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# CHANGES IN INTER- AND INTRA DYFFERON SKIN TISSUES HETEROMORPHISM UNDER CONDITIONS OF INFLUENCE OF SILVER NANOPARTICLES OF 20, 30, 70 NM

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**ЗМІНИ МІЖДИФЕРОННОЇ ТА ВНУТРІШНЬОДИФЕРОННОЇ ГЕТЕРОМОРФІЇ ТКАНИН ШКІРИ ЗА УМОВ ВПЛИВУ НАНОЧАСТИНОК СРІБЛА РОЗМІРОМ 20, 30, 70 НМ**
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Метою дослідження є розробка морфологічних критеріїв оцінки реакцій біологічних тканин на металеві наночастинки методом змін внутрішньо- й міждиферонної гетероморфії тканин, які взаємодіють із наночастинками. Вивчення тканинної гетероморфії забезпечує комплексну оцінку функціонального стану тканини, дозволяючи об'єктивно оцінити реакцію біологічних тканин при взаємодії з наночастинками металів. За допомогою кількісних гістологічних методик описані реактивні зміни внутрішньодиферонної гетероморфії клітин базального шару епідермісу і фібробластів дерми. Виявлені розмірозалежні ефекти впливу наночастинок срібла.
**Ключові слова:** гетероморфія тканин, наночастинки.

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The study is focused on developing of morphological criteria of biological tissue reactions to metal nanoparticles by detecting changes of tissues heteromorphism interacting with NPs. The study of heteromorphism tissue provides an integrated assessment of functional state of the tissue, allowing objectively evaluate the response of biological tissues in metal nanoparticles. Size-dependent effects of silver nanoparticles were identified, namely depending on the nanoparticles size recovery rate of basement membrane structure differs; the increase of mitotic index of the epidermal basal cells; changes of dermal fibroblasts's heteromorphism, such as increasing of number of functionally active fibroblasts; and the number of collagen fibers of the dermis. Reactive changes of intradifferon heteromorphism of epidermal basal cells and the dermal fibroblasts was described using quantitative histological methods.
**Key words:** heteromorphism tissue, nanoparticles.

Active development of nanoscience and the possible application of nanoparticles (NPs) in medicine has led to the creation of new areas of fundamental research, studying the effects of the interaction of nanoparticles and biological objects [1; 2]. Despite the increase in morphological research on the biological effects of nanoparticles of metal, most of them dedicated to the interaction of the NPs at the cellular level and carried out on cell cultures [3; 4]. However, the reduction of the problem only to the cellular level of structural organization of living things, prevents the study and prediction of histogenesis, assessment of tissue structure changes and features of cellular interactions, that is, the response of tissue as a complex self-organizing system, in which each of the components has all emergent properties of the whole. The absence of a methodological approach of morphological studies of the interaction of tissues and NPs complicates the analysis and systematization of results and leads to unsystematic findings [5].

**The aim** of this study is to develop a method for the morphological evaluation of tissue reactions to metal NPs by identifying changes in inter- and intra differons tissue heteromorphism in contact with silver NPs in different sizes.

## Materials and Methods

### *1. Model of the experiment*

Experimental studies conducted on 261 Wistar rats weighing 0.18–0.24 kg. Animals were kept in standard conditions of experimental biological clinic of the Odessa National Medical University,

according to the scientific and practical recommendations on laboratory animals management and working with them [6]. The experiment was carried out according to observation of the Law of Ukraine "On protection of animals from abuse" as well as common ethical principles of animal experiments and the Code of Ethics of Ukraine scientists.

Animals were divided into 5 groups: the intact group, animals treated with Ag NPs 30 nm, animals treated with Ag NPs 20 nm, animals treated with Ag NPs 70 nm, animals treated with a colloidal solution of silver ions. Administration of 0.01 ml solution was carried out in the area of the withers strictly subcutaneously to a depth of 120 microns using an insulin syringe. Effect was evaluated after administration of 1, 3, 7, 14, 21, 30, 45 hours. Removal of animal from experiment was conducted by an overdose of ether anesthesia.

### *2. Obtaining and Characterization of Ag NP*

In this paper, for Ag NP, citrate method was applied. When we use the synthesis parameters with the use of sodium citrate silver nanoparticles of spherical shape were obtained.

The formation of silver nanoparticles corresponds to the appearance of absorption bands of the spectrum, the maximum of which is determined by the size of the nanoparticles. The nature of this band is associated with a local surface plasmon resonance (LSPR) [7]. Fig. 1 shows the absorption spectra of silver nanoparticles of different sizes. The inset in Fig. 1 shows the experimentally determined dependence of the position of the maximum of LSPR sizes on silver nanoparticles. We have obtained Ag nanoparticles, **LSPR** maximum of which is localized at wavelengths of 400, 413 and 445 nm, which corresponds to the size of silver nanoparticles 20, 30 and 70 nm.

### *3. Technique*

For morphological studies the back skin was taken. The material was fixed in 10 % neutral formalin and embedded in Histamix (BioVitrum, Russia) in accordance with standard histological procedure permanent histological specimens were prepared. Sections 5–7 microns in thickness were stained with hematoxylin — eosin, PAS-reaction [8, 9].

Permanent histological specimens were examined by light microscopy, microscope "Zeiss" "Axiostar plus", equipped with a video analysis system images. The morphometric study of obtained images was performed using "VideoTest — Morphology Master" program (VideoTesT, Russia).

Statistical processing of the results was performed using the "Microsoft Office Excel" computer program. For mathematical data analysis of variance was used. If the null hypothesis is rejected Newman-Keyls criterion was used for further analysis using.

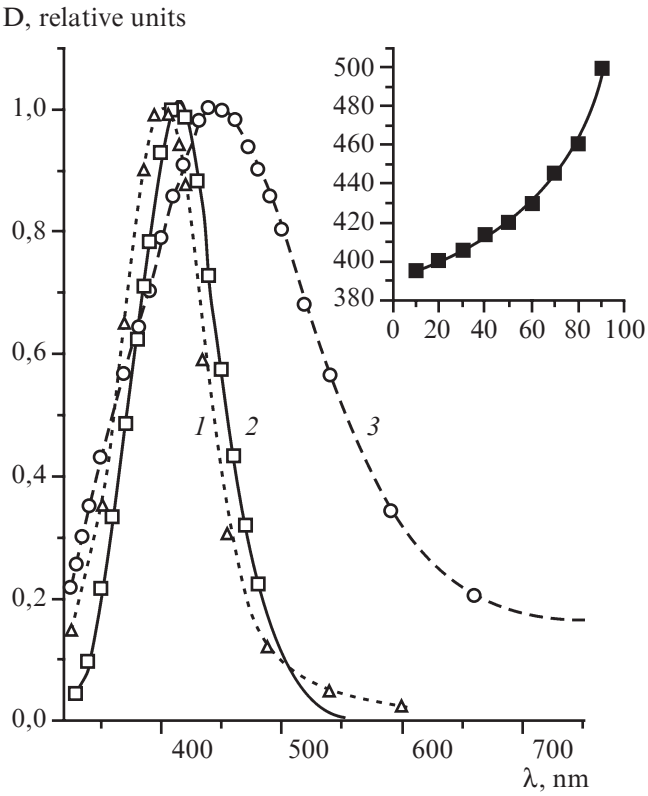



*Fig. 1.* Absorption spectra of silver nanoparticles size, nm: *1* — 20; *2* — 30; *3* — 70. The inset shows the position of the maximum LSPR on the size of the nanoparticles

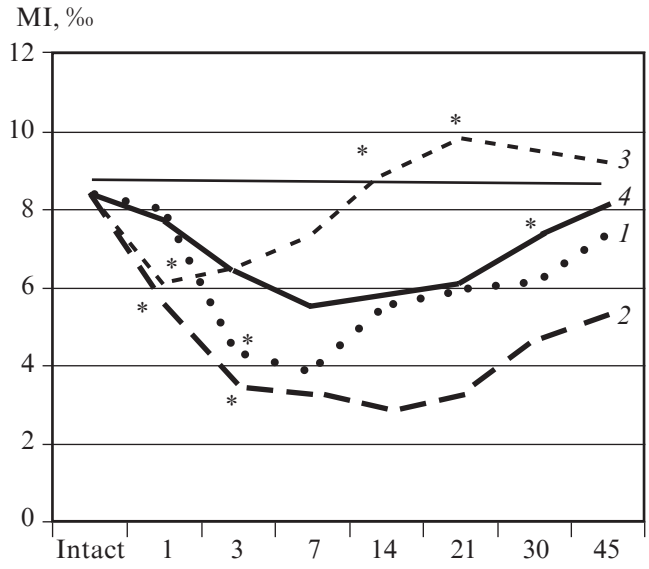



*Fig. 2.* Dynamics of changes in the mitotic index epidermis basal cells when administered silver NPs of different sizes: *1* — ions; *2* — 20 nm; *3* — 30 nm; *4* — 70 nm; * — p<0,05 as compared to the previous period of observation

Size-dependent effects of silver nanoparticles were identified, namely depending on the nanoparticles size recovery rate of basement membrane structure differs (the fastest — under conditions of 30 nm silver nanoparticles influence, under conditions of 20 nm silver nanoparticles influence, regeneration was ab-

*Table 1*

**Distribution of fibroblastic cells differon at maturity with the introduction of silver NPs of different sizes, M±m, n=6 %**

| Days | Undifferentiated | | | Differentiated | | | Mature fibroblasts | | |
|---|---|---|---|---|---|---|---|---|---|
| | 30 nm | 20 nm | 70 nm | 30 nm | 20 nm | 70 nm | 30 nm | 20 nm | 70 nm |
| Intact | 5.7±0.3 | | | 9.8±0.7 | | | 85.3±0.5 | | |
| 1 | 6.3±0.4 | 6.4±0.7 | 7.3±0.4$^{\#}$ | 10.1±0.6 | 10.7±0.6 | 8.1±0.6 | 84.5±0.7 | 82.2±0.7 | 86.1±0.7 |
| 3 | 11.2±0.5$^{\#*}$ | 8.9±0.8$^{\#*}$ | 10.2±0.5$^{\#*}$ | 21.1±0.7$^{\#*}$ | 14.5±0.7$^{\#*}$ | 15.8±0.7$^{\#*}$ | 70.4±0.6$^{\#*}$ | 77.5±0.2$^{\#*}$ | 75.4±0.6$^{\#*}$ |
| 7 | 17.9±0.6$^{\#*}$ | 9.4±0.4$^{\#}$ | 13.6±0.6$^{\#*}$ | 32.6±0.2$^{\#*}$ | 17.5±0.2$^{\#*}$ | 23.6±0.2$^{\#*}$ | 56.2±0.9$^{\#*}$ | 75.4±0.8$^{\#*}$ | 66.5±0.9$^{\#*}$ |
| 14 | 15.4±0.1$^{\#*}$ | 8.0±0.4$^{\#*}$ | 15.9±1.1$^{\#}$ | 40.6±0.9$^{\#*}$ | 22.6±0.9$^{\#*}$ | 30.1±0.9$^{\#*}$ | 49.5±0.1$^{\#*}$ | 69.3±0.2$^{\#*}$ | 59.5±0.1$^{\#*}$ |
| 21 | 14.3±0.1$^{\#*}$ | 5.9±0.2* | 16.3±0.$^{\#}$ | 39.5±0.7$^{\#}$ | 16.5±1.7$^{\#*}$ | 28.5±0.7$^{\#}$ | 55.5±0.3$^{\#*}$ | 79.5±3.8* | 54.5±0.3$^{\#*}$ |
| 30 | 12.3±0.8$^{\#*}$ | 4.5±0.3$^{\#*}$ | 12.5±0.8$^{\#*}$ | 19.6±0.5$^{\#*}$ | 12.8±0.5$^{\#*}$ | 19.0±0.5$^{\#*}$ | 73.3±0.2$^{\#*}$ | 82.9±0.2$^{\#}$ | 69.3±0.2$^{\#*}$ |
| 45 | 8.2±0.6$^{\#*}$ | 2.2±0.6$^{\#*}$ | 9.2±0.6$^{\#*}$ | 11.5±0.1$^{\#*}$ | 8.8±0.5* | 11.7±0.2$^{\#*}$ | 80.4±0.5$^{\#*}$ | 89.1±0.8$^{\#*}$ | 78.4±0.5$^{\#*}$ |

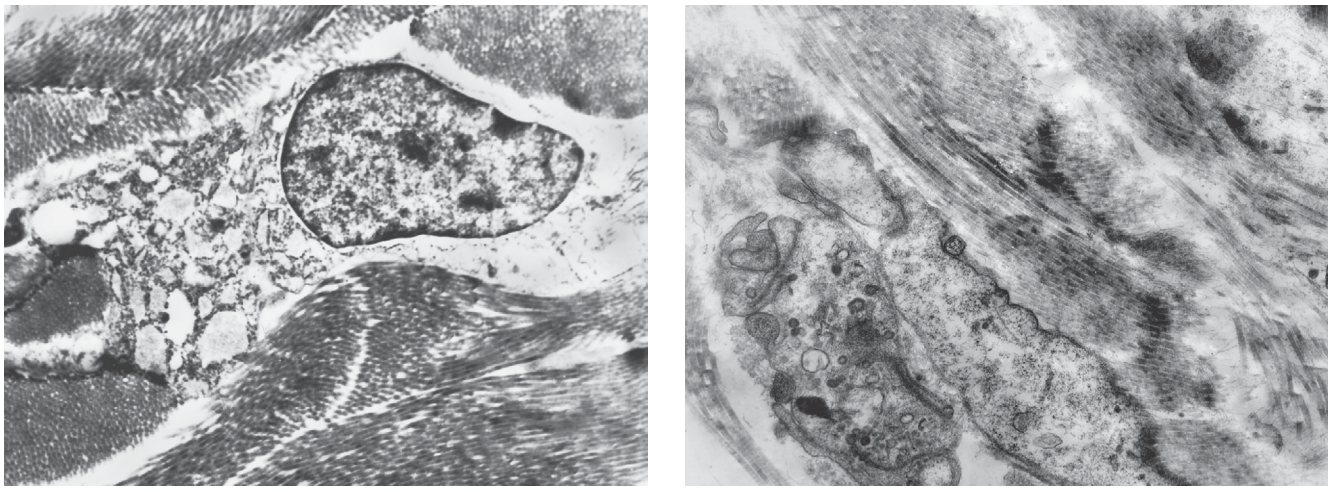
*Note.* * — p<0.05 compared with the previous period of observation; # — p<0.05 compared to intact.

sent); the increase of mitotic index of the epidermal basal cells (the highest under conditions of 30 nm silver nanoparticles influence) (Fig. 2); changes of dermal fibroblasts's heteromorphism, such as increasing of number of functionally active fibroblasts (the highest under the conditions of 30 nm silver nanoparticles influence) (Table 1); and the number of collagen fibers of the dermis (the highest under conditions of 30 nm silver nanoparticles influence).

Morphometric data were verified by electron microscopy. It showed an increase in number of synthetically active differentiated fibroblasts in the group treated with silver NPs size of 30 nm (Fig. 3).

Analyzing the process of morphological changes in the skin tissue by introducing silver NPs one should note that regardless of the size of silver NPs in skin tissue distinctive changes arise.

The leading violation is damage of the basement membrane structure in epidermis, which leads to disruption of transfer and the development of degenerative processes in the basal cells of the epidermis and reduce of their mitotic activity. As you can see, violation of the basement membrane structure, namely, its homogenization occurs under the influence of both ions and silver NPs of all sizes, that means, this reaction does not depend on the particle size. The only term of the restoration of the basal membrane structures vary depending on the size. Under exposure conditions 30 nm and 70 nm NPs basal membrane structure is reduced and under influence of NPs 20 nm structure does not recover during the entire experiment. Moreover, the introduction of NPs 30 nm restoration of the basement membrane is noted faster than when exposed to the NPs 70 nm. Lack of recovery of the basal membrane structure can be explained by proven toxicity of silver ions and NPs 20 nm, in the absence of toxicity in the NPs 30 and 70 nm. There is dependence of the rate of reparative processes, depending on the NPs size. Similar results were obtained in the study by Kwan and colleagues,



*a* *b*

*Fig. 3.* Ultrastructure of rat dermis on the 7th day after the administration of the Ag NPs. Transmission electron microscopy. 10000×: *a* — mature differentiated fibroblast; *b* — fibroblast with an increased number of organelles

which proved the dependence of the rate of wound healing by NPs size [10].

Restoration of mitotic activity and reduced degenerative phenomena in the cells of the basal layer of the epidermis, which is noted under the influence of silver NPs sizes 30 and 70 nm, is not associated with the effect of the NP, and is due to the restoration of the basement membrane structure. This is confirmed by the coincidence of the term of recovery and restoration of the membrane structure of the mitotic activity. Although, there are studies confirming the low impact on the proliferative activity of keratinocytes due to the effect on epithelial growth factors [11].

We decribed morphological and functional changes in the dermis in experiment are similar to those that occur in the dermis of the skin under aseptic inflammation caused by the implantation of a foreign body [12]. Introduction of silver NPs in the skin causes a similar reaction as the introduction of allogenic biomaterial leads to such changes in the cellular composition. At the same time the greatest changes occur in number of macrophages [13]. This confirms the data [12] of the main regulatory role of macrophages in tissue regeneration in the intercellular and stromal-cell interactions, which indicate that the nature of inflammation, followed by the regeneration process or disregeneration in the connective tissue determines different intensity macrophage stage, which depends on the the characteristics of the material introduced.

In addition it takes place the influence of silver NPs on mast cell degranulation. Similar results were obtained by Gunasekaran [14] in a study on the influence of NPs on wound process. But this study demonstrates decrease of mast cell degranulation in a silver NPs exposure, whereas we had reduced degranulation index only under influence of NPs 20 and 30 nm, compared with the effect of silver ions. These differences can be explained by the fact that the studies used different in size NP. This requires further immunohistochemical studies depending on the activity of tissue basophils of silver NPs size.

**Conclusions.** The study of changes in inter- and intra differons heteromorphism of tissue is a promising method for assessing the impact of silver NPs on the morpho-functional state of the skin tissue.

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