

Effect of Upconversion Nanoparticles on Erythrocytes

Anna A. Doronkina^{1*}, Alexander B. Pravdin¹, Artyom M. Mylnikov², Vyacheslav I. Kochubey¹, and Irina Yu. Yanina¹

¹ Saratov State University, 83 Astrakhanskaya str., Saratov 410012, Russian Federation

² Saratov State Medical University named after V.I. Razumovsky (Razumovsky University), 112 B. Kazachya str., Saratov 410012, Russian Federation

*e-mail: annador95@bk.ru

Abstract. We have shown experimentally that the interaction of upconversion nanoparticles with erythrocytes leads to changes in cell size and shape. One of the reasons for this is thought to be a change in the viscosity of erythrocyte membrane. Using a fluorescent probe, pyrene, we have shown that this nanoparticle-induced change in membrane viscosity depends on the nature of the nanoparticle shell (cover). © 2024 Journal of Biomedical Photonics & Engineering.

Keywords: erythrocytes; nanoparticles; morphology; membrane viscosity.

Paper #9059 received 16 Jan 2024; revised manuscript received 8 May 2024; accepted for publication 8 May 2024; published online 29 Jun 2024. [doi: 10.18287/JBPE24.10.020309](https://doi.org/10.18287/JBPE24.10.020309).

1 Introduction

The widespread prevalence of oncological diseases requires the search for and development of various treatment options. Chemotherapy, radiotherapy, surgical treatment, and photodynamic therapy are among the existing ones [1]. Photodynamic therapy is based on the generation of reactive oxygen species using visible or near infrared (IR) radiation in combination with a photosensitizer [2].

In photodynamic therapy, the main problem is the delivery of the photosensitizer to the area to be treated. There is also a need for direct measurement of thermal fields within the tissue in the immediate vicinity of the treatment area, as intensive radiant heating of the tissue can cause negative effects.

One way of solving this problem is to use of nanoparticles, which can act as both photosensitizer carriers and nanothermometers at the same time. The use of nanoparticles in medicine plays an important role in the development of drugs and their delivery routes. One of the main advantages of nanoparticles is their ability to deliver drugs directly to the desired cells and organs of the body. Because of their size, nanoparticles can penetrate biological barriers such as cell membranes, allowing targeted drug delivery. In addition, nanoparticles can be designed so that their luminescence parameters depend on the ambient temperature, with the most promising nanothermometers being ZnCdS [3], CuInS₂ [4], and NaYF₄Er,Yb [5, 6].

Today's technologies allow the synthesis of nanoparticles of various sizes from ~ 5 to 100 nm raising the question of their safe application in medicine. The application of anticancer drugs usually starts with an injection into the bloodstream to be transported to the tumour site. Such nanoparticles can then penetrate and accumulate in various organs: brain, liver, kidney, lung, and spleen [7].

There are a number of requirements for nanoparticles used in medicine, including biocompatibility, safety of use, and low toxicity being among them.

When introduced into the bloodstream, nanoparticles can attach to or penetrate the membrane blood cells. The most common blood cells are red blood cells. They represent a natural carrier for various bioactive substances and contrast agents due to their unique properties such as biocompatibility, membrane flexibility [8].

Nanoparticles have previously been shown to interact with erythrocytes and alter cell structure and function [3, 4]. Nanoparticles interacting with erythrocyte membranes alter the microviscosity and mechanical stresses of the lipid bilayer [3, 4]. As a result, the changes that occur in the membranes affect some functions of erythrocytes, such as the ability of cells to pass through microcapillaries [9].

Therefore, when planning a therapeutic application of nanoparticles, it is necessary to be sure that the interaction of nanoparticles with the erythrocyte

membrane does not lead to fatal morphological and physiological consequences.

The aim of this work is to observe the change of erythrocyte membrane properties under the action of NaYF₄ nanoparticles with different surface conditions.

2 Materials and Methods

The synthesis of nanoparticles was carried out according to the following protocol.

Solutions 1 M of Y(NO₃)₃ (3 mL), Yb(NO₃)₃ (0.6 mL), and Er(NO₃)₃ (0.04 mL) were mixed. Then solutions of sodium citrate (Na₃C₆H₅O₇, 1 M, 4 mL) and citric acid (C₆H₈O₇, 3.66M, 50 mL) were added successively. At the resulting concentration of citrate groups, the rare earth (RE) citrate complexes are water soluble, so the solution was stirred until completely clear. Then 38 mL of 2 M sodium fluoride (NaF) solution was added and the resulting gel-like solution was stirred for 30 min.

As a result, the following molar ratios were obtained: Y:Yb:Er = 1:0.2:0.013, RE: fluorine = 1:19, RE:citrate = 1:51.4. Near stoichiometry was achieved for sodium.

The solution was poured into a 100 mL reactor, hermetically sealed, and heated at 180 °C for 18 h. After synthesis, the reactor was allowed to cool naturally. The synthesized nanoparticles were washed three times with distilled water with intermediate precipitation by centrifugation (centrifugation: Eppendorf 5804 (Eppendorf Germany), operating mode 6000 rpm = 4200 rcf, 15 min, T = 22 °C) and then air dried. To partially replace the citrate ions with BF⁻⁴, the nanoparticles were mixed with a solution of NaBF₄ in dimethylformamide at a ratio of 60 mg NaBF₄ to 25 mg of nanoparticles. The mixture was stirred for 20 h using a PTR-25 mini-rotator (Russia). The nanoparticles were then washed three times with ethanol and dried.

To coat the surface of the nanoparticles with SiO₂ shell, 80 mg of synthesised NaYF₄:Er,Yb particles were added to 1 mL of isopropyl alcohol and treated with ultrasound. Distilled water (7 mL), 13% ammonia solution NH₃(aq.) (1 mL), suspension of NaYF₄:Er,Yb particles in alcohol (1 mL), and tetraethoxysilane (TEOS) (80 µL) were added to of isopropyl alcohol (31 mL). The resulting mixture was stirred for 2.5 h. The white precipitate was then separated by centrifugation, washed three times in water, and dried at 70 °C for 20 h.

To coat the nanoparticles with an albumin shell, 1 mL of 2 mg/mL solution of commercial human serum albumin (HSA) (Sigma-Aldrich, USA) conjugated with the photodynamic dye Cy₃ was added to 10 mL of nanoparticle suspension (40 mg) in 0.05 M phosphate buffer at pH 6.5. The suspension was incubated for 0.5 h with vigorous stirring using a magnetic stirrer. Then 30 µL of 3% hydrogen peroxide solution was added and stirred for a further 0.5 h. The nanoparticles were precipitated by centrifugation (centrifugation: Eppendorf 5804 (Eppendorf, Germany), operating mode 6000 rpm = 4200 rcf, 10 min, T = 22 °C). The precipitate was resuspended in washing buffer and then precipitated

again. The procedure was repeated three times, after which the nanoparticles were suspended in 10 mL of buffer solution.

The attachment of folic acid to the albumin-coated nanoparticles was carried out by standard EDC/NHS covalent binding. For this purpose, 40 mg of folic acid (C₁₉H₁₉N₇O₆), 14 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 10.4 mg of N-hydroxysuccinimide (NHS) were dissolved in 2.8 mL of dimethyl sulfoxide (DMSO). The solution was stirred for 30 min and then added dropwise to the nanoparticle suspension under vigorous stirring. The resulting mixture was then stirred for 3 h at room temperature. The nanoparticles were precipitated by centrifugation, washed three times with saline solution, and then resuspended in this solution at the concentration of 4 mg/mL. The resulting nanoparticle suspension, which acquired a yellow colour characteristic of folic acid was stored in the dark at 4–6 °C. The presence of folic acid on the surface of the nanoparticles was monitored by IR spectroscopy.

The interaction of nanoparticles with erythrocyte membranes was studied in white Wistar rats. All the work with experimental animals was performed in accordance with the research protocol, which does not contradict the Geneva Convention of 1985 on “International Principles of Biomedical Research Using Animals” and the Helsinki Declaration of 2000 on the humane treatment of animals, as well as in accordance with the provisions of the Order № 755 of the Ministry of Health of the USSR dated August 12, 1977. The experiments were carried out on the basis of the Collective Use Centre (CUC) of the Research Institute of Basic and Clinical Urology of V. I. Razumovsky Saratov State Medical University of the Ministry of Health of the Russian Federation.

Experiments were performed in rats with liver cancer and healthy controls. White laboratory rats, weighing 300 ± 50 g, were implanted subcutaneously in the region of the scapula with 0.5 ml of a 25% tumour (alveolar liver cancer strain PC-1) suspension in Hanks' solution. The experiment was started when the tumours reached a volume of 1 cm³. A suspension of NaYF₄:Er,Yb nanoparticles with different shells (coated 1) SiO₂, 2) human serum albumin (HSA), 3) HSA and folic acid, 4) HSA-folic acid-photodynamic dye Cy₃ complex) was injected intravenously, and blood samples were taken one day after nanoparticle injection.

As a control, blood was taken from healthy and cancerous rats that had not been injected with nanoparticles.

Heparin-stabilized rat blood samples obtained from experimental or control animals were diluted with twice the volume of physiological solution and washed in a centrifuge for 5 min at 3000 rpm (centrifugation: ОПН-8УХЖ4.2 (Dastan, Kyrgyzstan), operating mode 3000 rpm = 930 rcf, T = 22 °C). After centrifugation, 0.02 ml of erythrocyte mass was removed from the bottom of the tube and added to a tube containing 1.8 ml of saline. Then, 0.5 ml of physiological solution was added to 0.5 ml of the previously obtained erythrocyte

suspension to obtain the working concentration of erythrocytes.

Observation of the changes in shape and size of erythrocytes in cancer control and after interaction with the nanoparticle suspensions was performed on the erythrocyte suspensions described above using a LOMO Mikmed-2 microscope. This microscope allows qualitative and quantitative assessment of the shape and internal structure of transparent biological microobjects. A Videoscan-415/P-USB camera with a 776×582 matrix was used to obtain the micrographs.

The recorded images were processed using the ImageJ software.

The following indices were used to characterize the size and shape of blood cells.

1) Area – the area of the horizontal projection of the erythrocyte,

2) Feret – maximum Feret diameter. It corresponds to the smallest diameter of the circle circumscribed around the object, *i.e.* the greatest length of the object. Instrumentally, this length is obtained by measuring the length with a caliper, and, *e.g.*, in pomology, for example, it is called the largest linear diameter, the longitudinal diameter. In cell microscopy, it is the diameter of the circle circumscribed around a cell of any shape.

3) MinFeret – minimal Feret diameter. It corresponds to the smaller diameter of the circle inscribed in the object. It can be obtained by measuring the transverse diameter of the object with a caliper.

We proposed to introduce a coefficient equal to the ratio of the diameters of the inscribed and circumscribed circles $k = \text{MinFeret}/\text{Feret}$. This coefficient indicates the degree of shape change of erythrocytes. When the shape of the erythrocytes changes to a stellate shape, *i.e.* the echinocytes are formed, the size of the circumscribed circle increases, while the diameter of the inscribed circle decreases, in which case the ratio of the diameters characterizes the degree of shape change.

In order to analyse the reasons for changes in erythrocyte shape, experiments were proposed to assess the structural and dynamic state of cell membranes using a fluorescent probe.

To analyse the microviscosity of erythrocyte membranes, we used a fluorescent probe, pyrene. In membranes, pyrene can be incorporated into the lipid bilayer. Upon photoexcitation, pyrene monomers fluoresce in the range of 370–400 nm. Also upon photoexcitation, pyrene monomers are able to form excimers with emission maximum at 470 nm. The ratio of the fluorescence intensity of excimers and pyrene monomers, *i.e.* the pyrene excimerization coefficient (F_e/F_m), depends on the lateral diffusion rate of the probe in the bilipid layer. The higher is the diffusion rate of the probe, the lower is the microviscosity of the lipid bilayer. The F_e/F_m ratio is therefore inversely proportional to the lipid microviscosity. Fluorescence spectra at 282 nm excitation are used to determine the microviscosity of ring lipids (in the region of protein-lipid contacts) and at 334 nm to determine the viscosity of the lipid bilayer [10, 11].

The polarity of the pyrene microenvironment was estimated by the ratio of the intensity of the first maximum of the pyrene fluorescence spectrum (I_1) to the intensity of the third maximum (I_3) [10].

To determine erythrocyte membrane microviscosity, a heparin – stabilized blood sample taken from a laboratory animal was centrifuged at 3000 rpm (centrifugation: ОПН-8YXJ14.2 (Dastan, Kyrgyzstan), operating mode 3000 rpm = 930 rcf, $T = 22^\circ\text{C}$) for 10 min. Plasma and upper layer of leukocytes were carefully pipetted and removed. The erythrocytes were washed three times with an isotonic solution containing 0.145 mol/L NaCl in 0.02 mol/L Tris-HCl buffer (pH 7.6 at 20°C), the cells were precipitated each washing round in the same mode (3000 rpm for 10 min). Erythrocyte membranes were obtained by hypoosmotic hemolysis using 0.02 mol/L Tris-HCl buffer (pH 7.6 at 20°C), a volume of washed erythrocytes was rapidly and vigorously mixed with a 2-fold volume of haemolytic solution and incubated for 15 min. Then the mixture was centrifuged at 8000 rpm (centrifugation: ОПН-8YXJ14.2 (Dastan, Kyrgyzstan), operating mode 8000 rpm = 6600 rcf, $T = 22^\circ\text{C}$) for 15 min. The supernatant was removed, and the membrane precipitate was washed with hypotonic solution of Tris-NS buffer (pH 7.6 at 20°C) until the haemolysate was washed away, each time precipitating the membranes in the same mode [12].

Freshly prepared membrane suspensions were used for fluorescence measurements. Membrane suspension (2 mL) was titrated with 20 μL of 8 $\mu\text{mol/L}$ pyrene alcoholic solution, followed by recording of fluorescence spectra at excitation wavelengths of 282 and 334 nm. The luminescence intensity of pyrene monomers and excimers was determined at 393 and 470 nm (the band maxima), respectively.

3 Results and Discussions

To assess the changes in erythrocyte shape and aggregate formation in pathology and after interaction with nanoparticles, we visually analysed the preparations (erythrocyte suspensions) of collected blood samples using optical micrographs.

Figs. 1a and 1b show microphotographs of erythrocytes from healthy and diseased (liver cancer) rats.

The formation of echinocytes is clearly observed in the blood sample from the diseased animal. It is known that various diseases affect the morphology and aggregation of erythrocytes [13, 14]. The formation of echinocytes (erythrocytes with an altered shape) in liver cancer leads to the decrease in the k coefficient compared to the erythrocytes from healthy rats, while the cell area increases. The corresponding data are shown in the graph (Fig. 2). The observed decrease in the coefficient k depends on the shape of the echinocytes: the larger and sharper are the rays, the larger is the size of the circle circumscribing the cells and the smaller is the inner diameter.

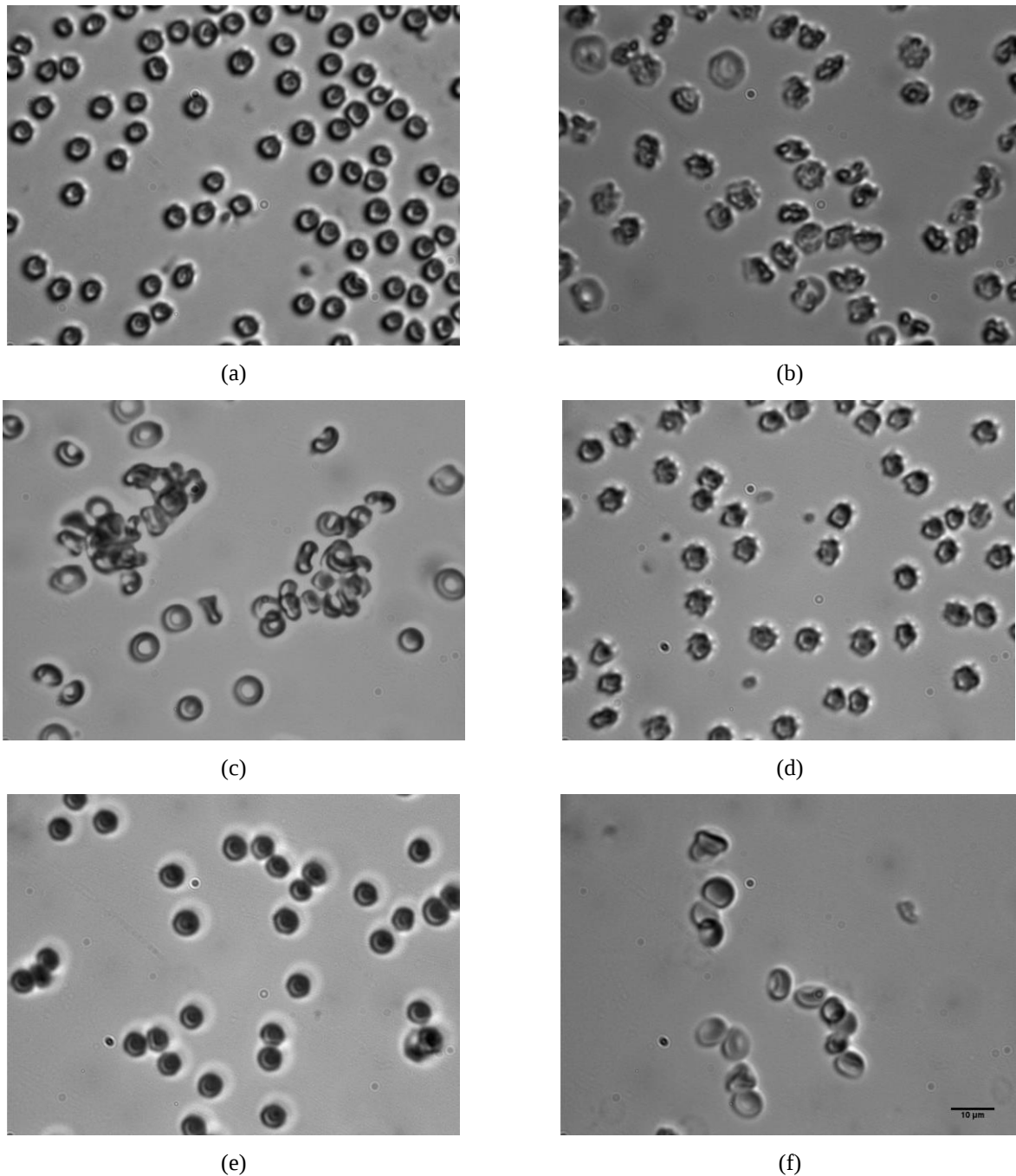


Fig. 1 Microscopic images of the rat erythrocytes samples: (a) healthy animal; (b) diseased animal (DA); (c) (DA) with $\text{NaYF}_4+\text{SiO}_2$ nanoparticles administered; (d) (DA) with NaYF_4+HSA nanoparticles administered; (e) (DA) with $\text{NaYF}_4+\text{HSA}+\text{FA}+\text{folic acid}$ nanoparticles administered; (f) (DA) with $\text{NaYF}_4+\text{HSA}+\text{folic acid}+\text{Cy}_3$ nanoparticles administered.

When nanoparticles are injected into the bloodstream of diseased rats, a change in erythrocyte morphology is also observed. However, the coefficient k increases, *i.e.* the formation of echinocytes decreases. The change in size and shape is the result of interaction between erythrocyte and nanoparticles, which is due to the fact that the nanoparticles are embedded in the outer or inner layer of the membrane [15]. In our case, the nanoparticles are embedded in the outer layer of the membrane, due to the size of the nanoparticles ~ 80 nm, which leads to subsequent membrane stretching and cell shape change.

Calculated from micrographs, the mean area of erythrocytes with NaYF_4 nanoparticles coated with SiO_2 is larger than that of the healthy group ($37 \pm 2.5 \mu\text{m}^2$), but coincides within the error with the control group (diseased rats) ($61 \pm 2.5 \mu\text{m}^2$ and $51 \pm 2 \mu\text{m}^2$, respectively). The coefficient k is comparable to the values of erythrocytes from diseased rats, the change in the morphology of erythrocytes: formation of protuberances, stellate shape, *i.e.* formation of echinocytes is observed in Fig. 1c.

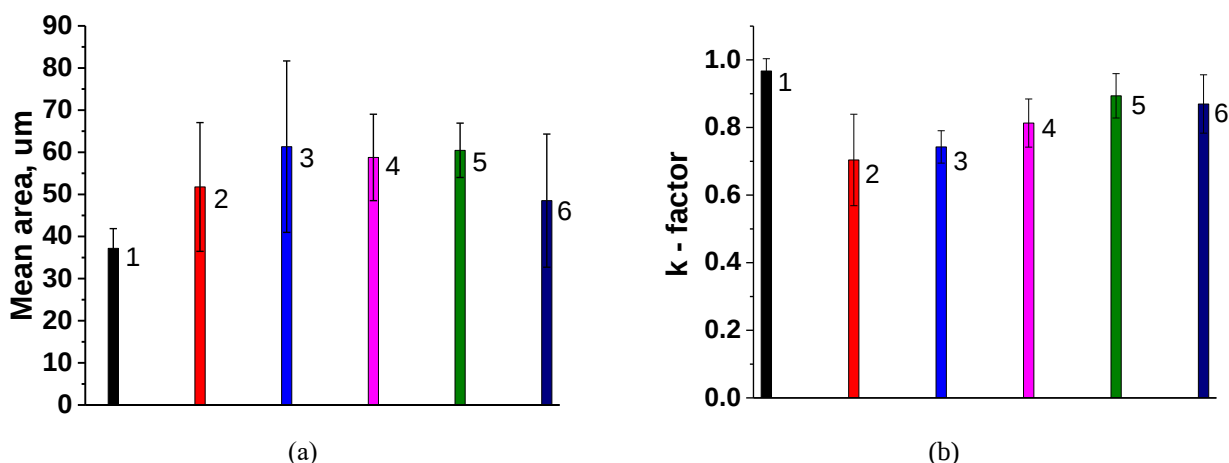


Fig. 2 Results of measurements on microscopic images: (a) mean cell area and (b) mean k-factor of erythrocytes, where erythrocyte preparations: 1) healthy animal; 2) diseased animal (DA); 3) (DA) with $\text{NaYF}_4+\text{SiO}_2$ nanoparticles administered; 4) (DA) with NaYF_4+HSA nanoparticles administered; 5) (DA) with $\text{NaYF}_4+\text{HSA}+\text{FA}+\text{folic acid}$ nanoparticles administered; 6) (DA) with $\text{NaYF}_4+\text{HSA}+\text{folic acid}+\text{Cy}_3$ nanoparticles administered. The calculation is performed for 70 for each of 20 rats.

In addition to the change in shape, strong aggregation is also observed as a result of the interaction, which is a negative indicator for the use of such a coating, as the formation of strong aggregates can be the cause of capillary blockage.

To overcome the problem of aggregation associated with the use of NaYF_4 nanoparticles, we tried an HSA shell for the nanoparticles. It is known [16] that positively charged nanoparticles have haemotoxic effects superior to those of analogous anionic nanoparticles. According to our measurements, the surface potential of NaYF_4 nanoparticles coated with SiO_2 is -0.77 mV, while it is -12.3 eV for nanoparticles with HSA shell. Functionalization of the nanoparticle surface with negatively charged groups cancels the aggregation effect of these nanoparticles on erythrocytes [17].

Since albumin is a blood component (endogenous protein, 35–50 g/L in human serum), it does not show immunogenicity, so such a shell ensures the biocompatibility of the nanoparticles with body cells [18].

After injection of NaYF_4+HSA , as expected, no aggregation was detected, also it was so in the samples with $\text{NaYF}_4+\text{HSA}+\text{FA}$ and $\text{NaYF}_4+\text{HSA}+\text{FA}+\text{Cy}_3$. However, in the case of NaYF_4+HSA , the changes in the shape of the erythrocytes were still observed (Fig. 1d), as evidenced by the values of the coefficient k which increased slightly (Fig. 2b).

However, when $\text{NaYF}_4+\text{HSA}+\text{FA}$ or $\text{NaYF}_4+\text{HSA}+\text{FA}+\text{Cy}_3$ nanoparticles were introduced into the bloodstream, the change in the shape of erythrocytes (in relation to erythrocytes from healthy animals without nanoparticles) was much less pronounced than in erythrocytes from diseased rats without nanoparticles. The values of the coefficient k of the erythrocytes with nanoparticles coated with albumin, folic acid, and dye are higher than those of the erythrocytes from the diseased rat without nanoparticles

and approach the values of the healthy rat cells without nanoparticles, Fig. 2b.

When nanoparticles coated with albumin-based shells were administered into the bloodstream, the values of the mean area of erythrocytes in the microimages were close within the error (Fig. 2a): for NaYF_4+HSA the mean was $58 \pm 1 \mu\text{m}^2$; for $\text{NaYF}_4+\text{HSA}+\text{FA}$ – $60 \pm 2.5 \mu\text{m}^2$; but for $\text{NaYF}_4+\text{HSA}+\text{FA}+\text{Cy}_3$ – $48 \pm 1.5 \mu\text{m}^2$. The values are close to the area values of healthy rat erythrocytes.

In patients with malignant tumours of various localization (lung cancer, gastric cancer, liver cancer), alterations in erythrocyte membranes, lipid bilayer disorders, and changes in the viscosity of intermolecular protein-lipid and lipid-lipid interactions are observed [19]. The interaction of nanoparticles with erythrocyte membrane can also lead to its structural changes in the membrane due to the redistribution of lipids, which is reflected in the microviscosity of the membrane.

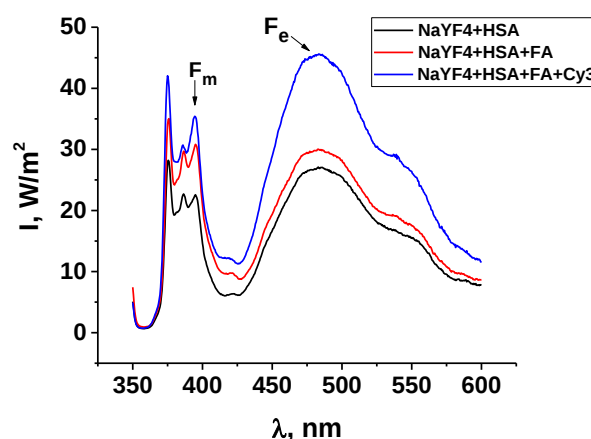


Fig. 3 Fluorescence spectra of pyrene in erythrocyte membranes with different nanoparticles.

Table 1 Spectral characteristics of pyrene fluorescence in erythrocyte membranes with different nanoparticles.

Membranes	F_e/F_m $\lambda_{ex} = 282nm$	I_1/I_3 $\lambda_{ex} = 282nm$	F_e/F_m $\lambda_{ex} = 334nm$	I_1/I_3 $\lambda_{ex} = 334nm$	$(F_0-F)/F_0$
Healthy animal erythrocytes without nanoparticles	1.36 ± 0.07	1.25 ± 0.17	0.57 ± 0.3	1.43 ± 0.16	0.75 ± 0.28
Diseased animal erythrocytes without nanoparticles	2.49 ± 0.42	1.24 ± 0.07	0.92 ± 0.16	1.43 ± 0.07	0.85 ± 0.12
Diseased animal erythrocytes with NaYF ₄ +SiO ₂ nanoparticles	1.3 ± 0.37	1.52 ± 0.13	0.53 ± 0.23	1.64 ± 0.07	–
Diseased animal erythrocytes with NaYF ₄ +HSA nanoparticles	1.57 ± 0.28	1.23 ± 0.04	1.1 ± 0.19	1.22 ± 0.02	0.5 ± 0.033
Diseased animal erythrocytes with NaYF ₄ +HSA+FA nanoparticles	1.25 ± 0.06	1.17 ± 0.03	1.07 ± 0.06	1.2 ± 0.036	0.46 ± 0.0
Diseased animal erythrocytes with NaYF ₄ +HSA+FA+Cy ₃ nanoparticles	2.14 ± 0.02	1.3 ± 0.016	1.33	1.41 ± 0.07	0.56546

Based on the obtained fluorescence spectra of pyrene incorporated into the erythrocyte membrane (Fig. 3), we calculated the degree of excimerization and estimated the polarity of the microenvironment of the pyrene molecules. The results are summarized in Table 1.

Table 1 shows that the differences in the values of F_e/F_m ($\lambda_{ex} = 282nm$) and F_e/F_m ($\lambda_{ex} = 334nm$) ratios for the erythrocyte membranes of diseased and healthy rats were 83% and 61%, respectively. This indicates a strong change in the viscosity of erythrocyte membranes from diseased rats, which affects the change in erythrocyte morphology (shape), as observed in Fig.1b.

When nanoparticles of different surface types are introduced into the bloodstream of diseased rats, the values of the F_e/F_m coefficients ($\lambda_{ex} = 282nm$), measured in the spectra obtained on erythrocyte membrane preparations, decrease by up to 50% compared to the coefficient values for diseased rats without nanoparticle introduction, but are close to the values for erythrocyte membranes from healthy rats.

At the same time, the values of F_e/F_m ($\lambda_{ex} = 334nm$) for NaYF₄+HSA nanoparticles increase by $20 \pm 4\%$ in relation to the values of the coefficient for the diseased rat without introduction of nanoparticles. However, in membrane samples with NaYF₄+HSA+FA+Cy₃ the coefficient increases by 40%, and with NaYF₄+SiO₂ it decreases by 57%. The decrease in the value of the polarity coefficient around the pyrene probe indicates an increase in membrane viscosity and hydrophobicity. Disease has no effect on polarity. Samples with NaYF₄+SiO₂ show the strongest polarity changes at I_1/I_3 ($\lambda_{ex} = 334nm$) and at I_1/I_3 ($\lambda_{ex} = 334nm$).

In the samples with NaYF₄+HSA and NaYF₄+HSA+FA, I_1/I_3 values ($\lambda_{ex} = 334nm$) are 15% lower than in membranes from diseased rats without

nanoparticles, and there is almost no change in I_1/I_3 ($\lambda_{ex} = 282nm$).

Thus, the changes in polarity upon nanoparticle introduction mainly affect lipid bilayer compounds, while ring lipids experience only an upward trend in lipid polarity.

Pyrene eximerization ratio data suggest that nanoparticles affect the microviscosity of erythrocyte membranes:

NaYF₄+SiO₂ promotes an increase in microviscosity (decrease in fluidity) of both total and annular lipids of erythrocyte membranes, as the degree of pyrene eximerization is inversely related to the microviscosity of the lipid phase. The increase in polarity may be due to the hydrophobicity of the NaYF₄+SiO₂ nanoparticle.

NaYF₄+HSA and NaYF₄+HSA+FA contribute to a decrease in microviscosity (increase in fluidity) in the ring lipids of erythrocyte membranes, but contribute to an increase in microviscosity (decrease in fluidity) in total lipids. There is virtually no change in the lipid-protein region and a decrease in polarity in the lipid-lipid region, which may be due to redistribution of pyrene binding to lipids.

NaYF₄+HSA+FA+Cy₃ contributes to a very small decrease in the microviscosity (increase in fluidity) of the ring lipids of erythrocyte membranes, but contributes to an increase in the microviscosity (decrease in fluidity) of the total lipids. The increase in polarity in the lipid-protein region, while there is virtually no change in the lipid-lipid region, may be due to pyrene binding to proteins.

All of the above indicates that due to the interaction of nanoparticles with erythrocyte membranes, there is a decrease in the polarity of the pyrene microenvironment in the lipid region of erythrocyte membranes, which

reduces the fluidity of the lipid bilayer and increases its viscosity. As a result, the membrane becomes harder, is able to keep its shape and does not change, which coincides with the data of the k coefficient calculation and is observed on microphotographs (Fig. 1).

The pyrene probe fluorescence parameter $(F_0-F)/F_0$, which characterizes the efficiency of electron excitation energy transfer from tryptophan residues of membrane proteins to pyrene, shows a significant decrease in the transfer efficiency. The decrease in the parameter $(F_0-F)/F_0$ may indicate structural rearrangements in erythrocyte membrane proteins associated with a decrease in the degree of immersion of proteins in the lipid bilayer or the formation of protein aggregates [20]. The decrease in the degree of immersion of proteins in the membrane lipid bilayer may be associated with an increase in membrane viscosity or changes in the structure of membrane proteins due to their interaction with nanoparticles.

4 Conclusions

The interaction of NaYF₄+SiO₂ nanoparticles with the erythrocyte membrane is accompanied by a change in cell shape and increased formation of aggregates, indicating a change in the properties of the cell

membrane surface. These nanoparticles also affect the state of erythrocytes: both cell size and membrane viscosity.

Nanoparticles coated with albumin without folic acid and albumin with folic acid cause changes in the viscosity of the protein-lipid contact zones of the membrane and the k coefficient tends towards the value for the healthy rat erythrocytes. The nanoparticles coated with albumin, folic acid, and dye have almost no effect on erythrocytes, both on cell size and membrane viscosity.

The results suggest that the HSA+FA+Cy₃ surface complex of NaYF₄ nanoparticles has better biocompatibility with erythrocytes and that the nanoparticles are embedded in the membrane so that they can be used in photodynamic therapy.

Acknowledgment

The study was supported by Russian Science Foundation, grant No. 21-72-10057.

Disclosures

The authors declare no conflict of interest.

References

1. K. Rhew, Y.-J. Chae, and J.-E. Chang, "Progress and recent trends in photodynamic therapy with nanoparticles," *Journal of Pharmaceutical Investigation* 52(5), 587–599 (2022).
2. M. Salimi, S. Mosca, B. Gardner, F. Palombo, P. Matousek, and N. Stone, "Nanoparticle-mediated photothermal therapy limitation in clinical applications regarding pain management," *Nanomaterials* 12(6), 922 (2022).
3. V. Nadtochenko, D. Cherepanov, S. Kochev, M. Motyakin, A. Kostrov, A. Golub, O. Antonova, Y. Kabachii, and S. Rtimi, "Structural and optical properties of Mn²⁺-doped ZnCdS/ZnS core/shell quantum dots: New insights in Mn²⁺ localization for higher luminescence sensing," *Journal of Photochemistry and Photobiology A: Chemistry* 429, 113946 (2022).
4. R. Marin, A. Vivian, A. Skripka, A. Migliori, V. Morandi, F. Enrichi, F. Vetrone, P. Ceroni, C. Aprile, and P. Canton, "Mercaptosilane-passivated CuInS₂ quantum dots for luminescence thermometry and luminescent labels," *ACS Applied Nano Materials* 2(4), 2426–2436 (2019).
5. A. A. Skaptsov, S. O. Ustaltkov, A. H. M. Mohammed, A. M. Zakharevich, A. A. Kozyrev, E. A. Sagaidachnaya, and V. I. Kochubey, "Application of upconversion luminescence of NaYF₄:Yb,Er nanoparticles to study protein coagulation dynamics," *Optics and Spectroscopy* 128(7), 952–958 (2020).
6. B. Zhang, X. Guo, Z. Zhang, Z. Fu, and H. Zheng, "Luminescence thermometry with rare earth doped nanoparticles: Status and challenges," *Journal of Luminescence* 250, 119110 (2022).
7. V. N. Postnov, E. B. Naumysheva, D. V. Korolev, and M. M. Galagudza, "Nanosized carriers for drug delivery," *Biotechnosphere* 6(30), 16–27 (2013). [in Russian].
8. C. H. Villa, A. C. Anselmo, S. Mitragotri, and V. Muzykantov, "Red blood cells: Supercarriers for drugs, biologicals, and nanoparticles and inspiration for advanced delivery systems," *Advanced Drug Delivery Reviews* 106, 88–103 (2016).
9. M. K. Borovskaya, E. E. Kuznetsova, V. G. Gorokhova, L. B. Koriakina, T. E. Kurilskaya, and Ju. I. Pivovarov, "Structural and functional characteristics of the erythrocyte membrane and its changes in pathologies of various genesis," *Acta Biomedica Scientifica* 3(73), 334–354 (2010). [in Russian].
10. G. E. Dobretsov, *Fluorescent probes in the study of cells, membranes and lipoproteins*, Nauka, Moscow (1989). [in Russian].
11. Y. A. Vladimirov, G. E. Dobretsov, *Fluorescent probes in the study of biological membranes*, Nauka, Moscow (1980). [in Russian].
12. V. G. Artyukhov, M. A. Nakvasina, *Biological membranes: structural organization, functions, modification by physicochemical aggregates: Textbook*, Voronezh State University Publishing House (2000). [in Russian].

13. V. V. Moroz, A. M. Golubev, A. V. Afanasyev, A. N. Kuzovlev, V. A. Sergunova, O. E. Gudkova, and A. M. Chernysh, “[The structure and function of a red blood cell in health and critical conditions](#),” General Reanimatology 8(1), 52 (2012). [in Russian].
14. O. Baskurt, B. Neu, and H. J. Meiselman, [Red Blood Cell Aggregation](#), CRC Press, Boca Raton (2011). ISBN: 9780429151132.
15. A. S. Goryunov, A. G. Borisova, S. P. Rozhkov, G. A. Sukhanova, and N. N. Rozhkova, “[Morphology and aggregation of erythrocytes in carbon nanodispersions](#),” Proceedings of the Karelian Scientific Center of the Russian Academy of Sciences 3, 30–37 (2009). [in Russian].
16. F. Zhao, Y. Zhao, Y. Liu, X. Chang, C. Chen, and Y. Zhao, “[Cellular uptake, intracellular trafficking, and cytotoxicity of nanomaterials](#),” Small 7(10), 1322–1337 (2011).
17. Y. Han, X. Wang, H. Dai, and S. Li, “[Nanosize and surface charge effects of hydroxyapatite nanoparticles on red blood cell suspensions](#),” ACS Applied Materials & Interfaces 4(9), 4616–4622 (2012).
18. F.-F. An, X.-H. Zhang, “[Strategies for preparing albumin-based nanoparticles for multifunctional bioimaging and drug delivery](#),” Theranostics 7(15), 3667–3689 (2017).
19. V. V. Novitsky, N. V. Ryazantseva, Ye. A. Stepovaya, T. S. Fyodorova, Ye. B. Kravets, V. V. Ivanov, T. V. Zhavoronok, N. Yu. Chasovskikh, O. M. Choudakova, V. N. Butusova, and N. M. Yakovleva, “[Molecular disturbances of erythrocytes membrane during pathology of different genesis are the typical reaction of the organism: contours of the problem](#),” Bulletin of Siberian Medicine 5(2), 62–69 (2006). [in Russian].
20. R. A. Khalilov, A. M. Dzhafarova, “[Intensity of free-radical processes and structural and dynamic parameters of erythrocyte membrane members in type 2 diabetes mellitus](#),” Medical and Pharmaceutical Journal Pulse 24(12), 23–28 (2022). [in Russian].