nanoparticles of 0.8 mg/ml and gold of 30 μg/ml were selected.

When preparing the ointments and gels studied the general rules for manufacturing semi-solid preparations were used. According to the results of the study the flowcharts of the ointment and gel production were developed.

To introduce silver and gold nanoparticles in semi-solid preparations the colloidal solution of nanoparticles was mixed with propylene glycol (ointment) or glycerol (gel) and then added by stirring to the base of the ointment or gel, respectively.

When manufacturing an ointment and a gel the bases were firstly prepared; they were a fusion of macrogols and carbopol gel, respectively, to which non-aqueous solvents and the colloidal solution of nanoparticles were introduced. The first step in the gel base preparation was swelling of carbopol in purified water at room temperature. Since in the case of using carbopol, gelling occurred after changing the pH of the carbopol solution to a neutral value, the gel base was thickened by 25 % solution of ammonium hydroxide to pH 7.0.

The experimental samples of semi-solid preparations in the laboratory conditions were prepared using a laboratory homogenizer at a stirring rate of 3000 rpm (ointment) and 2000 rpm (gel); the homogenization time was 15 min. The effect of the rate and time of emulsification on the stability and homogeneity of semi-solid preparations was determined visually. The samples of semi-solid preparations studied were assessed by organoleptic (appearance, color) and consumer properties (ease of application, stickiness, absorption rate, sensation and the skin condition after application of semi-solid preparations).

CONCLUSIONS

1. According to the results of the study conducted the marked complex action (antimicrobial, anti-inflammatory and regenerative) of the combination of AgNP and AuNP colloidal solutions in the treatment of purulent-inflammatory diseases of the maxillofacial area has been determined.

2. The composition and technology of three dosage forms based on the combination of silver and gold colloidal solution (ointment, gel and solution for wound cleansing) have been substantiated; it can be promising for the use in dentistry and surgery.

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