ORIGINAL ARTICLE

UDC 616.716.4-001.5-06-002.3-085-084:612.08

PROSPECTS OF USING SILVER AND GOLD NANOPARTICLES IN THE PREVENTION AND TREATMENT OF PURULENT-INFLAMMATORY DISEASES OF THE MAXILLOFACIAL AREA

Anna Rybachuk,
rybachuk_av@ukr.net

A. Rybachuk[1], L. Rieznichenko[2], S. Dybkova[2], T. Gruzina[2], Z. Ulberg[2], V. Malanchuk [1]

[1] Bohomolets National Medical University, Kyiv, Ukraine

[2] F.D. Ovcharenko Institute of Biocolloidal Chemistry NAS of Ukraine, Kyiv, Ukraine

Key words: *silver nanoparticles, gold nanoparticles, purulent-inflammatory diseases of the maxillofacial area, antimicrobial activity.*

Actuality. Purulent-inflammatory diseases of the maxillofacial area are complex in treatment and make up a significant part of the total number dental surgical patients. Prevail, purulent-inflammatory process in the form of abscesses and phlegmons, periostitis, osteomyelitis, suppuration of the bone wounds etc. Among them, the most common pathogens isolated an aggressive *MDR Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Micrococcus*, fungi of the genus *Candida albicans* etc [2, 3, 5, 15].

Of particular note is the fact of the growing number of life-threatening complications caused by the proliferation of pathogenic strains of pathogens resistant to wide range of one existing antimicrobial drugs, over saturation of pharmacological preparations, weakening the immune system and patient allergization [1, 7, 11, 16].

Effective solution in current situation requires the development and implementation of new alternative antimicrobial therapy drugs. Prospective in this way may be silver nanoparticles, because of their potent antimicrobial activity against a wide spectrum of pathogenic microorganisms. Enhancing the silver nanoparticles efficiency may be possible due to the addition of the gold nanoparticles, given their anti-inflammatory potential [8, 9, 12, 13, 14, 17].

Aim. The aim of this article was to identify the antimicrobial activity ofthe silver and gold nanoparticles, asan alternative method for mandibular fractures purulent-inflammatory complications treatment.

Materials and methods. We used silver and gold nanoparticles synthesized by chemical condensation in aqueous medium according to the original method developed at the F.D. Ovcharenko Institute of Biocolloidal Chemistry NAS of Ukraine.

The initial drug concentration of silver nanoparticles was 8.0 mg/ml by the metal, of gold nanoparticles – 193.0 mcg/ml by the metal, of their combination – 4.0 mg silver/96.5 mcg gold by the metal.

Visualization of the nanoparticles size and shape and their specific contact interaction with pathogenic test strains of microorganisms was performed by transmission electron microscopy (JEM-1230, "JEOL", Japan).

The antimicrobial activity of the new synthesized substances of silver and gold nanoparticles and commercial antimicrobial agents was determined by *in vitro* studies using serial dilutions in agar according to the Methodical Guidelines 4.2.1890-04, 2004 [6].

As pathogenic test cultures we used the strains of the following microorganisms: *Staphylococcus aureus* 209P, *Enterococcus faecalis* G35№4-410, *Escherichia coli* №25,

Pseudomonas aeruginosa ATCC27853 (F-51), *Candida albicans*, *Proteus vulgaris* HX 19 №222 from the State scientific-control Institute of biotechnology and strains collection (Kyiv).

As a comparison drug in assessing of the metal nanoparticles experimental substances antimicrobial activity we used commercial antimicrobials, which are using in common practice: Chlorhexidine and Furacilinum. The final concentration of Chlorhexidine in the environment definition was 5 mg/ml; Furacilinum – 0.13 mg/ml.

The clinical isolates of microorganisms were allocated from patients who were undergoing treatment in the department of maxillofacial surgery based on the Kyiv Clinical Hospital №12. Biopsy specimens was performed on patients of both sexes, aged from 18 to 70 years old with the diagnosis abscesses and phlegmons of various parts of the maxillofacial area.

In vivo studies were carried out using 70 certified laboratory *Wistar* rats weighing 250-300 g from National Scientific Centre "N.D. Strazhesko Institute of Cardiology" of NAMS of Ukraine vivarium.

All experiments on animals were carried out according to the "European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes".

Modeling of jaw abscess area was carried out according to the standard method [10]. By subcutaneous injection in the submandibular area of the laboratory animals with 0.5 ml suspension culture daily clinical isolates of *Staphylococcus aureus* ($5 \cdot 10^7$ CFU/ml) with activated carbon mass fraction powder in the suspension was 9%.

Expansion of the abscess was performed outside access on the 4th day after infection. The skin incision was performed over the infiltration center. The length of the section was not less than the length of infiltration.

Rinsing uncovered abscesses by experimental substances and preparations metal nanoparticles comparison was performed 1 time per day during 3 days. The disclosed abscesses were irrigated with spray at a rate 4 ml of each substance in 1 animal.

Experimental animals were divided into 7 groups:

Group 1 – control group – healthy animals;

Group 2 – control group – animals with untreated disclosed abscess;

Group 3 – experimental group – animals, which revealed abscess was irrigated with substance of silver nanoparticles (AgNP) at a concentration of 0.8 mg/ml by the metal;

Group 4 – experimental group – animals, which revealed abscess was irrigated with substance of gold nanoparticles (AuNP) at a concentration of 19.3 mcg/ml by the metal;

Group 5 – experimental group – animals, which revealed abscess was irrigated with substance of silver and gold nanoparticles (Ag/AuNP) combination at a concentration of 0.8 mg (Ag) / 19,3 mcg (Au) by the metal;

Group 6 – experimental group – animals, which revealed abscess was irrigated with a commercial drug "Decasan" (1 ml containing 0.2 ml decametoxine);

Group 7 – experimental group – animals, which revealed abscess was irrigated with a commercial drug "Chlorhexidine" (1 ml containing 0.5 ml of Chlorhexidine digluconate).

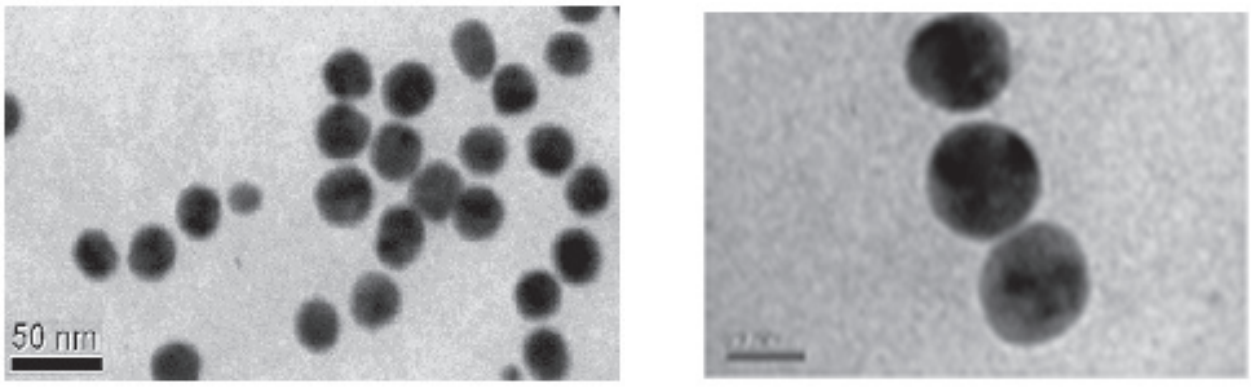
Slaughter of animals of the control and experimental groups was performed on the 5 day after last rinsing uncovered abscesses by decapitation using general anesthesia.

Microbiological control of the abscess condition was performed according to the standard technique [4]. The incubation was carried out at a temperature of crops 37^0C during 24 hours.

Results and their discussion. Silver and gold nanoparticles which we used in the work were synthesized by chemical condensation in aqueous medium, allowed to obtain monodisperse, stable in size, highly concentrated and sterile biocompatible nanoparticles as prospective pharmaceutical substance to create different dosage forms of antimicrobial agents.

According to the transmission electron microscopy was established that the synthesized silver (A) and gold nanoparticles (B) have spherical form and medium size 30 nm (Fig. 1).

Studies of the AgNP interaction in bacterial cells pathogenic test strains carried by electron microscopy and showed the ability of the studied pathogenic test strains actively accumulate on the surface silver and inside cells



a *b*

Fig. 1. Electron-microscopic images of the synthesized silver (A) and gold (B) nanoparticles average size of 30 nm.

nanoparticles of 30 nm. In Fig. 2 is shown a test strain *E. coli* № 25 on electron microscopy image illustrating the bacterial cells ability of pathogenic test strains connection of tested silver nanoparticles medium size 30 nm.

Data on the assessment of antimicrobial activity of the silver and gold nanoparticles experimental substances in relation to the test of strains – representatives of the main pathogens types of the maxillofacial area inflammatory diseases are presented in Tab. 1.

Thus, the definition of the silver nanoparticles substance peculiarities on pathogenic microorganisms test strains showed a significant level of antimicrobial activity relatively of all studied pathogenic test cultures in both concentrations. The nanoparticle concentration 0.16 mg/ml for the determination of metals in the environment observed complete inhibition of growth activity of all studied pathogenic test strains.

Growth of single colonies of the *Candida albicans* test strain was observed at a test strain dose 10^5 CFU/cm^3 at a concentration of silver nanoparticles 0.08 mg/ml by the metal in the environment definition.

Gold nanoparticles were characterized by less antimicrobial activity. The inhibition of growth activity under the influence of gold nanoparticles studied concentration was observed only against of test strains of *Staphylococcus aureus* 209P, *Enterococcus faecalis* G35№4-410, *Escherichia coli* №25 and *Proteus vulgaris* HX 19 №222.

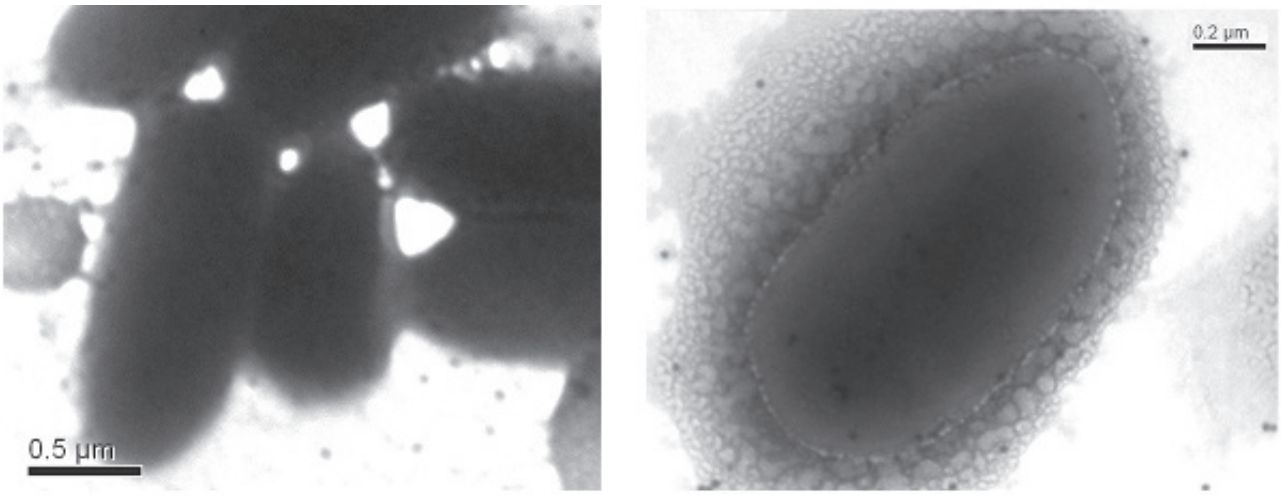


Fig. 2. Electron-microscopic images of bacterial cells test strain E. coli №25 accumulated with silver nanoparticles average size of 30 nm.

Table 1.

Test strain	Cultivated dose of the test strain CFU/cm^3	The final concentration of **AgNP** in the environment of definition, mg/ml by the metal		The final concentration of **AuNP** in the environment of definition, mcg/ml by the metal	Control growth of test strain
		0.08	0.16	96.5	
Staphylococcus aureus 209P	10^3	I	I	I	++++
	10^4	I	I	I	++++
	10^5	I	I	+	++++
Enterococcus faecalis G35 №4-410	10^3	I	I	I	++++
	10^4	I	I	I	++++
	10^5	I	I	I	++++
Escherichiacoli №25	10^3	I	I	I	++++
	10^4	I	I	I	++++
	10^5	I	I	+	++++
Pseudomonas aeruginosa ATCC 27853 (F-51)	10^3	I	I	+	++++
	10^4	I	I	++	++++
	10^5	I	I	++++	++++
Candidaalbicans	10^3	I	I	+++	++++
	10^4	I	I	+++	++++
	10^5	+	I	++++	++++
Proteus vulgaris HX 19 №222	10^3	I	I	I	++++
	10^4	I	I	I	++++
	10^5	I	I	+	++++

"I" – complete growth inhibition of the test strain;
"++++" – intensive growth of the test strain;
"+" – there is only a single colony growth on the cup.
"++" – marked increase growth inhibition compared with control;
"+++" – weakly growth inhibition compared with control;

Results evaluation of silver nanoparticles (AgNP) and their combination with gold nanoparticles (Ag/AuNP) experimental substances antimicrobial activity relatively to the clinical isolates of microorganisms – causative agents of the maxillofacial area inflammatory diseases are shown in Tab. 2.

Complete growth inhibition of all investigations of the clinical isolates was observed in the presence of microorganisms in the environment of the investigated substances silver nanoparticles and their combination with gold nanoparticles in concentration of 0.08 mg (Ag)/ml by the metal definition.

Evaluation results of antimicrobial activity of commercial antimicrobial drugs in relation to the spectrum allocated clinical isolates of microorganisms – causative agents of maxillofacial area inflammatory diseases are shown in Tab. 3.

Tab. 3 shows that the Chlorhexidine at a concentration of 5 mg/ml in the medium determines antimicrobial effectiveness only in a strain of *Micrococci*, while the clinical isolates strains of *E. coli*, *S. aureus*, *P. aeruginosa*, *C. albicans* fungi and *Yeast Fungi* have low sensitivity to this drug.

More strong antimicrobial efficiency has Furacilinum. For the investigated concentrations of this drug 0.13 mg/ml in medium definition slight increase was observed only for gram-positive strains of *S. aureus* and *Micrococcus*.

Thus, we established, that the experimental substance of silver nanoparticles (AgNP) and a combination of silver and gold nanoparticles (Ag/AuNP) has stronger bactericidal action relative to a wide range of clinical isolates of microorganisms – causative agents of the maxillofacial area inflammatory diseases, in comparison with traditional antimicrobial agents (Chlorhexidine and Furacilinum).

Therapeutic efficacy of silver nanoparticles antimicrobial action and their combinations with gold nanoparticles were determined on *in vivo* studies at the experimental model of laboratory rats jaw purulent abscess.

As a comparison product we have used Chlorhexidine and Decasan – the most common traditional antimicrobial drugs using in maxillofacial surgery.

On the 4th day after injection of *Staphylococcus aureus* experimental suspension culture with activated carbon powder, at the injection site was observed occurrence of inflammatory processes – abscess with expressed soft tissue swelling of the right submandibular area. The experimental animals in comparison with the control group were sluggish, prone to poor appetite. The skin over the existing infiltrate was tense and hyperemic (Fig. 3).

Expansion of abscess external access over the center of infiltration and jet rinsing with experimental metal

Table 2.

Clinical isolates	Strains growth in the presence of **AgNP** in the environment in determining the concentration 0.08 mg / ml by the metal	Strains growth in the presence of **Ag/AuNP** in the environment in determining the concentration 0.08 mg / ml by the Ag	Control the growth of the strain
Staphylococcus aureus	I	I	++++
Micrococcus	I	I	++++
Escherichia coli	I	I	++++
Pseudomonas aeruginosa	I	I	++++
Fungi genus Candida albicans	I	I	++++
Yeast Fungi	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	++++

"I" – complete growth inhibition of the test strain;
"++++" – intensive growth of the test strain;

Table 3.

Clinical isolates	Strains growth in the presence of **Chlorhexidinum** at a concentration 5 mg / ml	Strains growth in the presence of **Furacilinum** at a concentration 0.13 mg / ml	Control the growth of the strain
Staphylococcus aureus	+	+	++++
Micrococcus	I	+	++++
Escherichia coli	+++	I	++++
Pseudomonas aeruginosa	+	I	++++
Fungi genus Candida albicans	++	I	++++
Yeast Fungi	+++	I	++++

"I" – complete growth inhibition of the test strain;
"++++" – intensive growth of the test strain;
"+" – there is only a single colony growth on the cup.
"++" – marked increase growth inhibition compared with control;
"+++" – weakly growth inhibition compared with control;

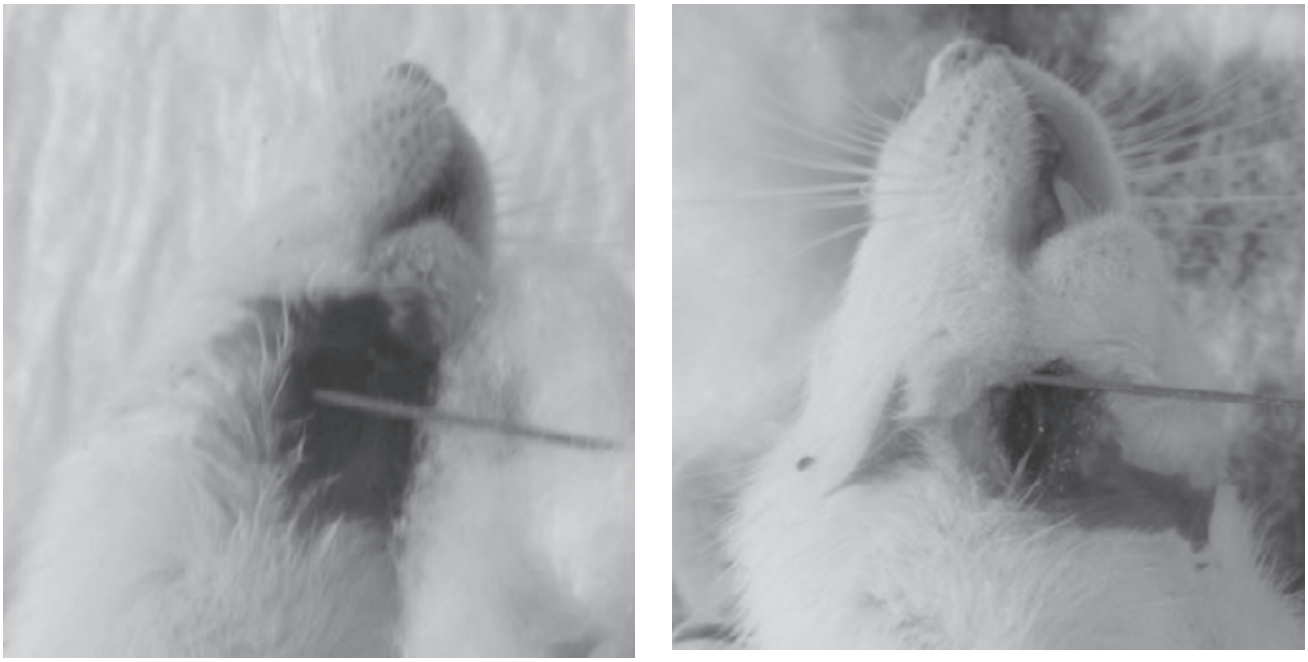
Fig. 3. Submandibular abscess formation at laboratory Wistar rats

nanoparticles substances (Fig. 4) and comparison products Chlorhexidine and Decasan was performed on the common practice in maxillofacial surgery technique.

Culturing of manure sterile swab taken from the lesion immediately after expansion abscess showed significant microbial contamination: the number of colonies of *S.aureus* in all investigated samples averaged $8 \cdot 10^8 - 11 \cdot 10^8$ CFU / cm^3.

After one day past the first rinsing of the uncovered abscesses in the control group with open untreated abscesses (group 2) the number of *S. aureus* colonies in the center of abscess remained at the same level, while after a single rinsing with metal nanoparticles experimental substances, showed a significant decrease in the number of *S. aureus* colonies (Tab.4).

Thus, according to bacteriological analysis conducted by the strong antimicrobial activity in the treatment of abscesses in animals has an experimental substance of silver and gold nanoparticles (Ag/AuNP) combination and silver



a *b*

Fig. 4. Inkjet irrigation of the disclosed abscess with Ag (A) nanoparticles and Ag/Au (B) combination substance.

Table 4.

Experimental groups	The number of colonies of S. aureus, which cultured in samples of material from the source of ignition, CFU / cm 3
Group 2 - control group - animals with untreated disclosed abscess	8×10^8 - 10×10^8
Group 3 - animals, which revealed abscess was irrigated with substance of silver nanoparticles (AgNP) at a concentration of 0.8 mg / ml by the metal	9×10^3
Group 4 - animals, which revealed abscess was irrigated with substance of gold nanoparticles (AuNP) at a concentration of 19.3 mcg / ml by the metal	6×10^8
Group 5 - animals, which revealed abscess was irrigated with substance of silver and gold nanoparticles (Ag/AuNP) combination at a concentration of 0.8 mg (Ag) / 19,3 mcg (Au) by the metal	3×10^3
Group 6 - animals, which revealed abscess was irrigated with a commercial drug "Decasan" (1 ml containing 0.2 ml decametoxine)	7×10^4
Group 7 - animals, which revealed abscess was irrigated with a commercial drug "Chlorhexidine" (1 ml containing 0.5 ml of Chlorhexidine digluconate)	2×10^5

(AgNP). Classic antimicrobials Chlorhexidine and Decasan used as a preparation of comparison, characterized by lower efficiency compared with the experimental substances nanoparticles Ag/AuNP and AgNP. Gold nanoparticles (AuNP) did not have good antimicrobial activity against clinical isolates of *S. aureus*, used in abscesses modeling of animal's submandibular area.

Conclusion. This article presents results, which demonstrate the significant effectiveness of the investigated silver and gold nanoparticles substances with average sizes 30 nm, as well as their combination, as a potential alternative remedies for using in oral and maxillofacial surgery for treatment and prophylaxis of the mandibular fractures purulent-inflammatory complications.

Reviewer: Corresponding Member NAS and NAMS Ukraine, professor I.S. Chekman

REFERENCES

1. Алешкин И.Г. Оптимизация комплексного лечения гнойно-воспалительных заболеваний челюстно-лицевой области: Автореф. дис. канд. мед. наук. / Алешкин И.Г. // – Иркутск, 1997. – 24 с.

2. Бернадский Ю.И. Очерки гнойной челюстно-лицевой хирургии / Бернадский Ю. И., Заславский Н. И. // Киев, 1978. – 211 с.

3. Дмитриева Н.А. Гнойно-воспалительные осложнения челюстно-лицевой области, структура возбудителей и возможные пути профилактики: Дисс. канд. мед. наук. / Дмитриева Н.А. // – Москва, 1993. – 111с.

4. Лабинская А.С. Микробиология с техникой микробиологических исследований / Лабинская А.С. // – М.: Медицина, 1978. – 394 с.

5. Маланчук В.О. Хірургічна стоматологія та щелепно-лицева хірургія; У 2 т. – Т.2. / Маланчук В.О., Логвіненко І.П., Маланчук Т.О. та ін. // К. – ЛОГОС, 2011. – 606 с. + 12 ст. кольор. вкл.

6. Методические указания МУК 4.2.1890-04 Определение чувствительности микроорганизмов к антибактериальным препаратам // Клин. микробил. антимикроб. химиотер. – 2004. – Т.6, №4. – С. 306-359.

7. Супиев Т.К. Гнойно-воспалительные заболевания челюстно-лицевой области / Супиев Т.К. // – М.: МЕДПресс, 2001. – 160 с.

8. Чекман І.С. Основи наномедицини / Чекман І.С., Маланчук В.О., Рибачук А.В. // – К.: Логос, 2011. – 250 с.

9. Чекман І.С. Нанонаука, нанобіологія, нанофармація / Чекман І.С., Ульберг З.Р. Маланчук В.О. та ін. // К. – Поліграф плюс, 2012. – 328 с.: іл.

10. Шалимов С.А. Руководство по экспериментальной хирургии / Шалимов С.А., Радзиховский А.П., Кейсевич Л.В. // – М.: Медицина, 1989. – 272 с.

11. Kyzas P.A. Use of antibiotics in the treatment of mandible fractures: a systematic review / Kyzas P.A. //J. Oral Maxillofac. Surg. – 2011. – Vol. 69(4). – P. 1129-1145.

12. Lansdown A.B. Silver in health care: antimicrobial effects and safety in use / Lansdown A.B. // Current problems in dermatology. – 2006. – Vol. 33. – P.17-34.

13. Mijnendonckx K. Antimicrobial silver: uses, toxicity and potential for resistance / Mijnendonckx K., Leys N., Mahillon J. et al. // Biometals. – 2013. – Vol. 26(4). – P. 609-621.

14. Morones J.R. The bactericidal effect of silver nanoparticles / Morones J.R., Elechiguerra J.L., Camacho A. et al. // Nanotechnology. – 2005. – Vol. 16. – P. 2346-2353.

15. Pham-Dang N. Etiology, distribution, treatment modalities and complications of maxillofacial fractures / Pham-Dang N., Barthülümy I.., Orliaguet T. et al. // Med Oral Patol Oral Cir Bucal. – 2014. – Vol. 19(3). – P. 261-269.

16. Schaefer E.H.[4th]. Antibiotic selection for open reduction internal fixation of mandible fractures / Schaefer E.H. [4th], Caterson E.J. // J. Craniofac. Surg. – 2013. – Vol. 24(1). – P. 85-88.

17. Shah M. Biological applications of gold nanoparticles / Shah M., Badwaik V., Dakshinamurthy R. // J. Nanosc. Nanotechnol. – 2014. – Vol. 14(1). – P. 344-362.

ПЕРСПЕКТИВЫ ИСПОЛЬЗОВАНИЯ НАНОЧАСТИЦ СЕРЕБРА И ЗОЛОТА ДЛЯ ПРОФИЛАКТИКИ И ЛЕЧЕНИЯ ГНОЙНО-ВОСПАЛИТЕЛЬНЫХ ЗАБОЛЕВАНИЙ ЧЕЛЮСТНО-ЛИЦЕВОЙ ОБЛАСТИ

А. Рыбачук[1], Л. Резниченко[2], С. Дибкова[2], Т. Грузина[2], З. Ульберг[2], В. Маланчук[1]

[1]Национальный медицинский университет имени А.А. Богомольца, г. Киев, Украина

[2]Институт биоколлоидной химии имени Ф.Д. Овчаренко НАН Украины, г. Киев, Украина

Резюме. Приведены полученные результаты, доказывающие значительную эффективность исследованного препарата наночастиц серебра и золота среднего размера 30 нм, синтезированного в Институте биоколлоидной химии им. Ф. Д. Овчаренко НАН Украины, как потенциальной противомикробной субстанции, перспективной для применения в хирургической стоматологии и челюстно-лицевой хирургии для лечения гнойно-воспалительных заболеваний челюстно-лицевой области и их осложнений.

Ключевые слова: наночастицы серебра, наночастицы золота, гнойно-воспалительные заболевания челюстно-лицевой области, антимикробная активность.

ПЕРСПЕКТИВИ ВИКОРИСТАННЯ НАНОЧАСТИНОК СРІБЛА І ЗОЛОТА ДЛЯ ПРОФІЛАКТИКИ ТА ЛІКУВАННЯ ГНІЙНО-ЗАПАЛЬНИХ ЗАХВОРЮВАНЬ ЩЕЛЕПНО-ЛИЦЕВОЇ ДІЛЯНКИ

А. Рибачук[1], Л. Рєзніченко[2], С. Дибкова[2], Т. Грузіна[2], З. Ульберг[2], В. Маланчук [1]

[1] Національний медичний університет імені О.О. Богомольця, м. Київ, Україна

[2] Інститут біоколоїдної хімії імені Ф.Д. Овчаренко НАН України, м. Київ, Україна

Резюме. Наведені отримані результати, що доводять значну ефективність дослідженого препарату наночастинок срібла та золота середнього розміру 30 нм, синтезованого в Інституті біоколоїдної хімії ім. Ф. Д. Овчаренка НАН Україні, як потенційної протимікробної субстанції, перспективної для застосування в хірургічній стоматології та щелепно-лицевої хірургії для лікування гнійно-запальних захворювань щелепно-лицевої ділянки та їх ускладнень.

Ключові слова: наночастинки срібла, наночастинки золота, гнійно-запальні захворювання щелепно-лицевої ділянки, антимікробна активність.