Диаметр частиц, фазовый состав, площадь поверхности и толщина пленки наночастиц меди как предикторы антибактериальной активности.

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Успешная разработка эффективных и безопасных фармацевтических препаратов на основе наноматериалов требует понимания того, как их свойства влияют на биологическую активность. Это исследование было сосредоточено на выявлении корреляции между физико-химическими свойствами наночастиц меди и их антибактериальным действием. Были протестированы шесть мазевых составов с монокристаллическими наночастицами. Частицы различались по размеру, составу кристаллической фазы металла и фазы оксида меди, толщине поверхностной оксидной пленки и площади поверхности. Методы линейной и нелинейной регрессии были использованы для оценки взаимосвязи между свойствами частиц и эффективностью их действия. Лучшая антибактериальная активность при тестировании с *E. coli* AB 1157 наблюдалась при увеличении площади поверхности, а также при большем количестве оксида CuO, более тонкой пленке и меньшем количестве кристаллической фазы. Улучшение антибактериальной активности при тестировании со *St. epidermidis* определялось уменьшением диаметра наночастиц, меньшим количеством оксида CuO, а также увеличением толщины пленки наночастиц и увеличением кристаллической фазы. Анализ, проведенный в этом исследовании, позволяет оценить, какие характеристики наночастиц играют наиболее важную роль в определении их активности, что далее будет применено для разработки наночастиц с желаемым фармакологическим действием.

Ключевые слова: *соединения меди, наночастицы, регрессионный анализ, антибактериальное действие*.

Particle diameter, phase composition, surface area and film thickness of copper nanoparticles as predictors of antibacterial activity.

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A successful design of effective and safe nanomaterial-based pharmaceuticals requires an understanding of how their properties influence biological activity. This research focused on identifying correlations between the physicochemical properties of copper nanoparticles and their antibacterial action. Six ointment compositions with monocrystalline nanoparticles were tested. The particles varied in size, crystalline metal phase and copper oxide phase composition, the surface oxide film thickness and surface area. Linear and non-linear regression methods were used to evaluate the relationship between the properties of particles and the effectiveness of their action. A better antibacterial activity when tested with *E. coli* AB 1157 was seen with an increase in surface area as well as bigger oxide CuO quantities, thinner film and smaller quantities of the crystal phase. An improvement in antibacterial activity when tested with *St. epidermidis* was determined with a decrease in nanoparticle diameter, smaller quantity of CuO oxide, as well as an increase in nanoparticle film thickness and a larger crystal phase. The analysis performed in this study allows evaluating, which nanoparticle characteristics play the most important role in determining their activity that would be used later for nanoparticle development with desired pharmacological activity.

Key words: copper compounds, nanoparticles, regression analysis, antibacterial effect.

1. Introduction

An important problem that arises in a clinical setting and in severe cases has an unmet medical need is wound healing. Currently, the annual number of patients suffering from painful injuries and chronic wounds exceeds eight million [1]. Likewise, there is an increase in bacterial resistance to standard pharmaceuticals including antibiotics. Chronic ulcers, bedsores, vascular, inflammatory, and rheumatic injuries are especially troubling since the number of elderly people and the proportion of diabetics who are at risk of developing skin diseases are growing worldwide [2]. Current treatments for local application are water-retaining bandages, sorbents, ointments, gels, antiseptics, wound dressings and coatings based on medical textiles, gels and hydrocolloids [3–7]. With the rise of nanotechnologies, an introduction of nanoparticles (NPs) into the field of pharmacology allowed for the development of metal nanoparticle-enriched antibacterial agents including silver, gold, copper, titanium, zinc, etc. [8–10]. Likewise, other nanostructures like nanocapsules, polymer and firm lipid NPs, polymer nanocomplex can be used as drug vehicles for drugs antibiotics improving targeting and, therefore, effectiveness [13].

The wound healing process is often delayed by bacterial infections resulting in severe aggravation of the disease. Therefore, the current strategy for treating wounds is to simultaneously stimulate tissue regeneration and fight against infections [3, 11, 13]. Nanocomposite gels with copper NPs demonstrate antibacterial activity for gram-positive (St. epidermidis) and gram-negative bacteria (E. coli AB1157) with preferential effectiveness against Staphylococcus species [11]. Apparently, the differences in the effects of Cu NPs on gram-positive and gram-negative bacteria are associated with the structure of the bacterial cell wall. Therefore, the interaction of the nanoparticles of different morphology coupled with differences in physicochemical properties was studied by assessing the relationship of the Cu NPs antibacterial effect and their physicochemical characteristics. Understanding the relationship between the physicochemical properties of synthesized nanomaterials and their biological activity is crucial for the development of effective strategies to design NPs with certain characteristics. The aim of this work is to analyze heterogeneous data collected from previous experiments studying antibacterial activity of Cu NPs obtained by directional modification of the Cu NPs surface by various factors (air, oxygen, water vapor). The featured analysis presents an exploration of data and attempts to describe physicochemical and biological properties of Cu NPs with linear and exponential trends.

2. Materials and Methods

2.1. Copper nanomaterial

The data obtained for the analysis was aggregated from a range of experiments with Cu NPs and Cu NPs gel ointments bearing various physicochemical characteristics carried out at the Laboratory of Biological Effects of Nanostructures from 2007 to 2020. These were manufactured from a 99.98 % copper wire using flow-levitation method developed at the Institute of Chemical Physics of the USSR Academy of Sciences by M.Gen and A. Miller in 1981 [14]. A molten drop of copper levitated in an electro-magnetic field of the counter-current high-frequency inductor operating at 440 KHz. As the material vaporized it was swept away from the drop surface by a continuous flow of the inert gas (high purity argon 99.99 %). The vapor was cooled down and, subsequently, condensed into the nano-scaled metal particles. Copper nanopowder was subjected in situ to water vapor or oxygen to synthesize the barrier surface layer. After the particle synthesis they are passivated by the atmospheric air. Oxygen is added into the air flow to manufacture copper oxide NPs. The shape and size of Cu NPs were determined by electron microscopy on a Joel JSM 7401F scanning electron microscope at 1 kV. The microphotographs were processed using Micran 25 by measuring the cross sections of at least a thousand particles to determine an average diameter. Likewise, the X-ray phase analysis of metal particles was carried out on an X-ray analyzer ADP-1 (Russia) in Co H-α radiation with a step of 0.05º and signal accumulation time from 8 to 10 min. The phase composition of metal NPs was determined by processing the interference peaks in Match 3.8.0.137 phase analysis software. The gel ointments were prepared by combining Cu NPs with preservatives MC-100, Twin-80, glycerin and para-hydroxybenzoic acid methyl ester (E218).

2.2. Antibacterial effect of Cu NPs

The antibacterial effect of gels with Cu NPs was studied on test cultures of gram-negative bacteria – *Escherichia coli* (*E. coli*) AB1157, a hospital strain obtained from the museum N. F. (Moscow, Russia) and on test cultures of gram-positive bacteria – *Staphylococcus epidermidis* (*St. epidermidis*) obtained from the Museum of the Department of Microbiology of Lomonosov Moscow State University (Gamaleya Research Institute of Microbiology and Epidemiology of the Russian Academy of Medical Sciences Moscow, Russia).

The activity of antibacterial ointments with Cu NPs as well as negative control was accessed by an in vitro method – disk diffusion analysis [15]. Tests were carried out with both *S. epidermidis* and *E. coli* AB 1157 strains. The antibacterial effect of gels was assessed by the size of the cell growth retardation zone in the solid nutrient media composed of peptone, yeast extract, sodium chloride, agar-agar (2 %). Paper disks with a 5 mm diameter were sterilized at a temperature of 180 °C for 2–3 h. After that, 0.2 g gel sample was placed and evenly distributed on each disk. Disks were placed on the Petri dish with bacteria (4 disks/1 dish). Then Petri dishes were placed in a thermostat at 37 °C for one day. The formation of a growth-retarding zone around the disk indicated that gel had antibacterial properties. The biological activity of the gel samples with/without Cu NPs was evaluated by the size of the cell growth retardation zone. The diameters of cell growth inhibition zone were measured in triplicates in Image J.

2.3. Mathematical modeling

The relationship between physicochemical properties and the performance of various Cu NPs was described using a range of regression models including linear regression ($y = a \cdot x + b$), exponential growth, and decay models. The model parameters were estimated against antibacterial data (cell growth inhibition zone for *S. epidermidis* and *E. coli* AB 1157 strains). The analyses were performed in R Statistics software 4.0.2 using an in-built *lm*() function from the *stats* package; the data was visualized using *ggplot2* package [16]. The successfully converged and adequately describing the data models were selected assessing the p-values for the estimated parameters as well as the R^2 score.

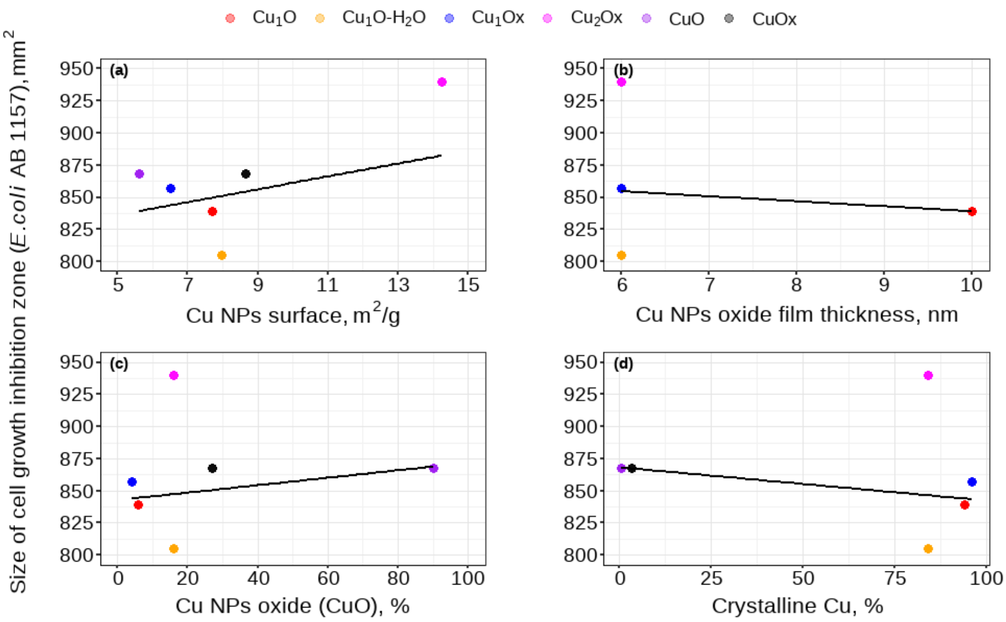

Fig. 1a. Antibacterial effect as the size of cell growth inhibition zone for *E. coli* AB1157 strain versus physicochemical properties of Cu NPs. Data (colored dots) are shown as well as the best fitted regression models (black solid line). (**a**) – Copper nanoparticles oxide film thickness, (**b**) – Percentage of CuO oxide phase in copper nanoparticles composition, (**c**) – Percentage of crystalline copper in nanoparticles composition, (**d**) – Copper nanoparticles surface.

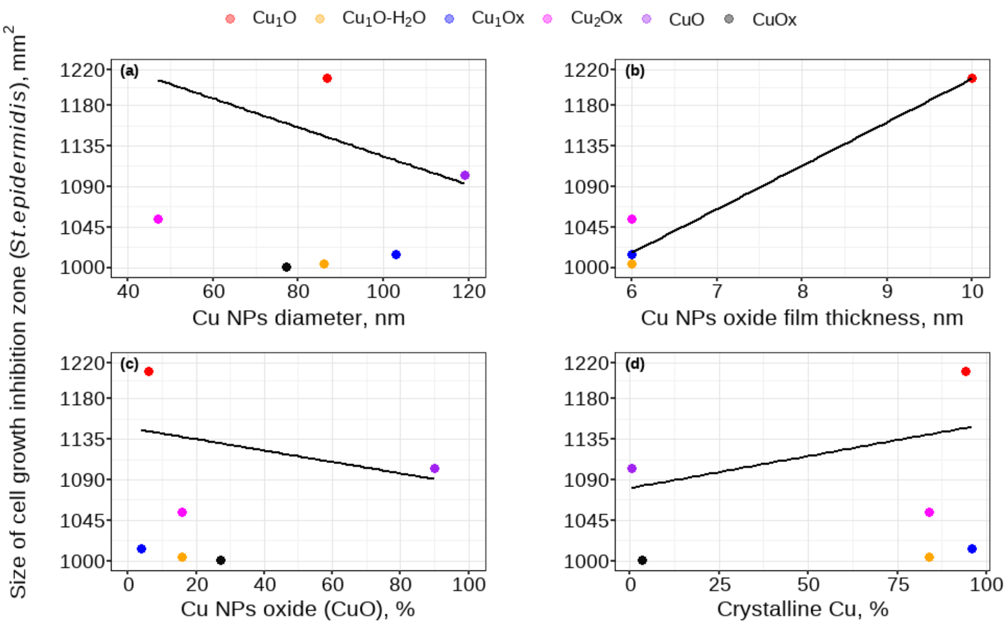

Fig. 1b. Antibacterial effect as the size of cell growth inhibition zone for *St. epidermidis* strain versus physicochemical properties of Cu NPs. Data (colored dots) are shown as well as the best fitted regression models (black solid line). (**a**) – Copper nanoparticles oxide film thickness, (**b**) – Percentage of CuO oxide phase in copper nanoparticles composition, (**c**) – Percentage of crystalline copper in nanoparticles composition.

3. Results and Discussion

3.1. Copper nanomaterial

We developed a range of Cu NPs that we proposed to possess antibacterial properties. Directed modification of Cu NPs by various factors during the nanoparticles synthesis was performed and their main physical and chemical characteristics determined. Manufactured Cu NPs had rounded monocrystalline structures covered with a translucent oxide film. Depending on the modification factor an average particle diameter ranged from 47.0 ± 0.6 nm to 119.0 ± 1.0 nm. According to the results of *X*-ray phase analysis the crystalline metal phase varied from 0.5 ± 0.03 to 96.0 ± 4.5 % and the copper oxide phase fluctuated from 86.6 % to 4.0 %. The nanoparticle oxide film surface thickness varied from 6 nm to 10 nm, the surface area ranged from 5.63 ± 1.23 m^2/g to 14.25 ± 3.2 m^2/g.

3.2. Assessment of Cu NPs antibacterial activity with different physicochemical characteristics

There are a few relationships determined based on the weighted linear regression analysis that showed significance. A better antibacterial activity when tested with *E. coli* AB 1157 was seen with an increase in surface area ($a = 4.99$; $b = 811.23$; $R^2 = 0.10$) as well as larger oxide CuO quantities ($a = 0.29$; $b = 842.69$; $R^2 = 0.14$), thinner film ($a = –3.88$; $b = 877.82$, $R^2 = 0.10$) and smaller quantities of the crystal phase ($a = –0.26$; $b = 868.17$; $R^2 = 0.16$). An improvement in activity when tested with *St. epidermidis* was determined with a decrease in nanoparticle diameter ($a = –1.60$; $b = 1283.77$; $R^2 = 0.07$), smaller quantity of CuO oxide ($a = –0.63$; $b = 1147.74$; $R^2 = 0.04$), as well as an increase in nanoparticle film thickness ($a = 48.41$; $b = 725.88$, $R^2 = 0.99$) and bigger crystal phase quantities ($a = 0.71$; $b = 1080.75$; $R^2 = 0.08$). These significant relationships are visualized in figures 1a and 1b.

Although statistically significant point estimates for the positive association between the amount of Cu_2O in the oxide phase and the bacterial growth were estimated, the slope was close to zero and therefore, had small practical relevance. As follows from the results, the obtained differences in the antibacterial action of copper NPs (including the top performance of Cu_2Ox or Cu_1O) on *E. coli* AB 1157 and *St. epidermidis* are related not only to structural differences in the bacterial cells but also to a different response of bacteria to the action of modified Cu NPs. Similar differences were observed in other cultures like *Vibrio cholerae*, that showed slowed growth in the presence of Au NPs of smaller size [17]. Likewise, growth inhibition of methicillin-resistant St. aureus was observed by ZnO NPs with an average diameter of 12 nm, while there was no growth inhibition with larger NPs over 100 nm in diameter [18]. Therefore, our research plausibly confirms previous studies and extends it into copper nanoparticle and their various physicochemical properties.

4. Conclusions

In this study we performed purposeful modification of the particles by various factors (oxygen, water vapor, and air) to obtain Cu NPs of different sizes and phase compositions. The modified Cu NPs were introduced into a therapeutic ointment and their antimicrobial activities were tested.

We used a linear regression approach to answer the question about the relationship between the physicochemical characteristics of Cu NPs and their biological activity. This approach made it possible to establish correlations between individual characteristics of Cu NPs and their antimicrobial activity.

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