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FULL PAPER

Assessment of biocompatibility and toxicity of basic copper carbonate nanoparticles stabilized with biological macromolecules

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The purpose of this work was to develop a synthesis technique and study the physico-chemical properties of copper carbonate nanoparticles (CC-NPs) stabilized with biopolymers to assess their biocompatibility and toxicity. For the synthesis of CC-NPs, $Cu(CH_3COO)_2$ and $CuCl_2$ were used as Cu precursors, while K_2CO_3, $(NH_4)_2CO_3$ and $(NH_4)_2CO_3$ were used as precipitators. The following biopolymers were used as stabilizing agents: chitosan, methylcellulose, hydroxyethyl cellulose and hyaluronic acid. The experimental data showed that $Cu(CH_3COO)_2$ is the optimal precursor and $(NH_4)_2CO_3$ is the optimal precipitator for the production of CC-NPs. The optimal sample consists of spherical aggregates with a diameter of 0.6 to 1.0 μm formed by nanoparticles with a diameter of 30 to 80 nm. This sample has a phase composition of $Cu_2(OH)_2CO_3$. All considered biopolymers were appropriate for stabilization of CC-NPs, but hyaluronic acid was selected as the optimal stabilizing agent. Interestingly, 10 mg of CC-NPs+HA powder caused 100% mortality of chicken embryos. The structural and surface properties of CC-NPs+HA powder allowed it to be absorbed on the surface of CAM while preserving the implantation site throughout the experiment. It is worth noting that the implantation of the CC-NPs+HA sample was accompanied by a clearly identified degradation of small and large vessels. Thus, the results of CAM assay indicate pronounced toxic changes in CAM under the action of CC-NPs+HA. Surprisingly, despite the fact that Cu is an essential trace element, the developed CC-NPs+HA complex is characterized by low biocompatibility and high toxicity.

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KEYWORDS

Trace element; CAM assay; biocompatibility; toxicity.

Introduction

The study of metal nanoparticles (Me NPs) is currently one of the promising scientific directions [1,2]. The Me NPs characteristics often depend on the shape, size, surface properties, etc. [3,4]. Currently, Me NPs have found wide application in many fields of human activity due to their unique physical and chemical properties, such as small size,

high specific surface area, high reactivity, etc. [5,6]. Special attention is paid to the study of the effect of biogenic metal nanopowders on the body, in particular copper, zinc, iron, the biological value of which is determined by the versatility of functions in complex biochemical processes and active participation in cellular metabolism, ensuring the normal functioning of the body [7,8].

Copper nanoparticles (Cu NPs) are actively used in medical preparations, cosmetic products, lacquers, paints, water filters, packaging, medical products, linen [9,11]. Due to Cu^{2+} ions, these particles have bactericidal properties and kill most harmful microorganisms without causing resistance in bacteria, unlike antibiotics [12,13]. Due to the above properties and functions, Cu-based compounds, such as copper carbonate, are widely used in various fields of medicine, including implantology [14,15]. However, their safety for the body remains in question. Researchers have evidence that Cu NPs can be toxic to various organs of the body – the brain, liver, lungs [16,18]. Despite the fact that the antibacterial properties of Cu have been known for a long time, and Cu NPs have been successfully used as antibacterial agents, the mechanism of their action has not been fully studied [19].

It is worth noting, that Cu-NPs have a higher activity compared to conventional Cu microparticles, are able to penetrate unchanged through cellular barriers, circulate, and accumulate in organs and tissues, causing more pronounced pathomorphological lesions of internal organs [20,21]. The toxicity of Cu-NPs is determined by their shape and size, while the smallest spindle-shaped particles cause more destructive effects in the body than similar spherical particles [22]. In this regard, the study of Cu-NPs cytotoxicity is an urgent topic and is of undoubted scientific and practical interest.

Nowadays a number of publications describe that Cu-NPs are capable of damaging the cell wall and cell membrane of bacteria, thereby leading to its death [23,24]. Moreover, Cu-based nanostructures can aggregate during their production and use. This is due to the low surface charge, which usually prevents the formation of particles [25,26]. One of the problems in the creation and use of such materials is the stabilization of the shape and size of NPs. The use of stabilizing agents, as the most promising currently being polymers, makes it possible to successfully solve this problem [27]. The increasing demand for products from the chemical, pharmaceutical, cosmetic, food, and other industries requires the creation of new ways to obtain Cu-NPs-based biologically active compounds. The correct selection of stabilizing agents will realize the unique properties of Cu-NPs. For instance, the use of natural biopolymers as stabilizing agent can decrease toxicity and expand biomedical application of Cu-NPs.

Interestingly, among all nanoscale Cu-containing form, copper carbonate nanoparticles (CC-NPs) are one of the most promising for application in medicine. Due to high initial discharge capacity, CC-NPs can be considered as a promising candidate for creation of functional scaffolds in medical implants [28,29]. At the same time, CC-NPs have comparable antibacterial activity, but are cheaper than Ag NPs, which are currently widely used in medicine [30]. CC-NPs can affect the activity of vitamins, hormones, and enzymes: it is necessary for the absorption and utilization of iron, participates in the formation of connective tissue, energy production at the cellular level, melanin formation, and hormonal regulation [31,32]. Notably, CC-NPs can promote a full-fledged synthesis of collagen and elastin, providing elasticity of the vascular wall and alveoli of the lungs, supporting skin elasticity and positively affecting the condition of hair and nails, having a restorative effect on bones and joints [33,34]. Thus, CC-NPs have a great potential for application in regenerative medicine. Therefore, a hypothesis was formulated that CC-NPs stabilized with biopolymers are less

toxic, biocompatible and can be used in regenerative medicine. Consequently, the purpose of this work was to develop a synthesis technique and study the physico-chemical properties of CC-NPs stabilized with biopolymers to assess their biocompatibility and toxicity.

Experimental

Materials

For the synthesis of CC-NPs, $Cu(CH_3COO)_2$ and $CuCl_2$ were used as Cu precursors, while K_2CO_3, $((NH_4)_2CO_3)$, and $(NH_3)_2CO_4$ were used as precipitators. The following biopolymers were used as stabilizing agents: chitosan, methylcellulose, hydroxyethyl cellulose and hyaluronic acid. All chemicals were purchased in LenReactive (St. Petersburg, Russia) at chemical grade. For biocompatibility test, fertilized chicken eggs of the Haysex Brown cross were purchased from the commercial supplier Agrokormservice Plus (Adygea, Russia). In vivo experiments were carried out using neutral buffered formalin solution (10%), sterile paraffin, isopropyl alcohol (≥99.7%), ethyl alcohol (≥99.7%) purchased from Dia-M, (Moscow, Russia)

Method of synthesis of CC-NPs in aqueous solutions of biopolymers

Synthesis of CC-NPs stabilized with biopolymers was performed as follows:

1) The required volume of 0.8 M solution of a copper-containing precursor was added to a 500 mL glass and stirred at 700-1000 rpm until completely dissolved.

2) The required volume of 1% biopolymer solution was added to copper-containing precursor.

3) The required volume of 0.8 M precipitator solution was poured into a drip funnel mounted on a tripod over a glass with a precursor solution. Precipitator addition was performed at a rate of 60 drops per minute.

4) The resulting solution was centrifuged for 5 minutes at 3000 rpm. After centrifugation, the filler liquid was carefully drained, avoiding loss of sediment.

5) 250 mL of distilled water was poured to the freshly deposited sediment and mixed. Solution was centrifuged for 5 minutes at 3000 rpm. After centrifugation, the filler liquid was carefully drained, avoiding loss of sediment.

6) Centrifugation was repeated till the filler liquid became transparent.

7) After the last centrifugation, the formed precipitate was carefully transferred into a porcelain crucible and dried at 110 °C for 8 h.

CC-NPs characterization

The average hydrodynamic radius of the particles was studied by the method of dynamic light scattering (DLS) on the Photocor-Complex device (Photokor, Russia) [35].

The microstructure of the obtained samples was studied by scanning electron microscopy (SEM) on the MIRA 3-LMH device (Tescan, Czech Republic) [36].

The phase composition of the samples was studied using an X-ray diffractometer (XRD) PANanytical Empyrean (PANanytical B.Y, the Netherlands) [37].

The elemental composition of samples of CC-NPs stabilized with chitosan, methylcellulose, hydroxyethyl cellulose and hyaluronic acid was analyzed by X-ray fluorescence spectroscopy (XFS) on a Bruker M4 Tornado X-ray fluorescence spectrometer (Bruker, Belgium). Elemental composition studies were carried out taking into account the fact that the device is sensitive to elements starting from Na [38,39].

CAM assay

The biocompatibility of powder samples was studied *in vivo* using the CAM (chorioallantoic membrane) model of a chicken embryo in accordance with the methodology described in

Coelho *et al.* [40], Shendage *et al.* [41], and Rzhepakovsky *et al.* [42] with some modifications. Notably, the use of chicken embryos is provided by Directive 2010/63/EU on the protection of animals used for scientific purposes [43]. An official conclusion of the ethics committee was not required for CAM assay, since chicken embryos used in the experiment had not reached the last third of embryogenesis (before the 15th day of incubation).

Fertilized chicken eggs of the Haysex Brown cross were purchased from the commercial supplier Agrokormservice Plus (Adygea, Russia). The Rcom Maru Deluxe Max 380 digital incubator (AUTOELEX CO., Korea) was used for incubation. Eggs treated with 70% ethanol were incubated at of 37.6 °C, humidity of 55% and automatic rotation of trays every hour. On the 3rd day of incubation, 3 mL of albumen was aspirated from the acute pole of the egg using a syringe with a needle in the box of the abacterial air environment BAVnp-01-"Laminar-C"-1.5 (LORICA, CJSC Laminar Systems, Russia). The hole was covered with sterile paraffin wax. A window with a diameter of 2.5 cm^2 was cut above the egg, covered with a transparent tape, and the eggs were returned to the incubator. On the 8th day of incubation, CC-NPs powder samples were placed through a window in the shell on the CAM surface between the embryo and the outer boundary of the CAM in the area of large vessels. Previously, samples of CC-NPs were subjected to UV disinfection using an ultraviolet irradiator "Azov" OBN-35-01 UHL 4.2 (St. Petersburg, Russia).

In accordance with Shendage *et al.* [41], CC-NPs powders were applied to the skin in an amount of 10 mg. Sterile silicone rings (thickness 1.5 mm, inner diameter 5 mm) were used as a reservoir and marker of the powder application site. The CAM with an implanted empty ring acted as a control. The window in the shell was closed again and incubation continued for another 6 days. Each experiment included ten embryos per group and was repeated twice. On the 14th day of incubation, the sealing tape was removed and CAM was photographed *in ovo*. Immediately after that, according to the recommendations for humane euthanasia [44], the embryos were sacrificed in a gas chamber (70% of CO_2 for 30 minutes). Further, the sections of the skin in contact with the CC-NPs samples were excised. Visualization of blood vessels on the reverse side of CAM was performed *ex ovo* using ARSTEK SZ0850 stereomicroscope (Beijing, China) with image fixation using digital camera (38 MP Samega V6) and the S-EYE2.0 software (YOUNG WIN Technology Co., Beijing, China).

Statistical data processing

Statistical data processing was performed using the Statistica 12.0 software package (StatSoft, Tulsa, USA) using nonparametric statistics methods. The results obtained are expressed in the form of median (Me), upper and lower quartiles (Q1–Q3). The differences were considered significant at $p<0.05$.

Results and discussion

Selection of optimal precursor and precipitator

At the first stage of the research, the optimal Cu-containing precursor and precipitator for the synthesis of CC-NPs were determined. As Cu precursors, copper acetate ($Cu(CH_3COO)_2$), and copper chloride $CuCl_2$ were used. At the same time potassium carbonate (K_2CO_3), ammonium carbonate ($(NH_4)_2CO3$) and sodium carbonate (Na_2CO_3) were used as precipitators. The obtained samples of CC-NPs were examined by X-ray diffractometry, scanning electron microscopy, and X-ray fluorescence spectroscopy. The interpretation of X-ray diffractograms of the obtained samples is indicated in Table 1.

TABLE 1 Interpretation of X-ray diffractograms of CC-NPs

Precursor	Precipitator	$Cu_2(OH)_2CO_3$	$Cu_2(OH)_3Cl$
$Cu(CH_3COO)_2$	K_2CO_3	+	–
$Cu(CH_3COO)_2$	$(NH_4)_2CO_3$	+	–
$Cu(CH_3COO)_2$	Na_2CO_3	+	–
$CuCl_2$	K_2CO_3	+	+
$CuCl_2$	$(NH_4)_2CO_3$	+	+
$CuCl_2$	Na_2CO_3	+	+

Analysis of the obtained X-ray diffractograms showed that the type of precursor significantly affects the phase composition of samples of CC-NPs. When using $Cu(CH_3COO)_2$, the basic copper carbonate with the chemical formula $Cu_2(OH)_2CO_3$ is formed. When using $CuCl_2$, a system consisting of two crystalline phases of $Cu_2(OH)_2CO_3$ and $Cu_2(OH)_3Cl$ are formed. It is worth noting, that the results obtained correspond to recent findings of other researchers [45,47].

The results of scanning electron microscopy are depicted in Figure 1.

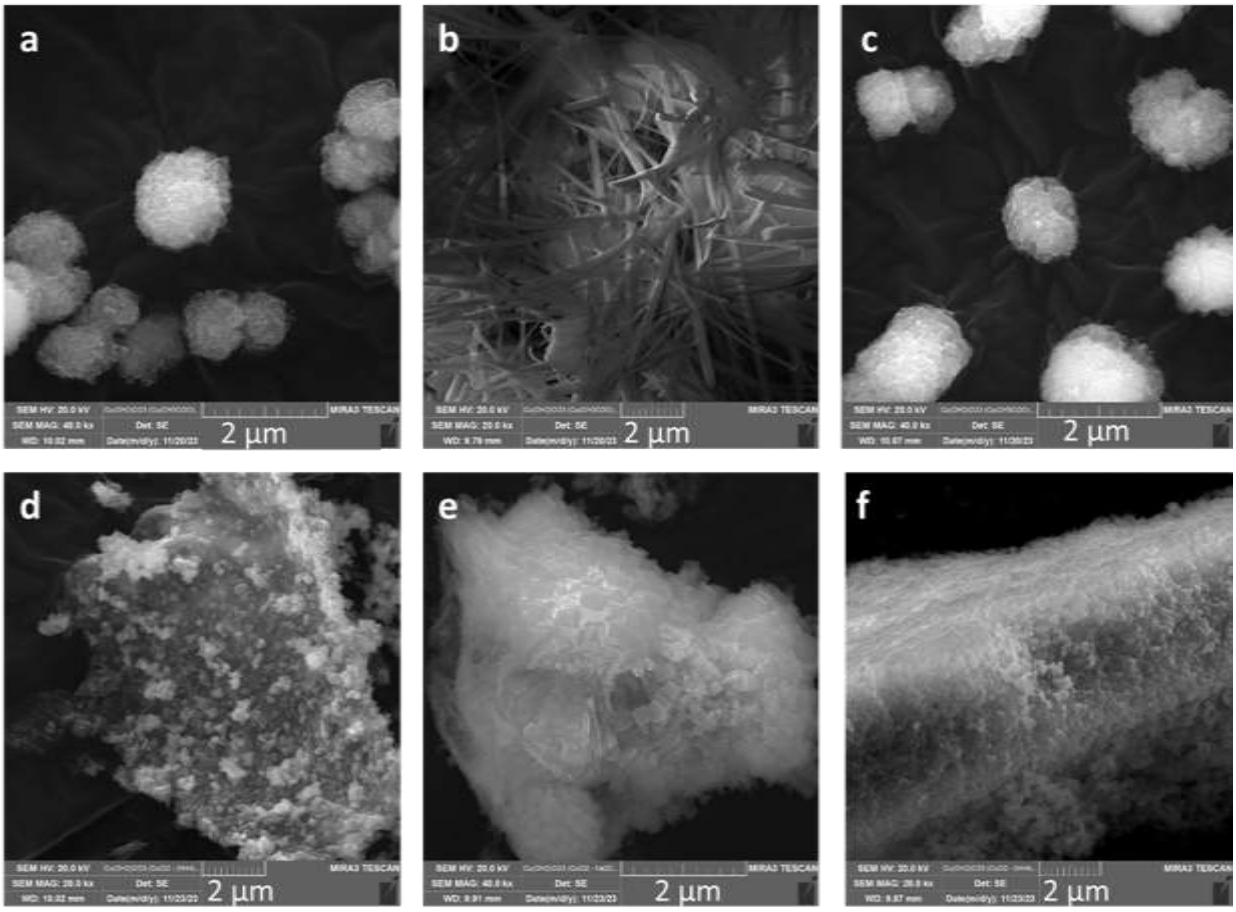

FIGURE 1 SEM-micrographs of CC-NPs synthesized from $Cu(CH_3COO)_2$ and $(NH_4)_2CO_3$ (a), $Cu(CH_3COO)_2$ and K_2CO_3 (b), $Cu(CH_3COO)_2$ and Na_2CO_3 (c), $CuCl_2$ and K_2CO_3 (d), $CuCl_2$ and Na_2CO_3 (e), $CuCl_2$ and $(NH_4)_2CO_3$ (f). Magnification ×40,000

It was found that CC-NPs synthesized from $Cu(CH_3COO)_2$ and $(NH_4)_2CO_3$ consists of spherical aggregates with a diameter of 0.6 to 1.0 μm formed by nanoparticles with a diameter of 30 to 80 nm (Figure 1a). Analysis of Figure 1b showed that filamentous crystals with a width from 80 to 350 nm are formed in samples of CC-NPs obtained from $Cu(CH_3COO)_2$

and K_2CO_3. In the CC-NPs sample obtained from $Cu(CH_3COO)_2$ and Na_2CO_3, spherical aggregates with a diameter of 1.0 to 2.0 μm formed by nanoparticles with a diameter of 30 to 70 nm (Figure 1c) are observed. Synthesis from $CuCl_2$ and K_2CO_3 leads to the formation of CC-NPs, represented as aggregates consisting of nanoparticles with a diameter from 25 to 60 nm (Figure 1d). The CC-NPs sample obtained from $CuCl_2$ and Na_2CO_3 consists of filamentous crystallites, which are based on a square (Figure 1e). The width of filamentous crystallites varies from 50 to 250 nm. The width of the square at the base of the crystallites lies in the range from 200 to 300 nm. Analysis of micrographs of CC-NPs obtained from $CuCl_2$ and $(NH_4)_2CO_3$ showed that the microstructure is represented as nanoparticles with a diameter of 40 to 80 nm (Figure 1f). Generally, the observed trends are in the line with data of Smith *et al.* [48] and Dong *et al.* [49].

At the next stage, the elemental composition of samples of CC-NPs was studied by X-ray fluorescence spectrometry on a Bruker M4 Tornado X-ray fluorescence spectrometer. The data obtained are depicted in Figure 2.

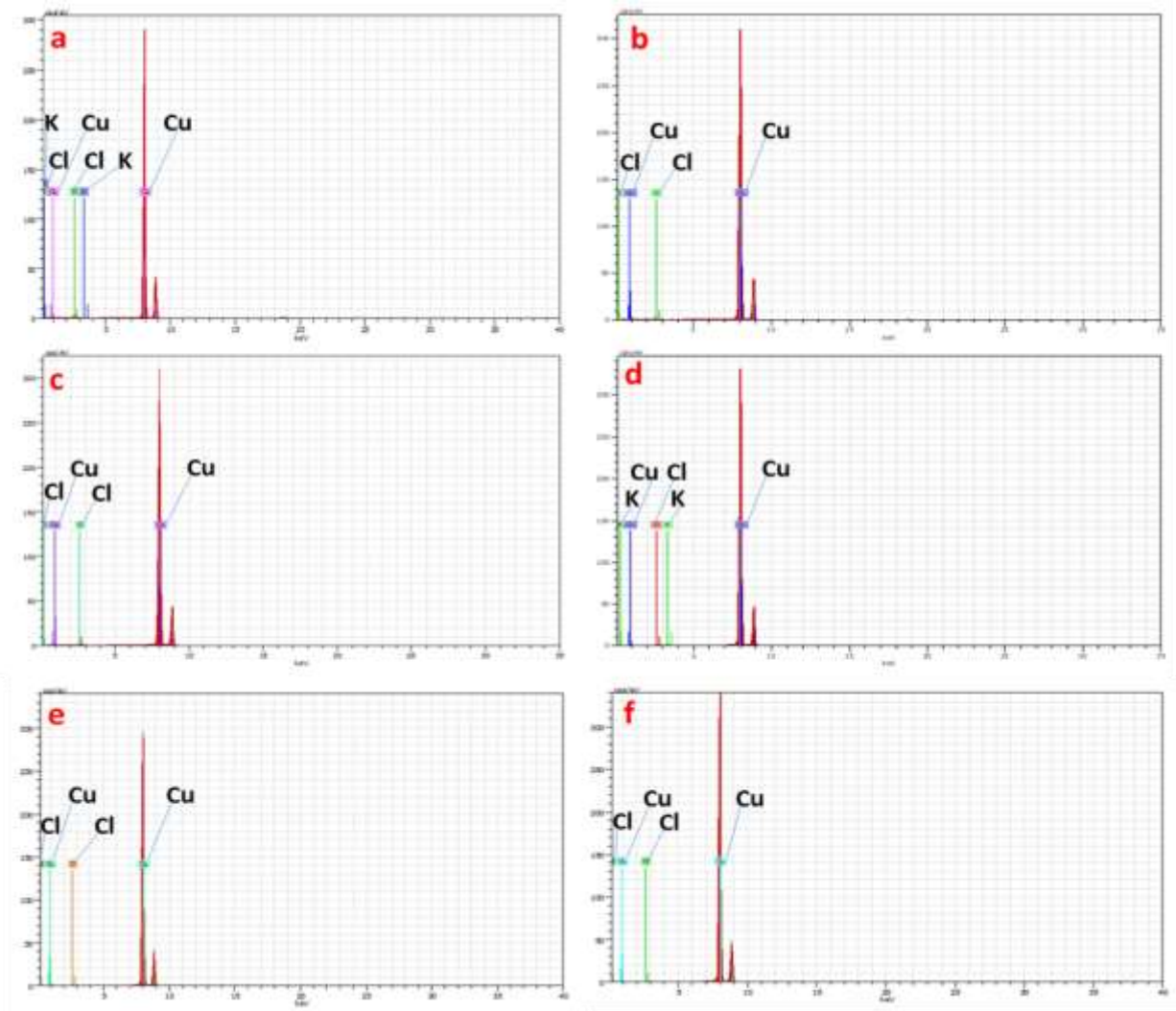

FIGURE 2 EDX spectra of CC-NPs synthesized from K_2CO3 and $CuCl_2$ (a), Na_2CO_3 and $CuCl_2$ (b), $(NH_3)_4CO_3$ and $CuCl_2$ (c), K_2CO_3 and $Cu(CH_3COO)_2$ (d), Na_2CO_3 and $Cu(CH_3COO)_2$ (e), $(NH_4)_2CO_3$ and $Cu(CH_3COO)_2$ (f)

The obtained spectra revealed that the Cu content in all samples is more than 90 wt. %. It can be seen that Cl with a content of more than 0.1 wt. % is observed in the spectra. The Cl content can be explained by the presence of a corresponding precursor and the fact that all measurements were carried out on paper, which also contains Cl in its composition [50]. It is worth noting that when K_2CO_3 is used as a precipitator, K appears in the spectra with a content from 0.91 to 3.94 wt. %. This makes impractical the use of this precipitator in the synthesis of CC-NPs.

Thus, the analysis of the data obtained showed that $Cu(CH_3COO)_2$ is the optimal precursor and $(NH_4)_2CO_3$ is the optimal precipitator for the CC-NPs production. The optimal sample consists of spherical aggregates with a diameter of 0.6 to 1.0 μm formed by nanoparticles with a diameter of 30 to 80 nm. This sample has a phase composition of $Cu_2(OH)_2CO_3$.

Characterization of CC-NPs stabilized with biopolymers

At the first stage, a sample of CC-NPs stabilized with chitosan was examined. SEM micrograph showed that the sample consists of spherical aggregates with a diameter of 3 to 6 μm formed by nanoparticles from 30 to 85 nm (Figure 3a). It was found that the sample has a monomodal size distribution. The average hydrodynamic radius of the particles is 41 ± 40 nm (Figure 3e). XRF showed that the sample consists of Cu on 100.00% (Figure 3i). There are no impurities in the sample. Next, a sample of CC-NPs stabilized with methylcellulose was examined. SEM micrograph showed that the sample consists of spherical aggregates with a diameter of 2 to 10 μm formed by nanoparticles from 45 to 90 nm (Figure 3b). It is important to note that spherical aggregates have a developed surface – nanocrystallites with a length of 40 to 250 nm are observed on the surface. Analysis of the resulting DLS histogram showed that the sample has a monomodal size distribution. The average hydrodynamic radius of the particles is 10 ± 8 nm (Figure 3f). XFS showed that the sample contains 99.98% Cu and 0.02% Ca, which may be associated with contamination of chemical dishes [51] (Figure 3j).

Analysis of Figure 3c revealed that sample of CC-NPs stabilized with hydroxyethyl cellulose consists of spherical aggregates with a diameter of 2 to 8 μm formed by nanoparticles from 50 to 150 nm. DLS histogram showed that the sample has a monomodal size distribution. The average hydrodynamic radius of the particles is 10 ± 6 nm (Figure 3g). XFS showed that the sample consists of Cu on 100.00% (Figure 4k). There are no impurities in the sample.

SAM showed that the CC-NPs stabilized with hyaluronic acid consists of spherical aggregates with a diameter of 2 to 5 μm formed by nanoparticles from 30 to 100 nm (Figure 3d). Analysis of the DLS histogram showed that the sample has a monomodal size distribution. The average hydrodynamic radius of the particles is 22 ± 16 nm (Figure 3h). XFS showed that the sample consists of Cu on 99.99% (Figure 3k). However, traces of chlorine were found in the sample, which is associated with the precipitator used.

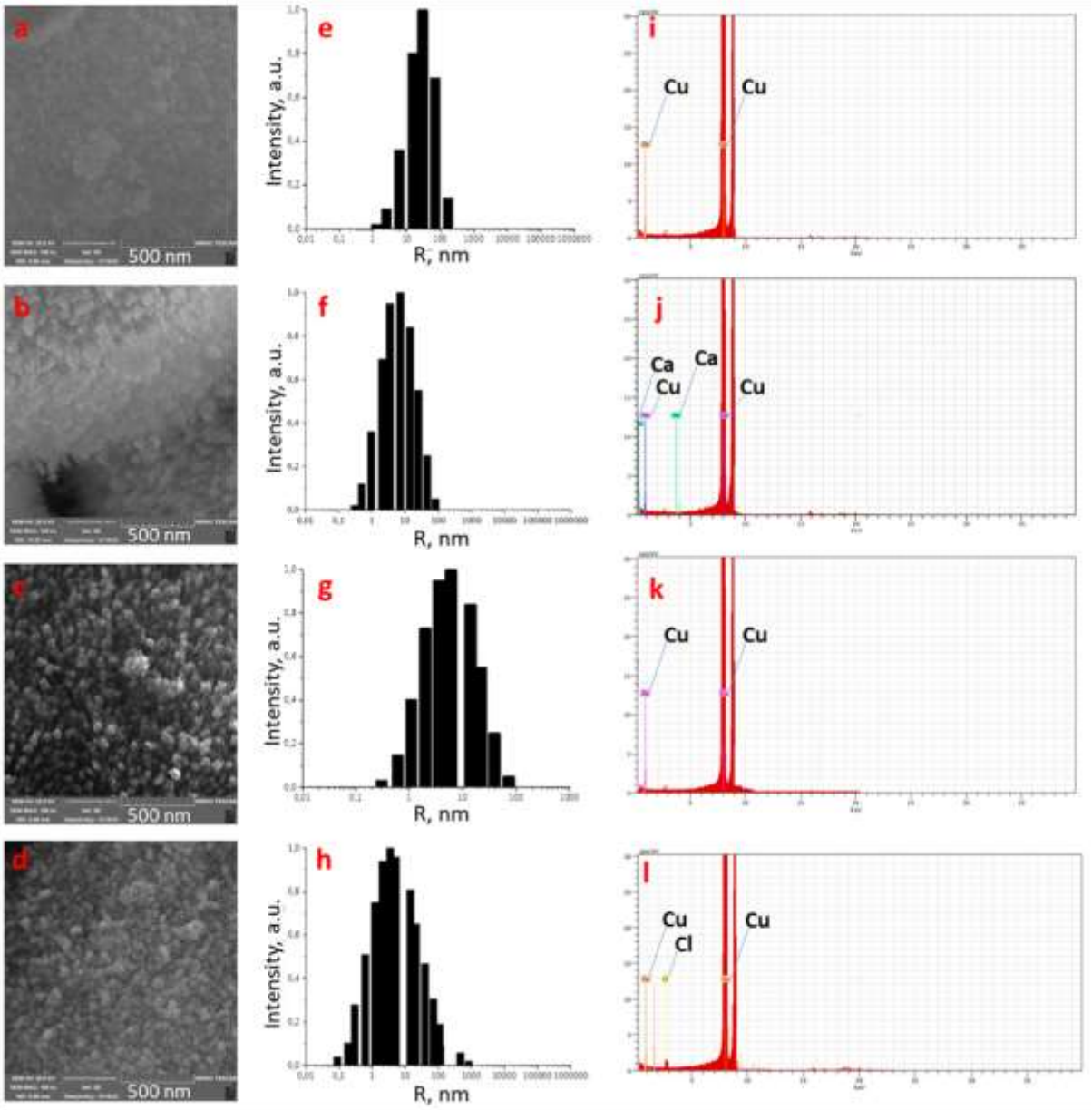

FIGURE 3 Characterization of complexes of CC-NPs with biopolymers: SEM-micrographs of CC-NPs stabilized with chitosan (a), methylcellulose (b), hydroxyethyl cellulose (c), hyaluronic acid (d); histograms of distribution of the hydrodynamic radius of CC-NPs stabilized with chitosan (e), methylcellulose (f), hydroxyethyl cellulose (g), hyaluronic acid (h) at magnification ×160,000; EDX spectra of samples of CC-NPs stabilized with chitosan (i), methylcellulose (j), and hydroxyethyl cellulose (k), hyaluronic acid (l)

Thus, the experimental data revealed that all considered biopolymers are appropriate for stabilization of CC-NPs. However, hyaluronic acid was selected as the optimal stabilizing agent because of the lowest aggregates size and more unified distribution of particles size. The next experiments were carried out with CC-NPs stabilized with hyaluronic acid.

CAM assay

The chicken embryo CAM system acts as a fully functional *in vivo* platform for studying tissue responses to various materials [52,55]. Taking into account the proven similarity of CAM tissue reactions with mammalian models, the primary biocompatibility and vascular

effects of CC-NPs stabilized with hyaluronic acid (CC-NPs+HA) were studied by applying samples to the surface of the CAM. Results of safety and biocompatibility assessment of CC-NPs+HA by CAM assay are demonstrated in Figure 4.

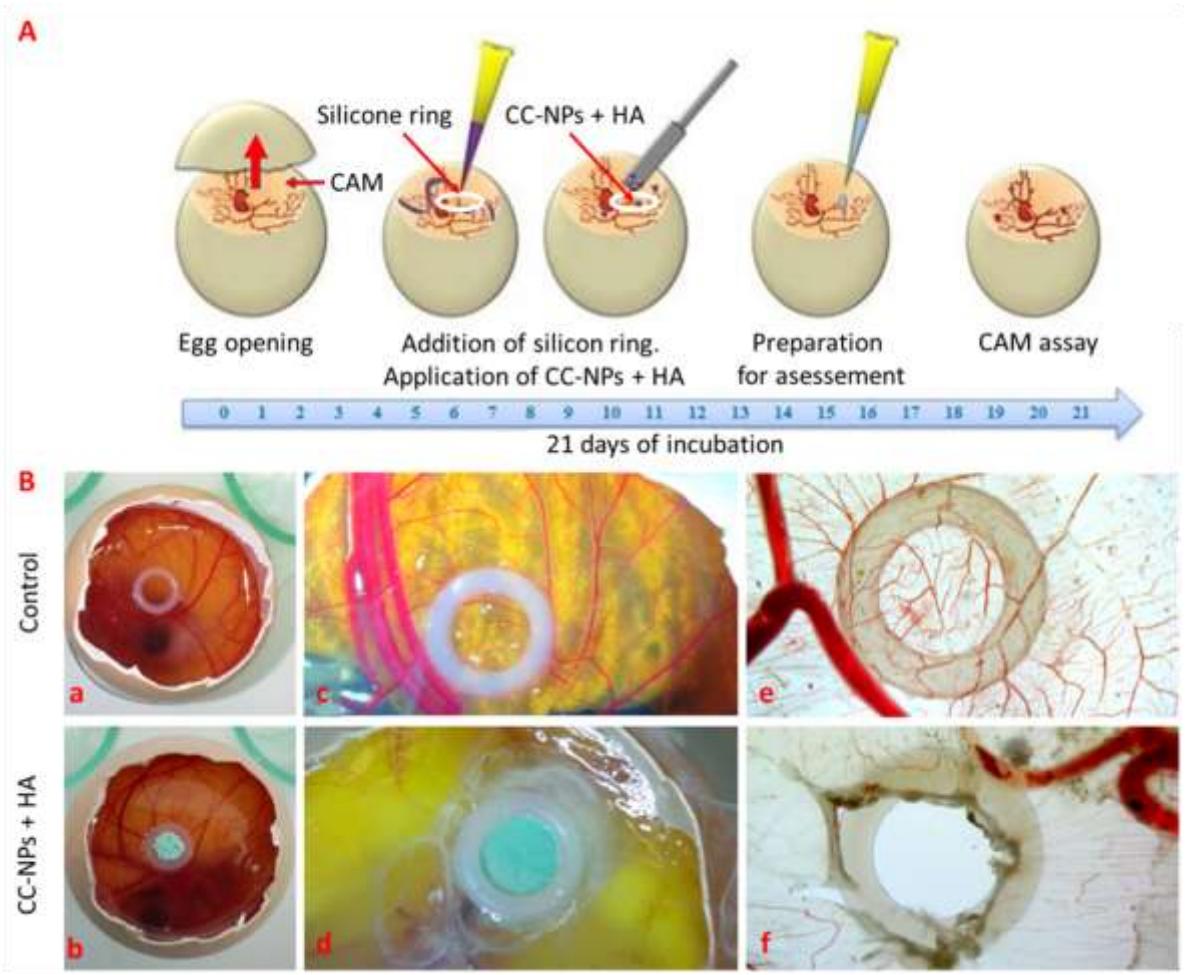

FIGURE 4 Results of safety and biocompatibility assessment of CC-NPs stabilized with hyaluronic acid (HA) by CAM assay. A – Scheme of CAM assay. B – Results of CAM assay: photographs of sterile silicone rings deposited on the surface of the CAM of chicken embryo on the 8th day of incubation (a – control, b – ring loaded with CC-NPs + HA); representative macroscopic photographs of CAM *in ovo* from the chorionic epithelium side 6 days after rings implantation (c – control, d – ring loaded with CC-NPs + HA); representative macroscopic photographs of CAM *ex ovo* from the allantois epithelium side 6 days after rings implantation (e – control, f – ring loaded with CC-NPs + HA)

In the control group, the percentage of embryo mortality was 10%, which corresponds to the reference range for the conditions of such experiments [56]. Interestingly, 10 mg of CC-NPs+HA powder caused 100% mortality of chicken embryos. The structural and surface properties of CC-NPs+HA powder allowed it to be absorbed on the surface of CAM while preserving the implantation site throughout the experiment [57]. For all groups, it was noted that a part of the implanted powders remained on the surface of the skin after 6 days of incubation. It is worth noting that the implantation of the CC-NPs+HA sample was accompanied by a clearly identified degradation of small and large vessels, which corresponds to results of Zhao *et al.* [58] and Näf *et al.* [59]. Thus, the results

of CAM assay indicate pronounced toxic changes in CAM under action of CC-NPs+HA. Surprisingly, despite the fact that Cu is an essential trace element, the developed CC-NPs+HA complex is characterized by low biocompatibility and high toxicity, which confirm finding of De Jong *et al.* [60].

Nanoscale Cu-containing compounds have a wide range of toxic effects with diverse clinical manifestations. Previously, it was found that CuO NPs, when added to the incubation medium, have an ambiguous effect on the mitochondrial function of fibroblast-like cells, which may be related to the biotic properties of Cu [61]. An important role in the mechanism of the toxic effect of copper is played by the ability of its ions to block SH groups of proteins, especially enzymes, and a high ability to increase the permeability of mitochondrial membranes [62]. Presumably, dissolved CC-NPs are capable of blocking Na^+ and K^+ ATPases [63]. A number of researchers pointed out that the Cu NPs toxicity to cells and tissues was caused by a significant increase in cell production of H^+ ions and an avalanche–like increase in the production of HCO_3^-, which exactly corresponds to CC-NPs [64,66]. Currently, there is no single picture that fully explains all the mechanisms of the toxic effects of nanoscale Cu-containing forms. In experiments, the negative effects realized were explained as the results of oxidant stress caused by the production of reactive oxygen species; direct damage to molecules by Cu NPs (RNA and DNA); variants of chemical interaction and biotransformation of macromolecules, including formation of chelates or metal substitution in metalloproteinases, as well as direct disruption of homeostasis in the cell as a result of the accumulation of excessive amounts of Cu^{2+} ions in cells [67,69].

Thus, this study needs further work on the synthesis and characterization of carbonates of other essential trace elements, such as Mn or Zn to achieve biocompatibility and low toxicity, which is necessary for medical use.

Conclusion

It was found that the type of precursor significantly affects the phase composition of samples of CC-NPs. When using $Cu(CH_3COO)_2$, the basic copper carbonate with the chemical formula $Cu_2(OH)_2CO_3$ is formed. When using $CuCl_2$, a system consisting of two crystalline phases of $Cu_2(OH)_2CO_3$ and $Cu_2(OH)_3Cl$ are formed. The analysis of the data obtained showed that $Cu(CH_3COO)_2$ is the optimal precursor and $(NH_4)_2CO_3$ is the optimal precipitator for the production of CC-NPs. The optimal sample consists of spherical aggregates with a diameter of 0.6 to 1.0 μm formed by nanoparticles with a diameter of 30 to 80 nm. This sample has a phase composition of $Cu_2(OH)_2CO_3$. Furthermore, it was found that all considered biopolymers are appropriate for stabilization of CC-NPs. However, hyaluronic acid was selected as the optimal stabilizing agent because CC-NPs stabilized with hyaluronic acid (CC-NPs+HA) have the lowest aggregates size and more unified distribution of particles size. Interestingly, 10 mg of CC-NPs+HA powder caused 100% mortality of chicken embryos. The structural and surface properties of CC-NPs+HA powder allowed it to be absorbed on the CAM surface while preserving the implantation site throughout the experiment. Notably, implantation of the CC-NPs+HA sample was accompanied by a clearly identified degradation of small and large vessels. Thus, the results of CAM assay indicate pronounced toxic changes in CAM under action of CC-NPs+HA. Surprisingly, despite the fact that Cu is an essential trace element, the developed CC-NPs+HA complex is characterized by low biocompatibility and high toxicity. This fact remains open for future discussion due to lack information on the use of CC-NPs in regenerative medicine. Thus, this study needs further experiments on the synthesis and characterization of carbonates of other essential trace elements, such as Mn and

Zn, to achieve biocompatibility and low toxicity, which is critically important for medical application. At the same time, revealed toxic effect of CC-NPs can be further investigated for study of their cytotoxic activity against different cancer cells, which open new way of their potential application.

Data availability

All raw data are available upon request from the corresponding author.

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Authors' Contributions

Andrey Blinov: Conceptualization, Methodology, Writing-original draft, Supervision; Andrey Nagdalian: Writing-review and editing, Validation, Project administration; Igor Rzhepakovsky: Methodology, Investigation, Writing-original draft; Zafar Rekhman: Investigation, Writing-original draft; Alina Askerova: Investigation, Writing-original draft; Vadim Agzamov: Visualization, Writing-original draft; Umedzhon Kayumov: Software, Writing-original draft; Dilshod Tairov: Resourses, Writing-original draft; Sobirzhon Ibrahimov: Formal analysis, Writing-original draft; Ilgiz Sayahov: Writing-original draft.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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