BBA - General Subjects 1869 (2025) 130777

Contents lists available at ScienceDirect

BBA - General Subjects

journal homepage: www.elsevier.com/locate/bbagen

Rod and spherical selenium nanoparticles: Physicochemical properties and effects on red blood cells and neutrophils

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ARTICLE INFO

Keywords:
Granulocytes
Erythrocytes
ROS
Nanorods
Nanospheres
Hemolysis
Cytotoxicity

ABSTRACT

The influence of selenium (Se) nanoparticles in the form of rods (SeNrs) and spheres (SeSps), synthesized by laser ablation, on the structural and functional properties of human blood erythrocytes and neutrophils was studied for anticancer activity *in vitro*. SeNrs and SeSps do not have cytotoxicity towards neutrophils and do not cause hemolysis. The elastic modulus and resistance of erythrocytes to HOCl-induced hemolysis increased after binding of Se nanoparticles to the plasma membrane. The interaction of Se nanoparticles with neutrophils is accompanied by their actin-dependent macropinocytosis, triggering intracellular signaling processes leading to the assembly and activation of NADPH oxidase. Comparative analysis of the effects of SeNrs and SeSps on cells showed that they have similar effects. This may be due to the fact that SeNrs interact with the cell surface with their end faces, and, therefore, have the same initial contact with the plasma membrane as SeSps. However, SeSps and SeNrs showed chronic cytotoxicity after 48 h incubation, indicating the need to find ways to reduce their toxicity further. Further use of Se nanoparticles in anisotropic form in biomedical research for the development of therapeutic agents seems promising.

1. Introduction

Selenium (Se) is a vital element in the human body [1]. Nanoparticles of Se have attracted the attention of researchers due to their high biological activities. These activities include adsorption capacity and low toxicity. An ability of Se nanoparticles to prevent the development of oxidative stress compared to Se in inorganic (selenite) or organic (selenocysteine, selenomethionine) forms was also described [2–5].

Se nanoparticles have antioxidant [6,7], antitumor [8,9], and antimicrobial [10,11], immunomodulatory [12], neuroprotective [13] and other properties. For example, in recent years, the antimicrobial activity of Se nanoparticles has been actively studied [14], which is due to a number of reasons. First, the resistance of pathogenic microorganisms to antibiotics is one of the main problems of global health. Of particular concern are bacteria with multiple drug resistance. Infections caused by these microorganisms affect the occurrence of acute and chronic diseases. In this regard, modern nanotechnology using antimicrobial peptides and nanoparticles represent a new strategy to combat pathogenic microorganisms, as an alternative to traditional antimicrobial drugs [15–18]. Nanoparticles can be used either as direct bactericidal agents [19,20] or as carriers that increase the effectiveness and delivery of known antibiotics [21–23]. Until recently, among the most studied nanomaterials exhibiting antimicrobial properties, nanoparticles of

Abbreviations: AFM, using atomic force microscopy; cyt b, cytochalasin; DPI, diphenyleneiodonium chloride; FS, front scatter; H_2DCFDA, dichlorodihydrofluorescein diacetate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NADPH, nicotinamide adenine dinucleotide phosphate; PI, propidium iodide; PMA, phorbol 12-myristate 13-acetate; ROS, reactive oxygen species; SEM, scanning electron microscopy; SeNrs, selenium nanorods; SeSps, selenium nanospheres; SS, side scatter; TEM, transmission electron microscopy.

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https://doi.org/10.1016/j.bbagen.2025.130777
Received 12 November 2024; Received in revised form 31 January 2025; Accepted 14 February 2025
Available online 19 February 2025

metals and metal oxides (silver, gold, copper, zinc *etc.*) were considered. However, a number of studies have noted the toxicity of metal nanoparticles [24] and the emergence of resistance to them in pathogenic microorganisms [25–27]. In this regard, in recent years, Se-based nanoparticles have attracted increasing interest due to their intermediate nature between metals and non-metals, which have a wide spectrum of activity against bacteria and fungi [28,29]. Their antimicrobial ability in some cases is due to an increase in the production of reactive oxygen species (ROS), leading to damage to cell membranes, inhibition of amino acid synthesis, and blocking of DNA replication [30].

Second, Se nanoparticles for anticancer activity is also currently being actively studied for anticancer activity [31]. It has been shown that the antitumor activity of Se nanoparticles is predominantly associated with the induction of receptor and mitochondrial signaling pathways of apoptosis, which leads to the death of cancer cells [31]. Also Se nanoparticles are promising systems for the delivery of various antitumor drugs, providing high efficiency and bioavailability of drugs to tumor cells [32].

The antimicrobial and antitumor properties of Se nanoparticles can be enhanced by their immunomodulatory action. Numerous studies have shown that Se nanoparticles influences the functioning of the immune system. For example, Se nanoparticles stimulate the production of cytokines by macrophages [33], modulate the activity of granulocytes [12,34], inhibit the growth and development of tumors by activating T-cells and tumor-associated macrophages [35]. The ability of Se nanoparticles to protect against immunosuppression during chemotherapy of oncological diseases has been shown [36]. Thus, Se nanoparticles can act as a universal therapeutic agent for the treatment of immune system dysfunctions in bacterial infections, oncology, and other diseases associated with immunosuppression, as well as in inflammatory processes [37–39].

It is worth noting that the vast majority of studies of Se nanoparticles have focused on spherical particles due to the ease of their synthesis. However, in recent years, special attention has been paid to obtaining anisotropic forms of Se nanoparticles in order to improve their physicochemical properties and biological activity. In this regard, nanoparticles in the form of rods or spheres may have different biodistribution, absorption by cells, toxicity and other properties [40]. At the same time, to date there are only a few studies of the effect of Se nanoparticles in the form of rods on biological systems [13]. In this regard, special attention should be paid to the study of the influence of Se nanoparticles on the structural and functional properties of blood cells, and especially leukocytes, on the activity of which the therapeutic effect of using Se nanoparticles will largely depend not only as drug carriers, but also in the development of agents with antimicrobial, antitumor and other activities based on them.

There are various methods for producing Se nanoparticles [11,41,42]. One of the most common methods for producing nanoparticles is the laser ablation method, in which the synthesized nanoparticles are usually homogeneous, have a narrow size distribution, and are virtually free of impurities. Another advantage of this method is that it allows one to obtain nanoparticles in anisotropic form, including rods, which have attracted much attention in recent years from the point of view of their use in biomedical research. Thus, the aim of this work was to study the effect of Se rod-shaped nanoparticles (SeNrs), synthesized by laser ablation, on the structural and functional properties of blood cells, and to compare their effects with those of Se nanoparticles in the form of spheres (SeSps).

2. Materials and methods

2.1. Reagents

The following reagents were used in the work: scopoletin, horseradish peroxidase, phorbol 12-myristate 13-acetate (PMA), SB 203580, histopac-1077, dextran T70, PP2, wortmannin, cytochalasin b (cyt b), amiloride, sodium hypochlorite (NaOCl), Percoll (Sigma-Aldrich, USA); 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA), propidium iodide (PI), Fura-2 AM, diphenyleneiodonium chloride (DPI), mounting medium Fluoromount (Molecular Probes, the Netherlands); NaCl, KCl, KH_2PO_4, $MgSO_4$, $CaCl_2$, (Reakhim, Russia), D-glucose, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), poly-L-lysine, paraformaldehyde, ethanol (Belmedpreparaty, Belarus).

The concentration of commercial NaOCl solutions was determined as OCl^- concentration measured spectrophotometrically at pH 12.0, taking the molar extinction coefficient (ε292) equal to 350 M^{-1} cm^{-1} [43]. Assuming that pKα for HOCl is ~7.5 and that at physiological pH about 50 % of HOCl exists in the protonated form, while the resting 50 % is in the dissociated form, hereinafter under the term "HOCl" is understood the HOCl/OCl^- mixture present in the test solution. The working solution of HOCl was prepared immediately before assay by dissolution of the commercial preparation in 10 mM Na-phosphate buffer pH 7.4 containing 140 mM NaCl.

2.2. Synthesis of selenium nanoparticles by laser ablation

Se nanoparticles were synthesized by laser ablation and fragmentation of a polycrystalline Se target (99.99 %) in various solvents. Spherical selenium nanoparticles were obtained using laser ablation and laser fragmentation techniques in aqueous solutions. The experiment used an experimental setup for laser ablation described in previous study [44]. The target was placed on the bottom of a 30 mL glass cuvette and filled with liquid. Deionized water (0.1 μS/cm) was used as the working fluid. The thickness of the liquid layer between the surface of the target and the liquid was 2–3 mm. A pulsed laser was used as a source of laser radiation. Nd:YAG laser NL300 (Ekspla, Vilnius, Lithuania). Pulse duration was 4 ns, repetition frequency was 1 kHz, wavelength was 532 nm, pulse energy was 2 mJ. Using a galvanomechanical scanner LScanH (Ateko-TM, Moscow, Russia) and an F-Theta lens with a focal length of 90 mm, the focused radiation beam was moved along the target surface. The spot size at the focus was 100 μm. The laser radiation energy density on the target surface was 25 J/cm^2. The beam movement speed was 3000 mm/s. The ablation time was approximately 25 min. At the next stage, a colloidal solution of selenium nanoparticles obtained as a result of laser ablation in the amount of 30 ml was placed in a glass cuvette with a transparent bottom and focused using an F-Theta objective at a distance of 1 cm from the bottom of the cuvette. The fragmentation time of the colloid of selenium nanoparticles was 60 min.

Elongated crystalline selenium nanoparticles (nanorods, SeNrs) were obtained using laser ablation and laser fragmentation techniques in organic solvents. The same methods and equipment were used in the process of obtaining crystalline selenium as in the case of spherical nanoparticles. Isopropyl alcohol was used as a working liquid in the experiments instead of water. The ablation time was 20 min, and laser fragmentation of the colloid was performed for 120 min. After obtaining elongated nanoparticles in an organic solvent, the liquid in the nanoparticle colloid was replaced with deionized water after several procedures of particle sedimentation in a centrifuge and solvent replacement. At the initial stage, the obtained selenium nanoparticles were sedimented in an LMC-4200 centrifuge (Biosan, Riga, Latvia). Sedimentation of nanoparticles in the centrifuge was carried out for 15 min at a speed of 4200 rpm, a rotor radius of 0.12 m, and a relative centrifugal force of ~2400 *g*. After centrifugation, the solvent was replaced with carbon tetrachloride (CCl_4). Then, the colloid was exposed to ultrasound in an ultrasonic bath ($p = 20$ W) for 10 min. The colloidal fluid was then successively replaced with dimethyl sulfoxide (C_2H_6OS), chloroform ($CHCl_3$), and acetone (C_3H_6O), using the described centrifugation and ultrasonication procedures at each step. After each colloidal centrifugation procedure, acetone was replaced with deionized water.