Supplementary Materials: $NaYF_4$:Yb,Er upconversion nanoparticles for imaging. Effect on red blood cells

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S1 – Synthesis and characterization of nanoparticles

Nanoparticle Synthesis Protocol:

Solutions of nitrates of rare earth elements, sodium citrate and citric acid with a concentration of 1M are preliminarily prepared. A 2M ammonium fluoride solution is prepared.

For synthesis, 3 ml of yttrium nitrate, 0.9 ml of ytterbium nitrate, 0.06 ml of erbium nitrate are mixed. The ratio of elements is Y/Yb/Er = 1/0.3/0.02. The mixture is stirred with a magnetic stirrer during 10 minutes. Then 4 ml of sodium citrate is added and stirred for 10 minutes until a homogeneous milky mass is formed.

50 ml of citric acid solution is added and stirred for 30 minutes until a completely clear solution is formed.

Then 38 ml of ammonium fluoride solution is added and stirred for 30 minutes until a homogeneous solution is formed. Immediately after the addition, a gel-like solution is formed, the viscosity of which decreases during mixing.

The molar ratio is NaF/Cit/RE=19/13/1.

All mixing was carried out at room temperature.

After stirring, it was poured into a 100 ml Teflon container, which was placed in an autoclave and kept for 18 hours at a temperature of 180°C.

After the autoclave had cooled to room temperature, the resulting white precipitate was separated by centrifugation and resuspended in water. The procedure was repeated three times. Then the particles were dried at a temperature of 70°C for 20 hours.

Removal of the citrate shell

The synthesized nanoparticles (NPs) $NaYF_4$:Yb,Er were heated for 3 hours at 550°C. Such heating, on the one hand, increases the intensity of the upconversion luminescence due to a decrease in the number of defects in the crystal lattice. On the other hand, heating removes the shell of citrate ions formed during the synthesis.

Method for creating the SiO_2 shell

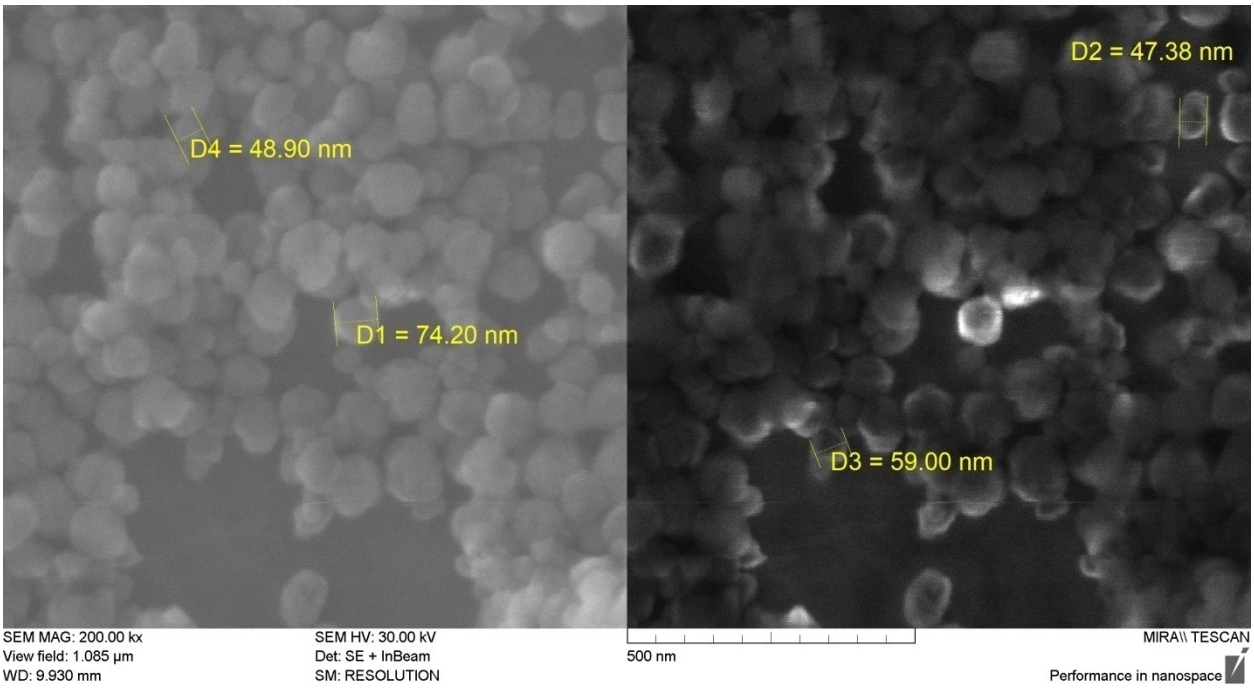
The synthesized NPs were dispersed in isopropyl alcohol (2n mg of NPs in 36 ml of alcohol). Then, 1 ml of an aqueous solution of ammonia (12 wt%) and 80 µl of TEOS (99 wt%) were successively added. The mixture was stirred using a PTR-25 minirotator for 1 hour. Then the NPs were separated by centrifugation, washed three times with distilled water, and resuspended. The resulting NPs were dried at 750 s for 12 hours.

Coating of upconversion nanoparticles with a protein shell

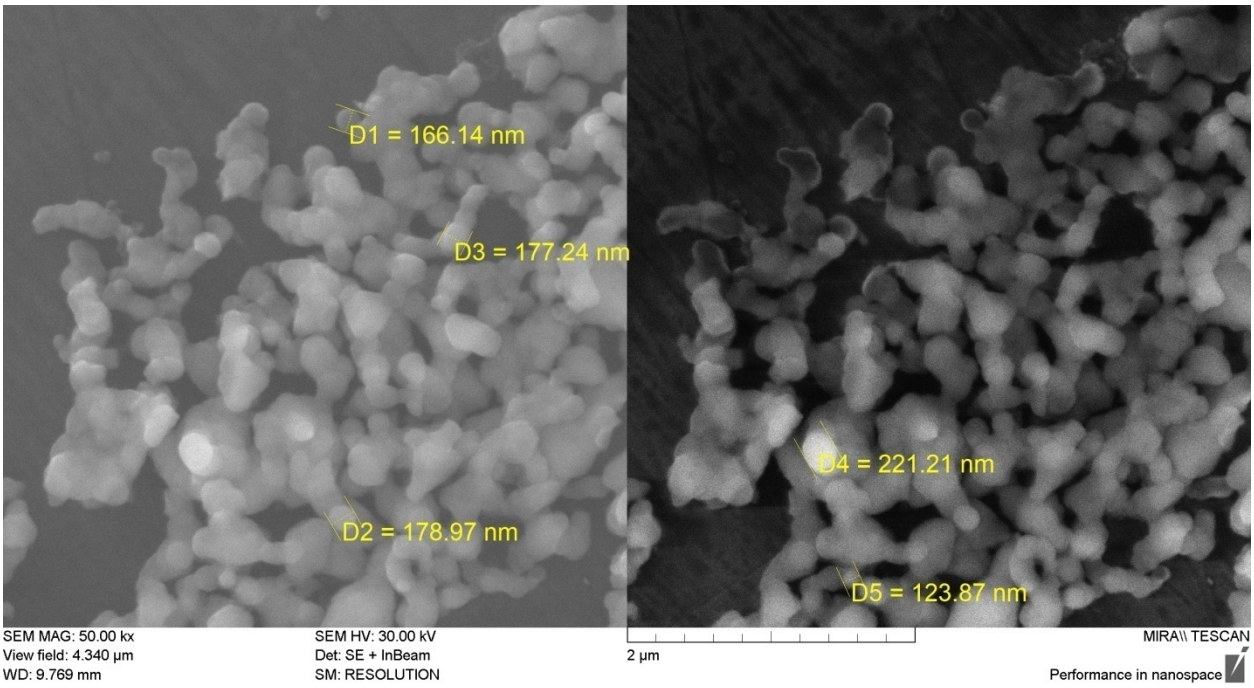
We used a simple method based on the spontaneous adsorption of proteins on the surface of NPs. The adsorption of proteins on the surface of materials, including NPs, and the formation of a protein crown are determined by thermodynamics in an aqueous medium. This occurs spontaneously at constant temperature and pressure in accordance with the Gibbs law of thermodynamics. The process of protein adsorption is mediated by ionic and van der Waals forces, hydrogen bonds and hydrophobic interactions. The change in the Gibbs free energy explains the adsorption of proteins even in the presence of electrostatic repulsion between the surface of the NP and the protein.

In an albumin solution, a protein corona is formed within a fairly short time (10–15 min). However, such a shell is dynamically unstable, so we carried out additional cross-linking of albumin molecules with each other in the presence of hydrogen peroxide.

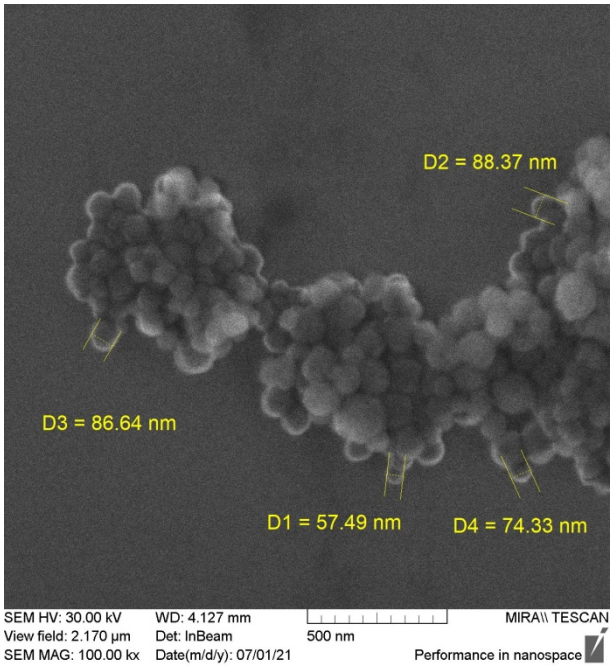
The synthesized NPs $NaYF_4$:Yb,Er were heated for 3 hours at 550°C. Then, 12 mg of dry NPs were added to 3 ml of a solution of a commercial HSA preparation (Sigma-Aldrich, USA) at a concentration of 2 mg/ml in 0.05 M phosphate buffer with pH 6.5. The suspension was kept for 0.5 hour with vigorous stirring with a magnetic stirrer. Then, 30 µl of a 3% hydrogen peroxide solution was added and the mixture was stirred for another 0.5 h. NPs were precipitated by centrifugation (6000 rpm, 10 min). The pellet was resuspended in wash buffer and precipitated again. The procedure was repeated three times. Synthesized particles can be stored in physiological solution at room temperature for more than one month without any change in luminescence intensity.



(a)



(b)



(c)

Figure S1-1. SEM images of $NaYF_4$: Yb^{3+}, Er^{3+} particles: a - unannealed; b - annealed at 550 ^oC; c - coated by SiO_2. Average size of NPs is 53 nm

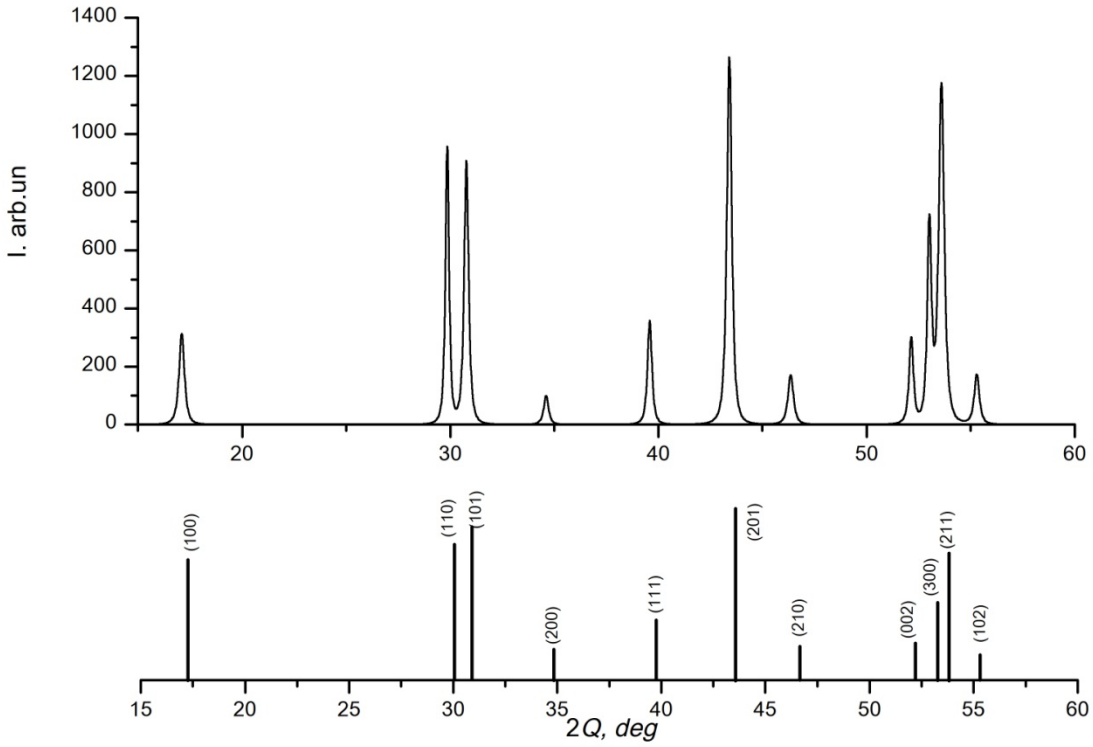


Figure S2-2. XRF spectrum of synthesized NPs.

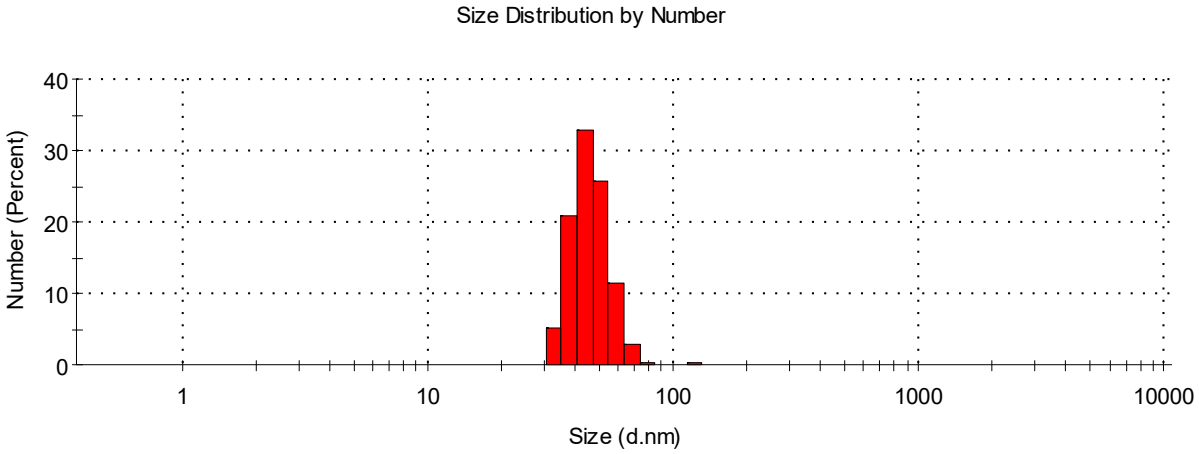
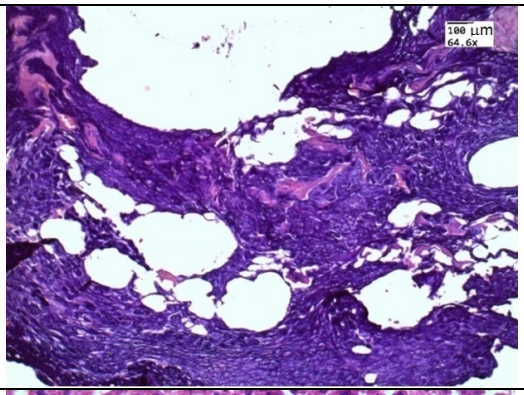
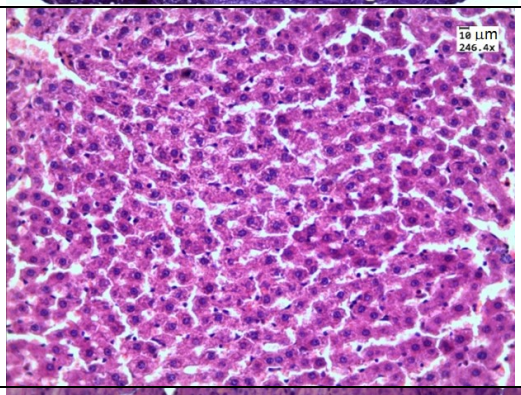
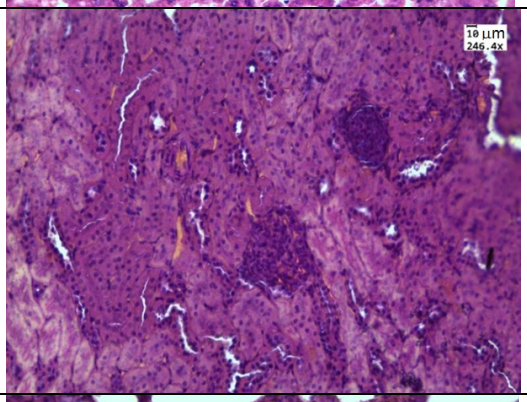
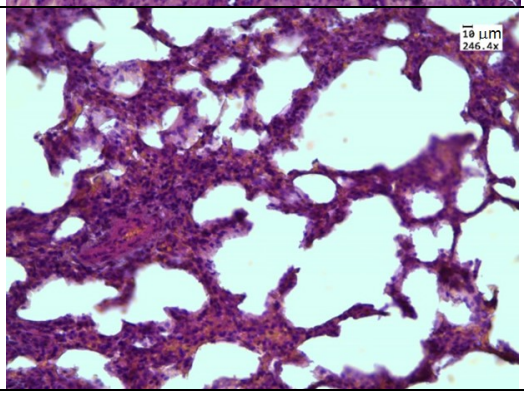
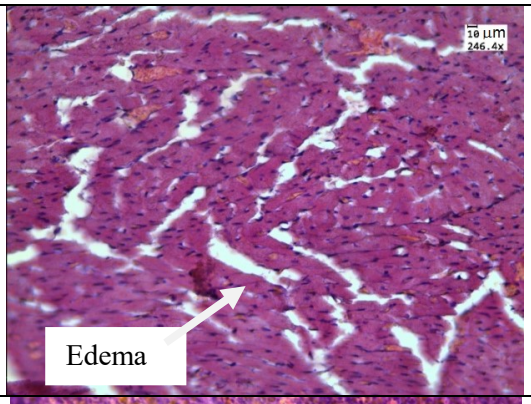
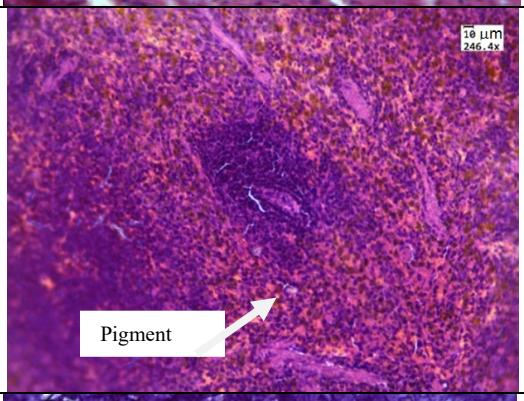
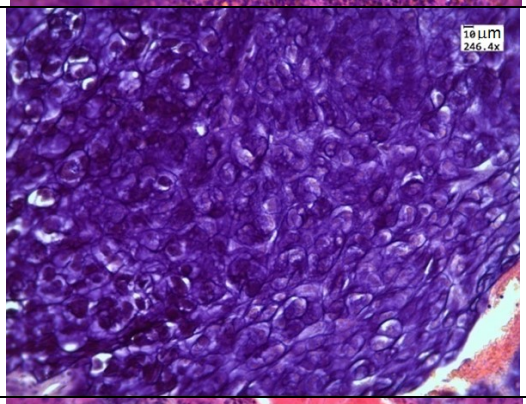
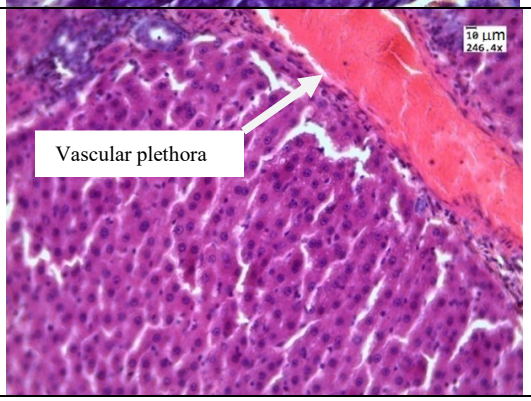


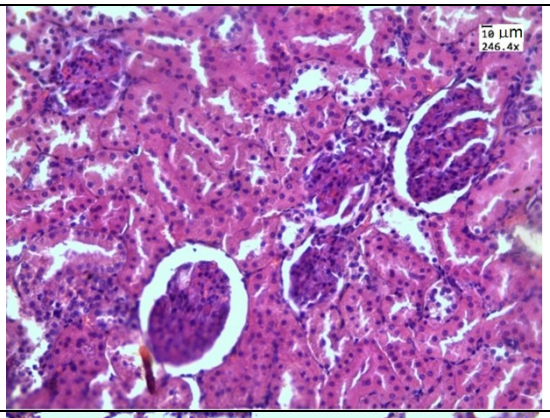
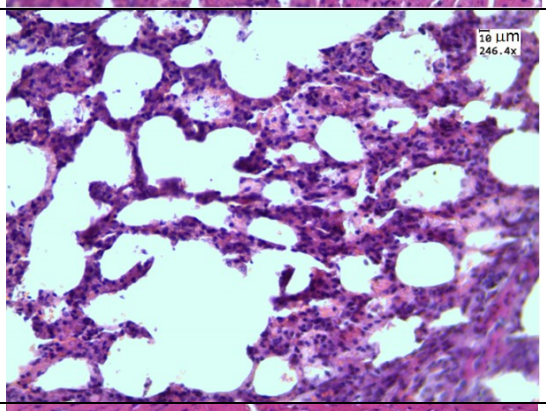
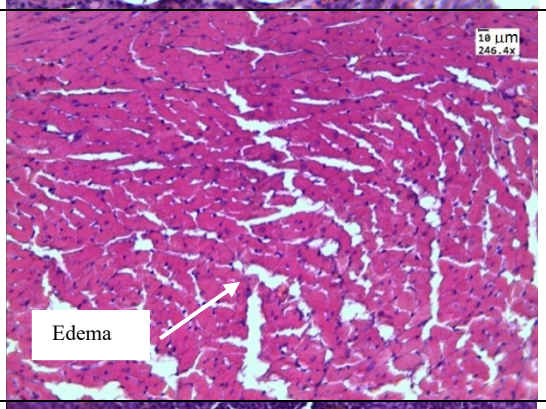
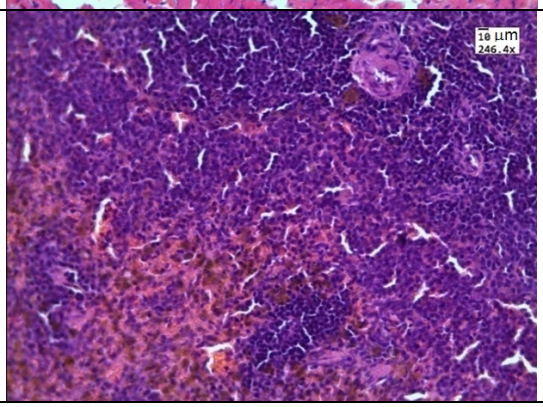
Figure S3-3. Data of DTS for synthesized NPs. Average size of NPs is 53 nm

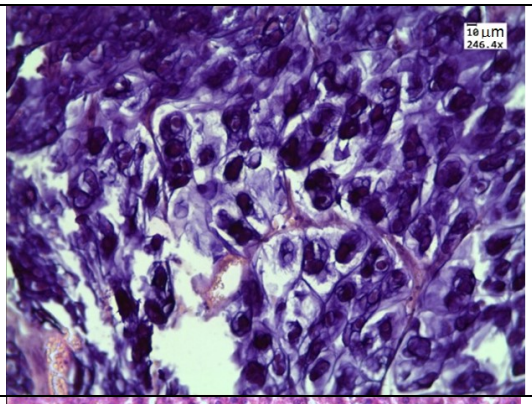
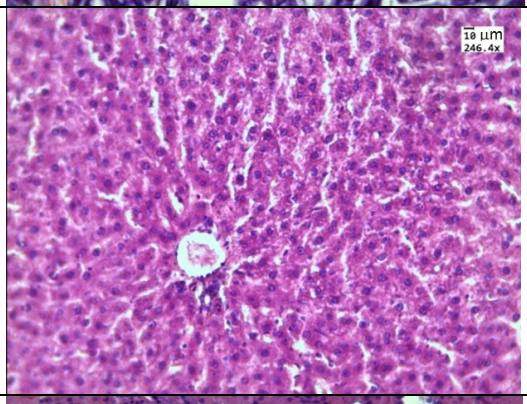
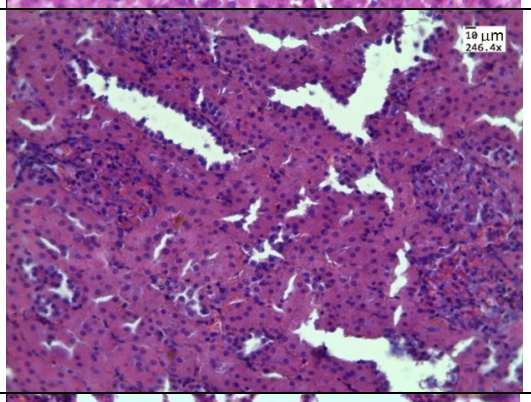
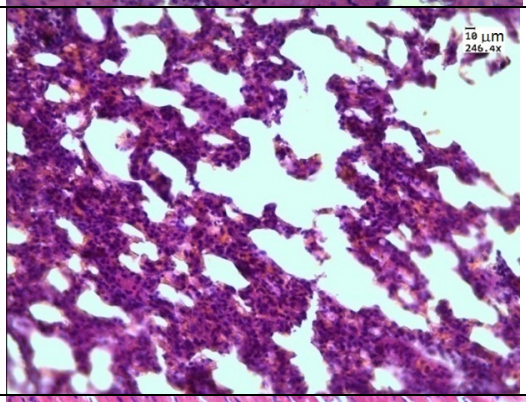
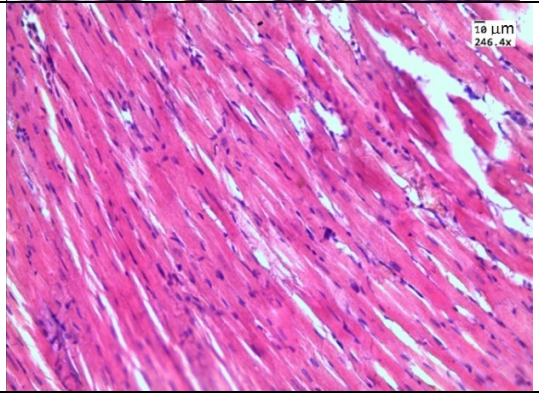
S2 – Results of histological studies of organs

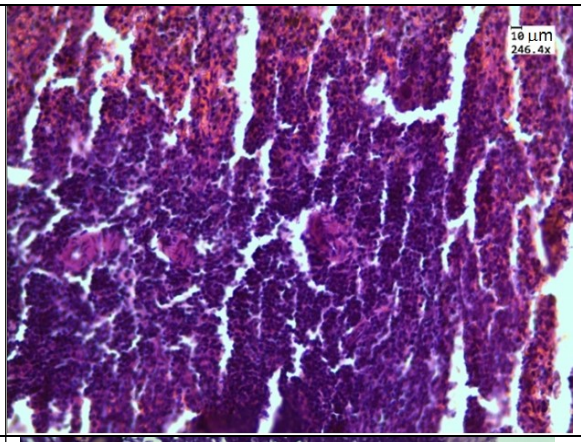
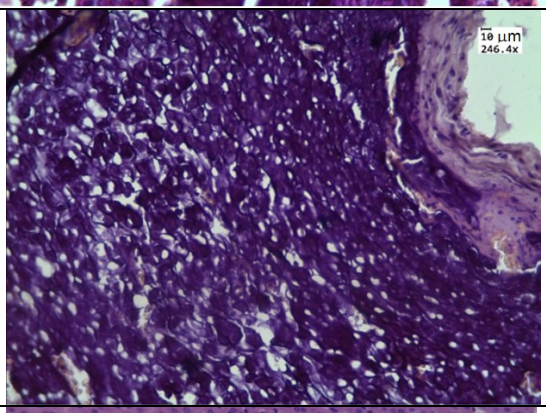
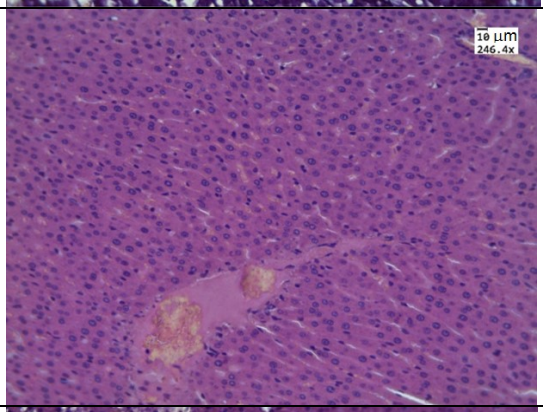
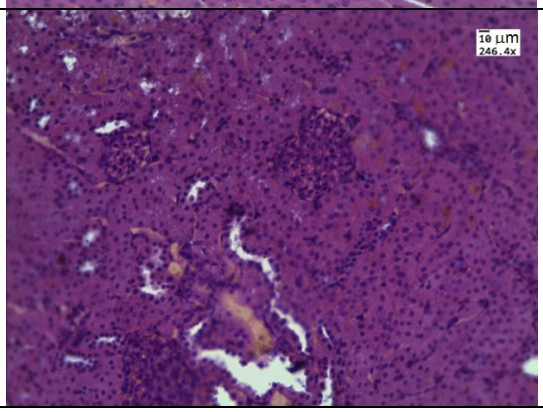
Table S2-1. Results of histological studies of organs.

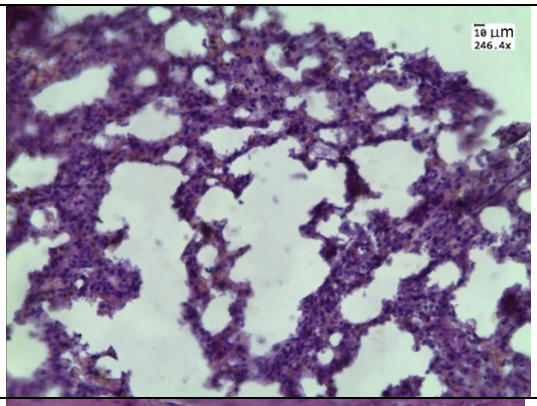
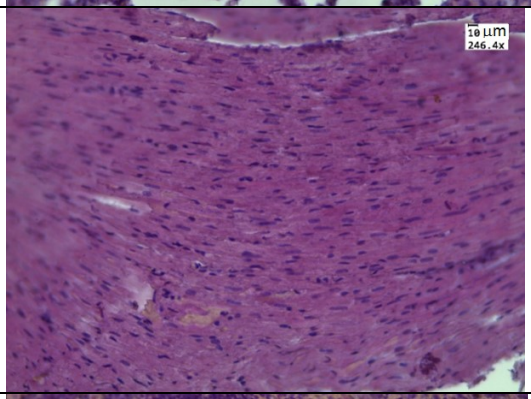
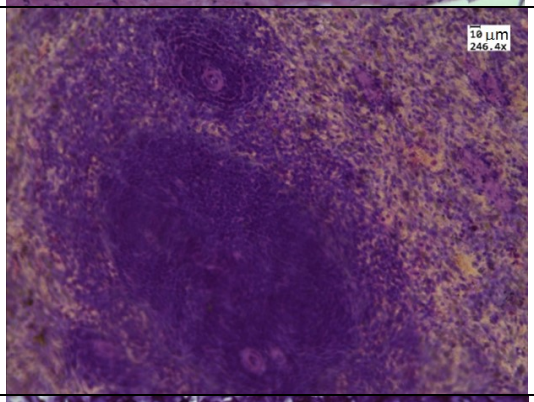
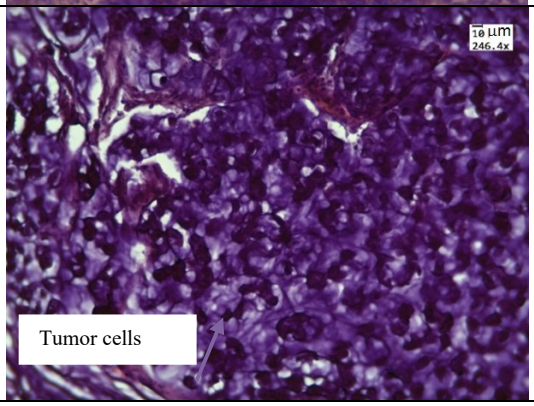
Type of particles, influence	Organ	Description	Photos
Type 1	tumor	Tumor necrosis up to 70-80% of the cut with the formation of cysts in the tumor tissue	
	liver	Moderate liver dystrophy	
	kidneys	Moderate plethora of glomeruli and swelling of convoluted tubules.	
	lung	Moderate thickening of the interalveolar septa, plethora of capillaries.	

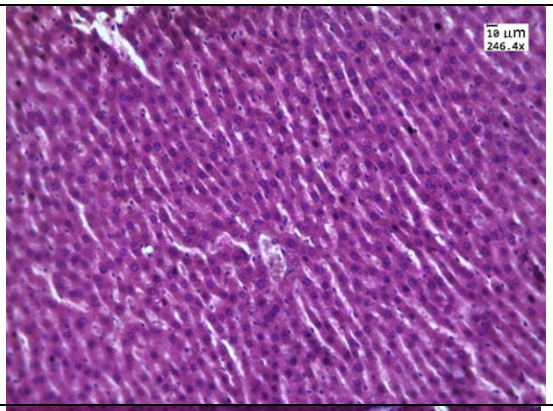
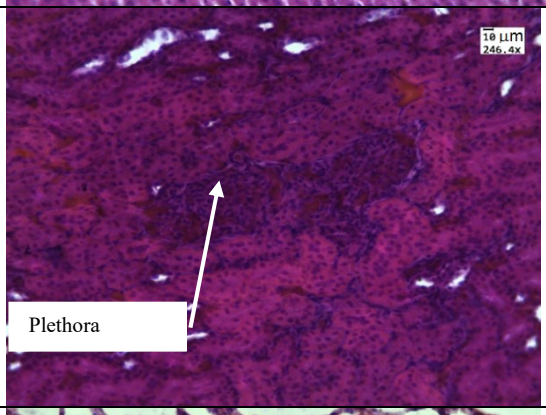
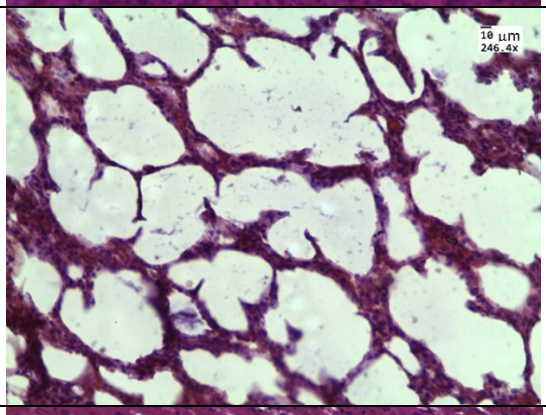
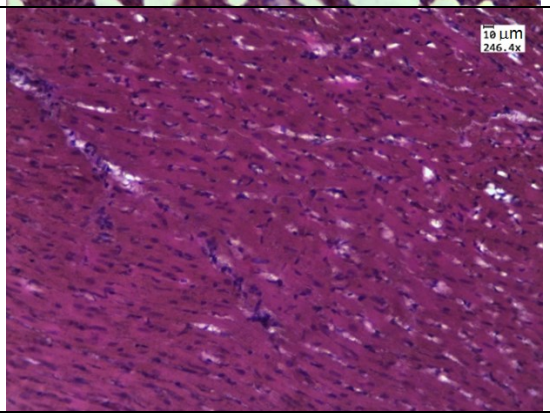
	Myocardium	Moderate stromal edema	
	Spleen	Clear boundaries between red and white pulp, large amount of pigment in the red pulp	
Type 2	tumor	Tumor necrosis up to 60% of the cut	
	liver	Vascular plethora	

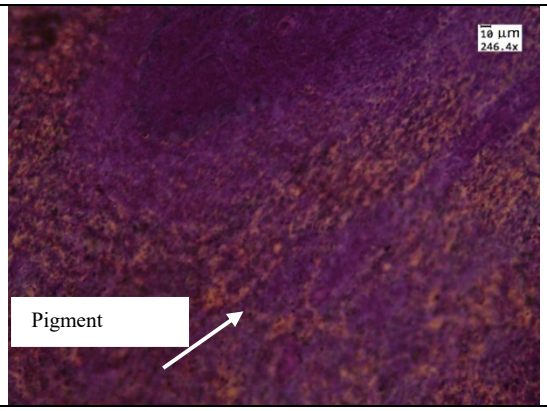
	kidneys	Moderate plethora of glomeruli, expansion of the lumen between the glomerulus and Shumlnsky-Bowman's capsule, swelling of the convoluted tubules.	
	lung	Slight thickening of the interalveolar septa, plethora of capillaries.	
	Myocardium	Moderate stromal edema	
	Spleen	Clear boundaries between red and white pulp, large amount of pigment in the red pulp	

Type 3	tumor	Tumor necrosis up to 60% of the cut	
	liver	Moderate degeneration of hepatocytes	
	kidneys	Moderate plethora of glomeruli and swelling of convoluted tubules.	
	lung	Thickening of the interalveolar septa, plethora of capillaries	
	Myocardium	Moderate stromal edema	

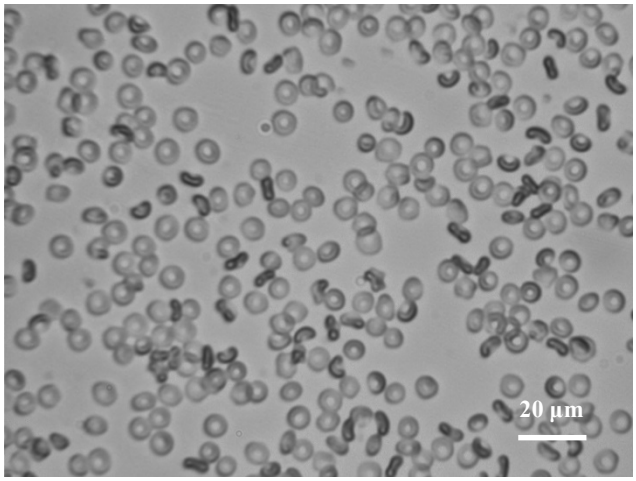
	Spleen	Congestion with red pulp, a large amount of pigment in the red pulp	
Type 4	tumor	Tumor necrosis up to 80% of the cut area, with thickening of the connective tissue septa	
	liver	Moderate plethora of liver vessels	
	kidneys	Moderate glomerular plethora and convoluted tubule swelling.	

	lung	Moderate plethora of capillaries, areas of emphysema.	
	Myocardium	Moderate plethora of blood vessels	
	Spleen	Clear boundaries between red and white pulp, large amount of pigment in the red pulp	
Control, without NPs	tumor	Necrosis up to 10% of the cut area, moderate vascular plethora	

	liver	Normal condition	
	kidneys	Moderate plethora of glomeruli and swelling of convoluted tubules.	
	lung	Moderate plethora of capillaries	
	Myocardium	Normal condition	

	Spleen	Clear boundaries between red and white pulp, large amount of pigment in the red pulp	

S3 – Analysis of the size and shape of red blood cells



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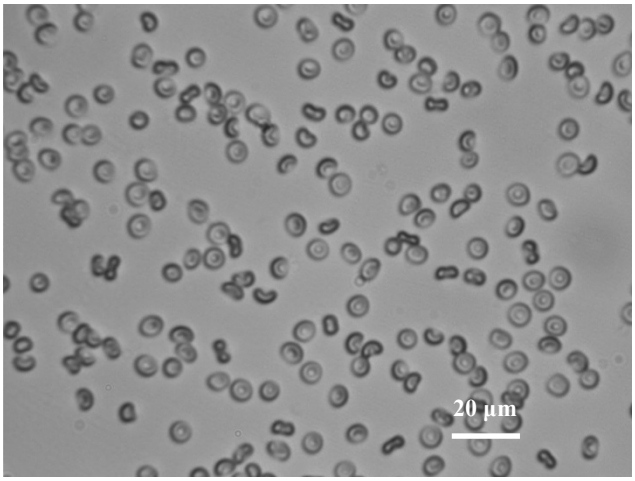

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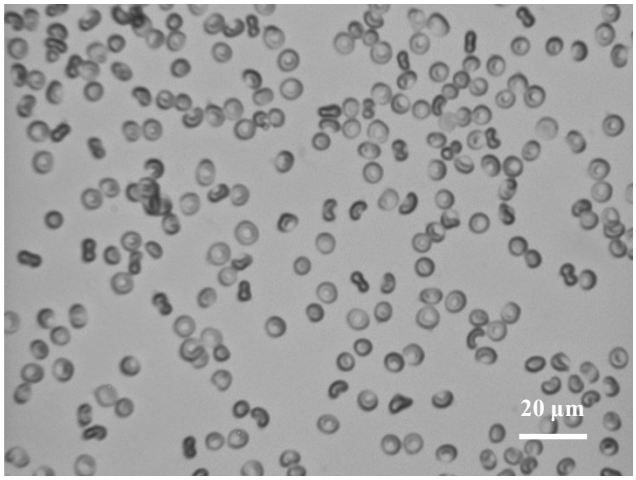

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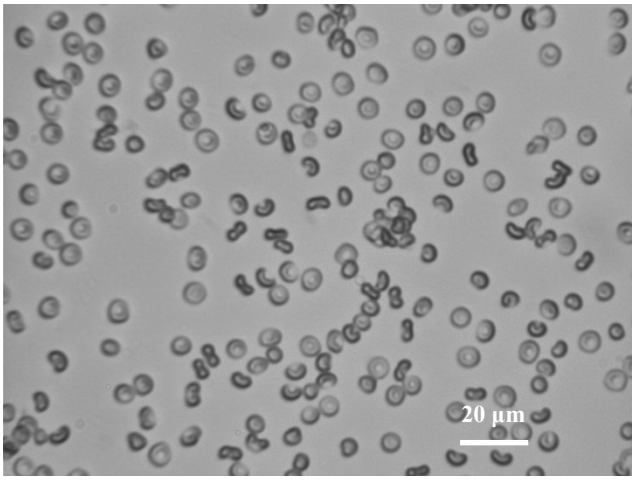
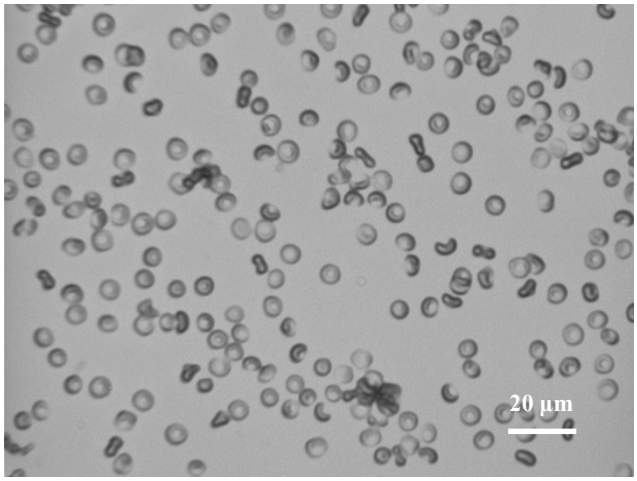
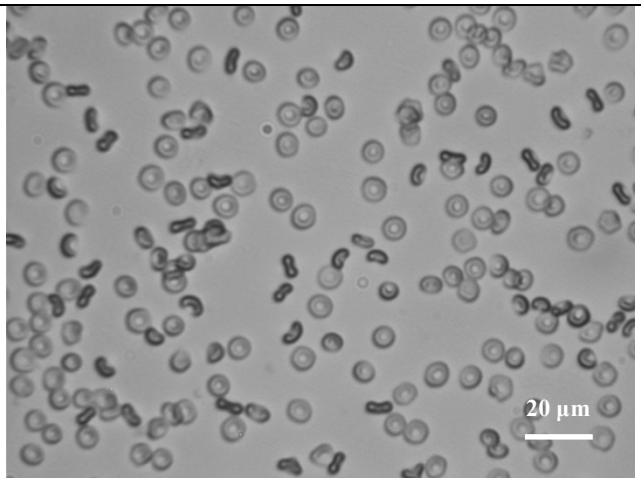


120 min



24 hours

(0.1 mg/ml).



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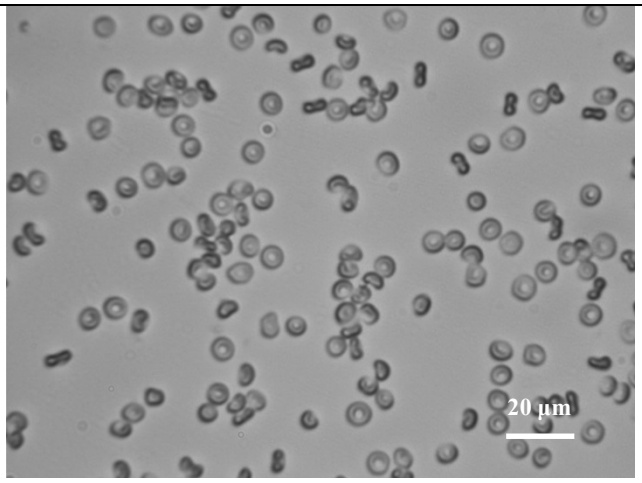

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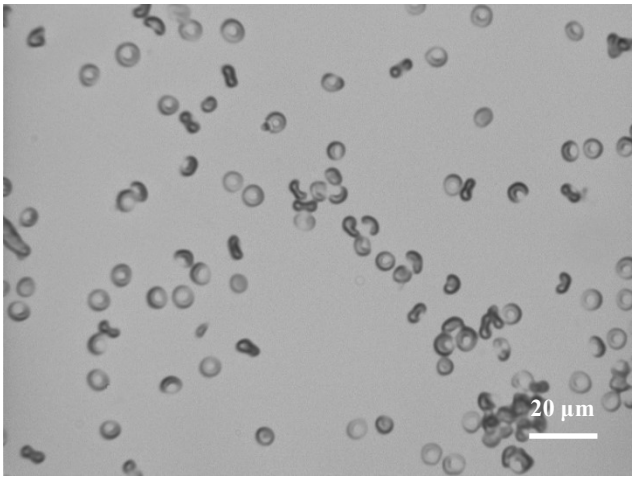

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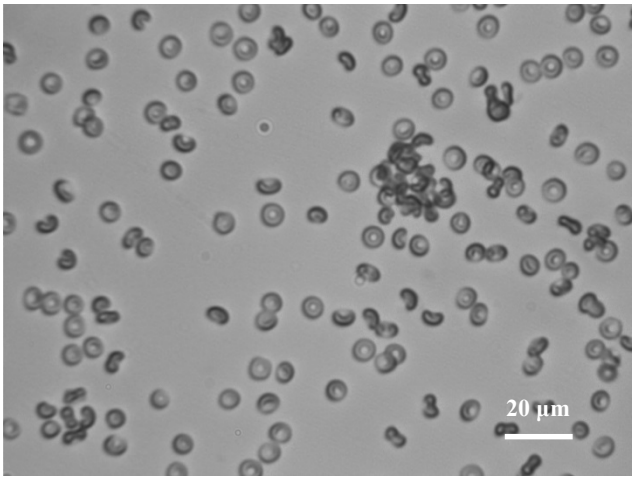

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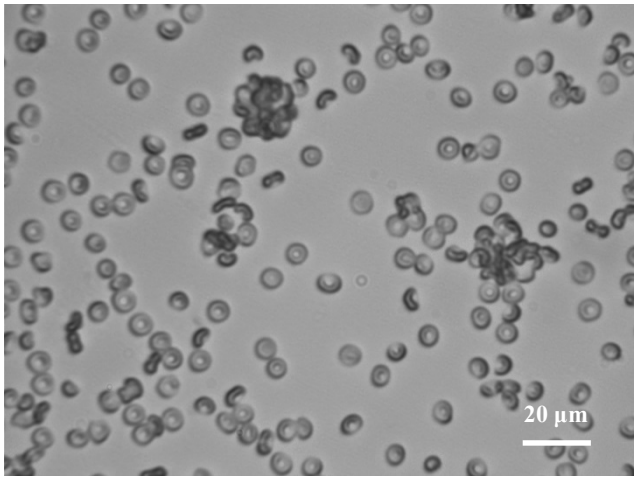
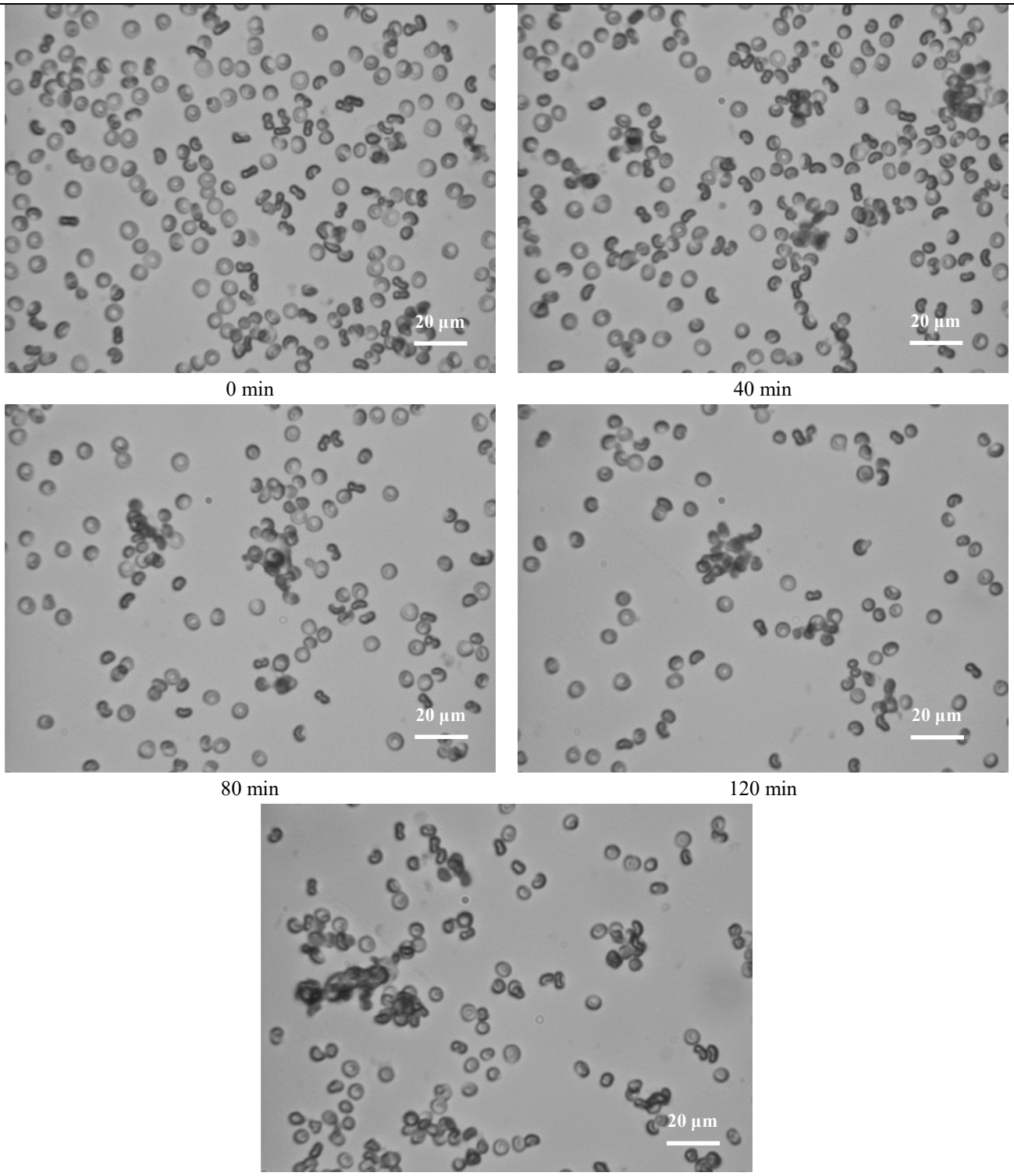


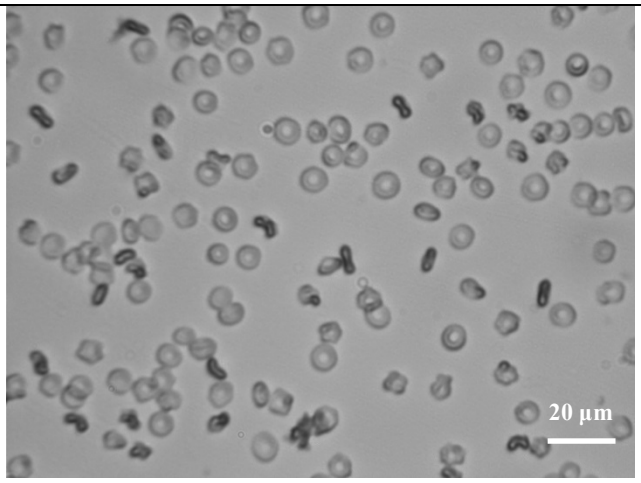
24 hours

(0.2 mg/ml).



(0.4 mg/ml)

Figure 1S3-1-1. Images obtained from 0.25% erythrocyte suspension in solution $NaYF_4$ type 1.
0.1 mg/ml: an increase in the number of aggregates can be seen during incubation time; 0.2 mg/ml: the aggregation is observed during the whole period of the incubation; 0.4 mg/ml: a strong increase in the size of the aggregates is observed during the incubation period.



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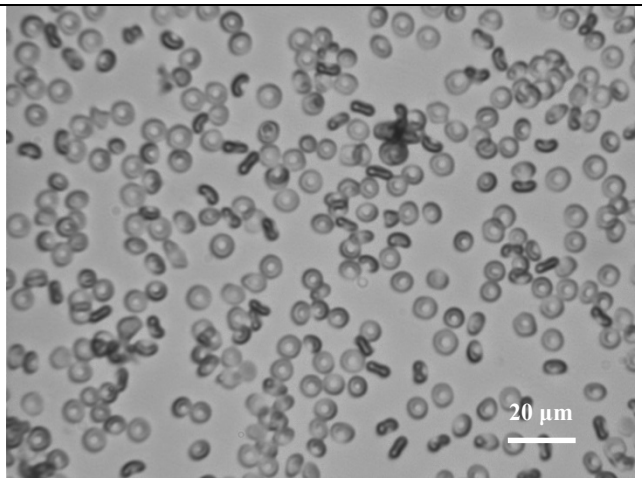
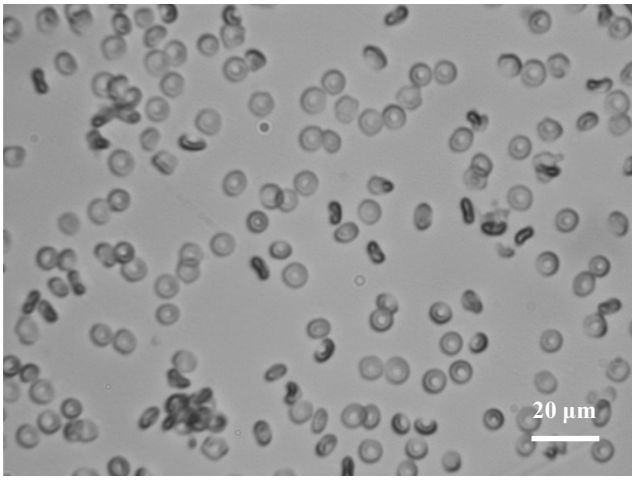
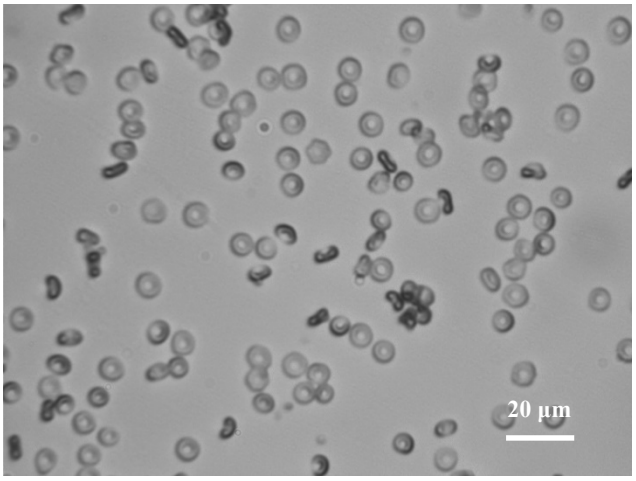


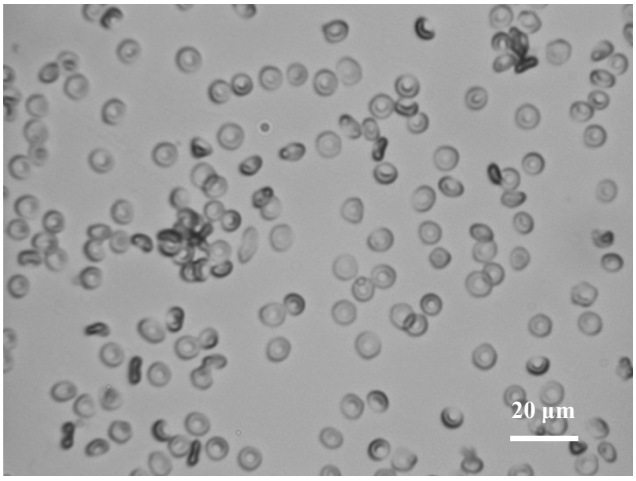
40 min



80 min

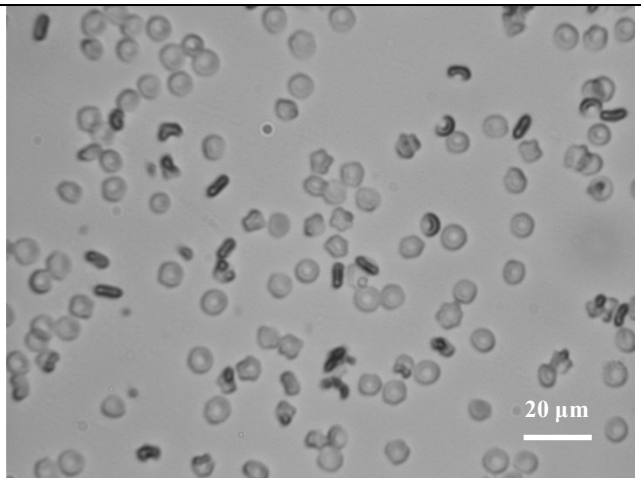


120 min

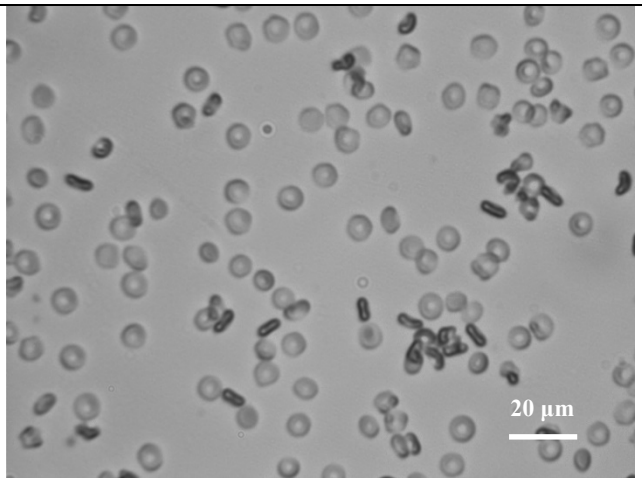


24 hours

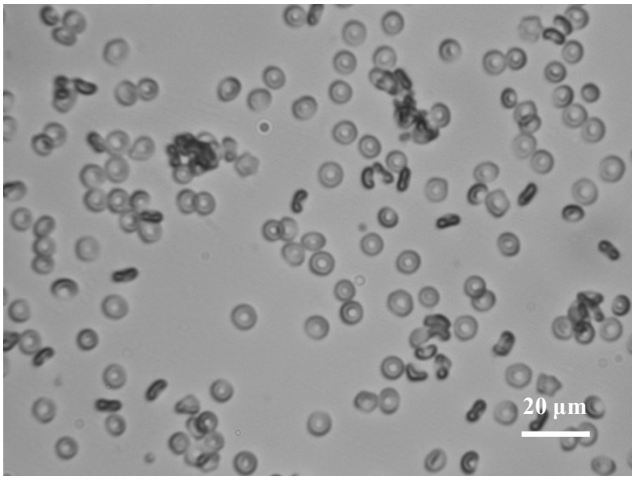
0.1 mg/ml



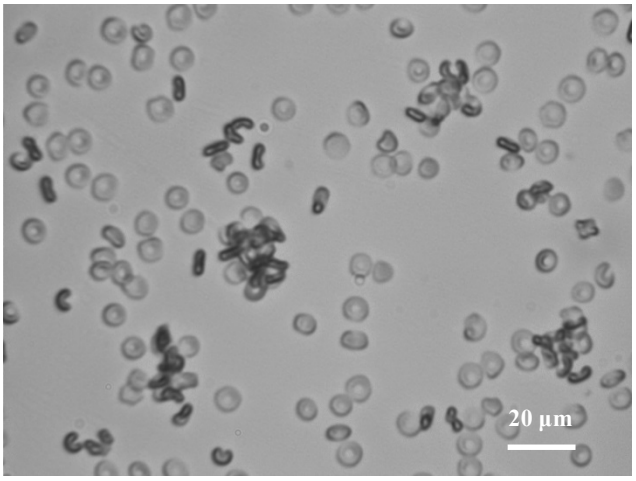
0 min



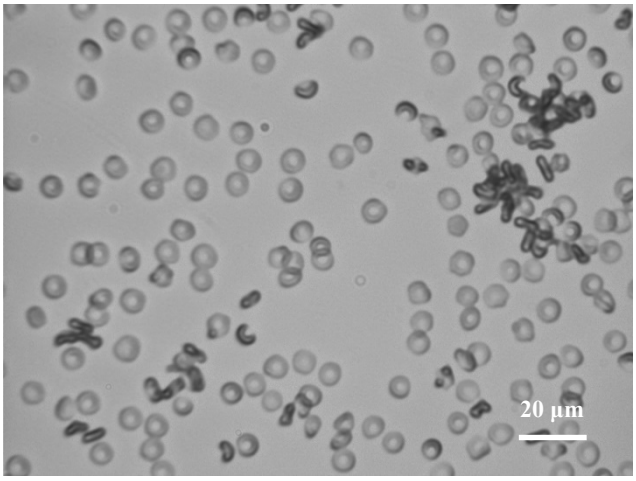
40 min



80 min



120 min



24 hours

0.2 mg/ml

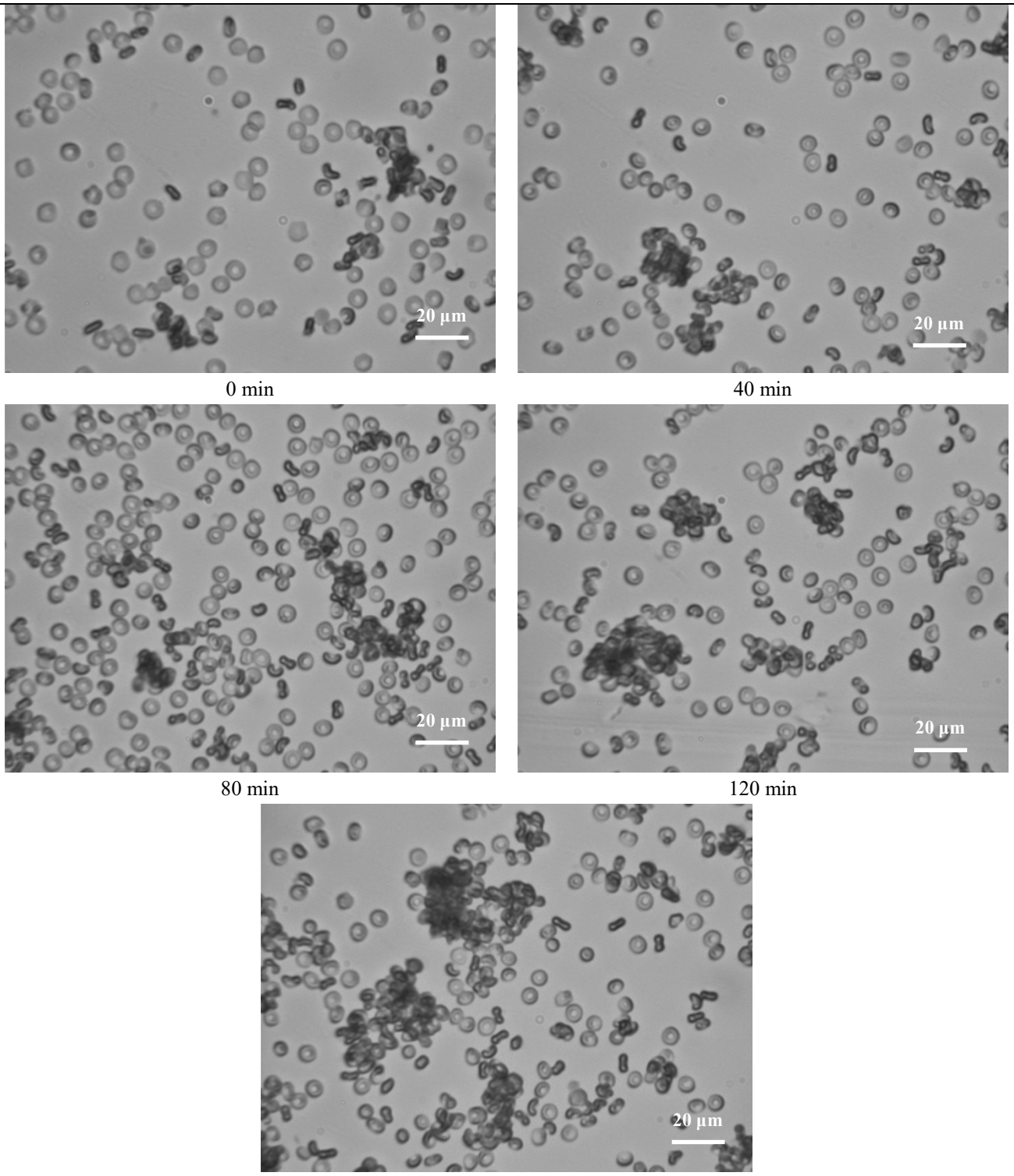
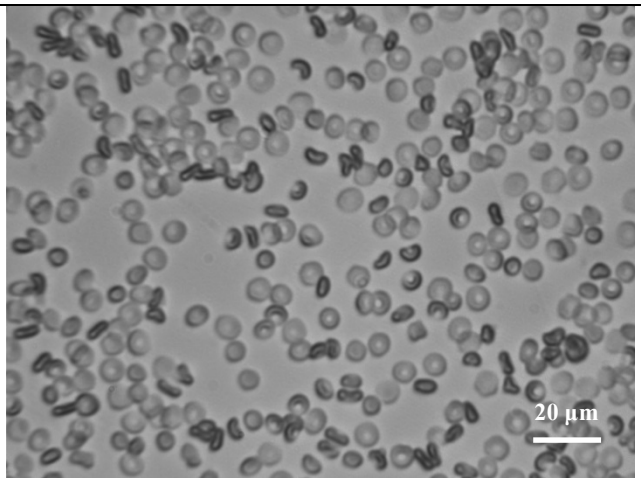
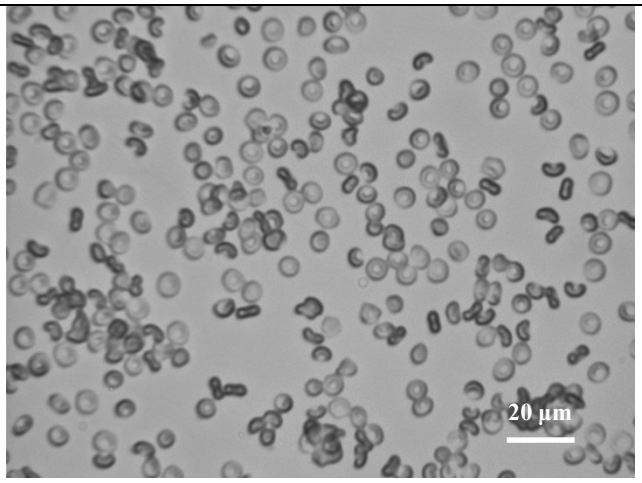


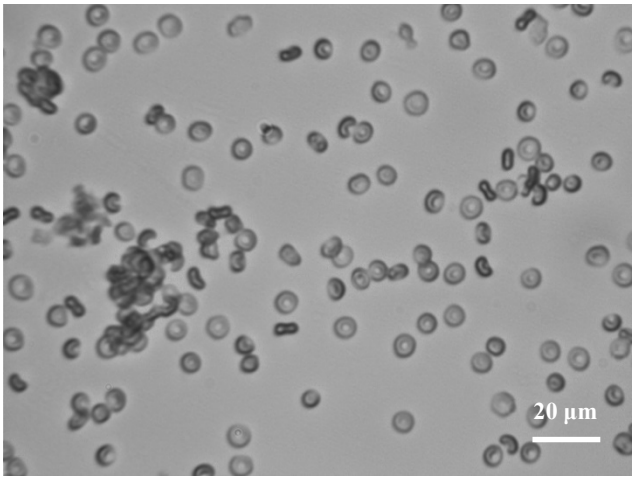
Figure 2S3-1-2. Images obtained from 0.25% erythrocyte suspension in solution $NaYF_4$ type 2. 0.1 mg/ml: the aggregation is observed during the whole period of the incubation; 0.2 mg/ml: an increase in the number of aggregates can be seen during incubation time; 0.4 mg/ml: increasing size and number of aggregates are observed as incubation progresses.



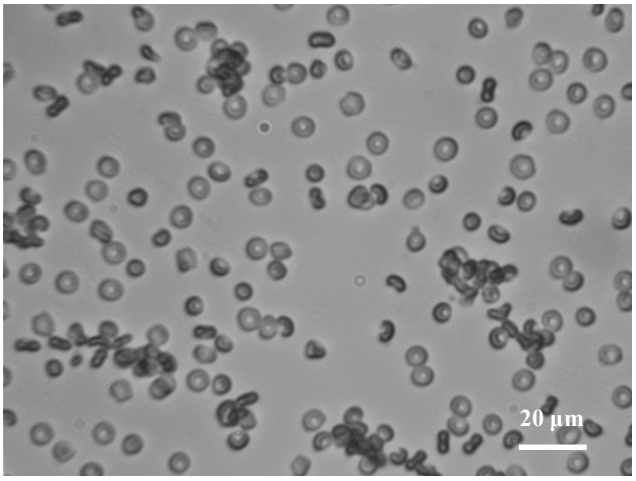
0 min



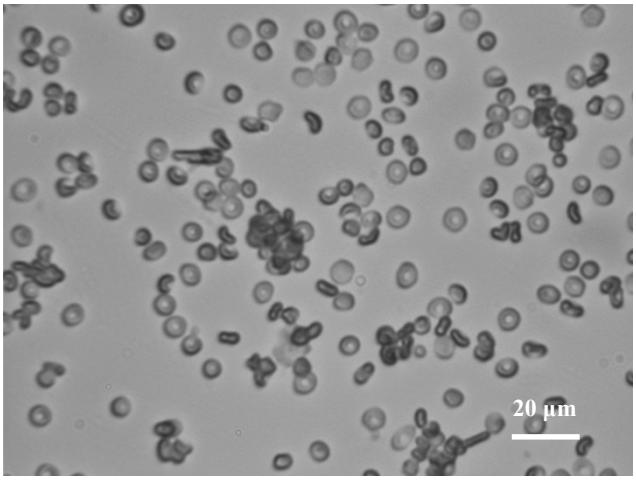
40 min



80 min

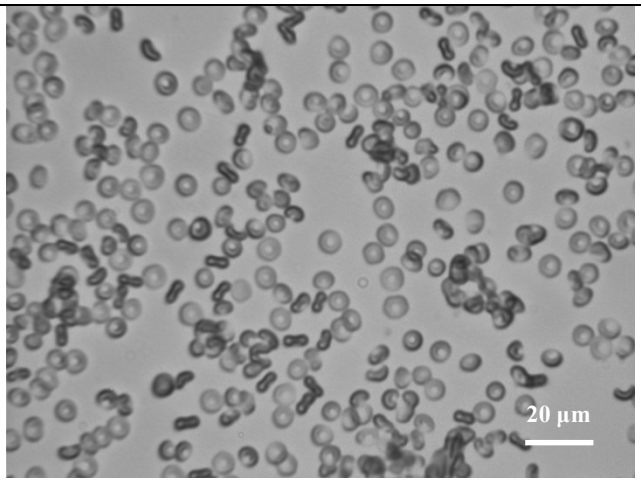


120 min

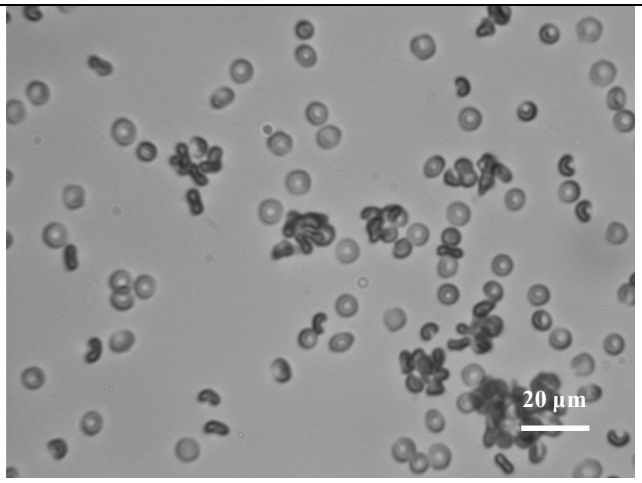


24 hours

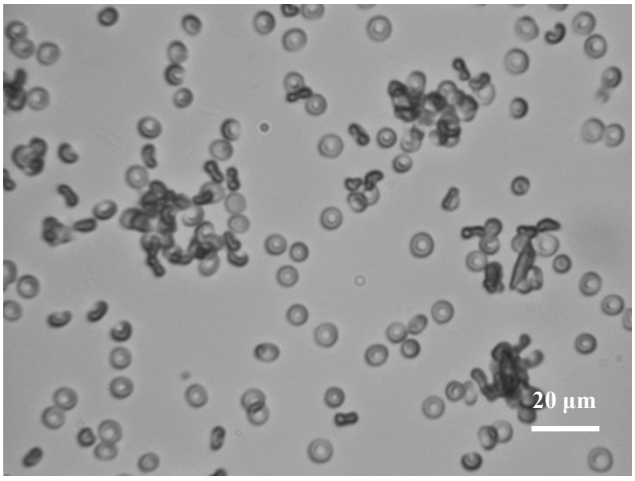
0.1 mg/ml



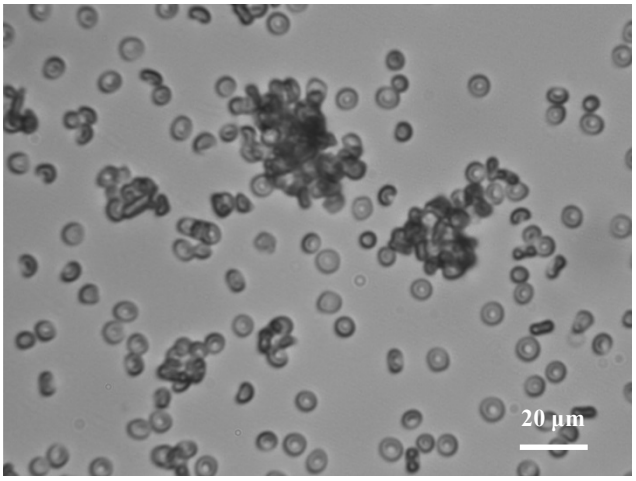
0 min



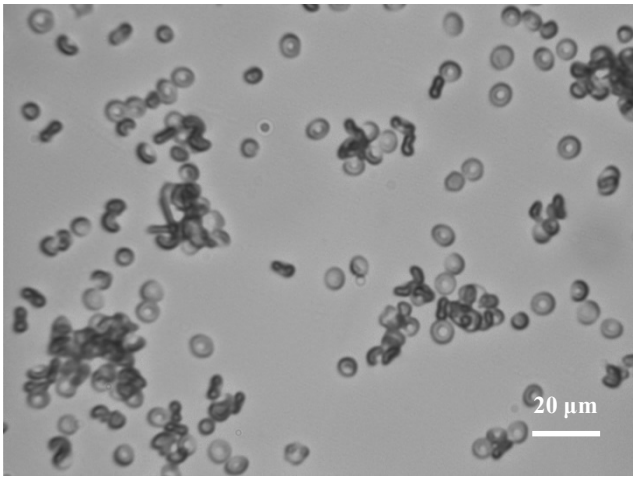
40 min



80 min



120 min



24 hours

0.2 mg/ml

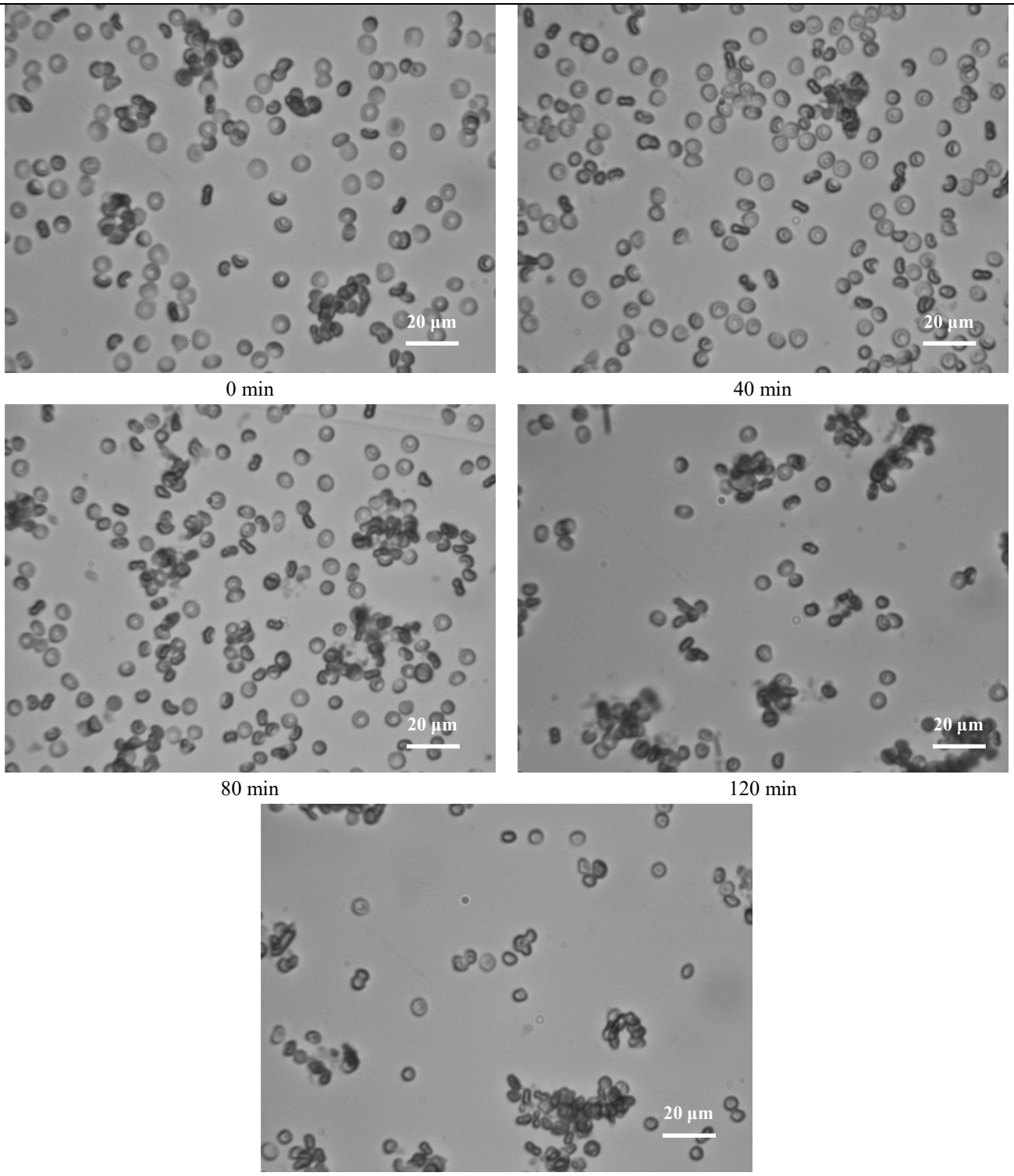
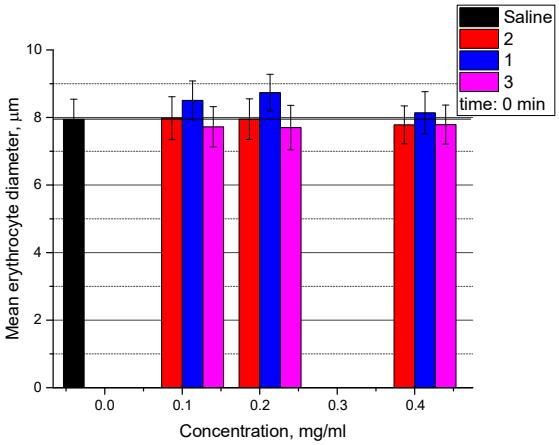
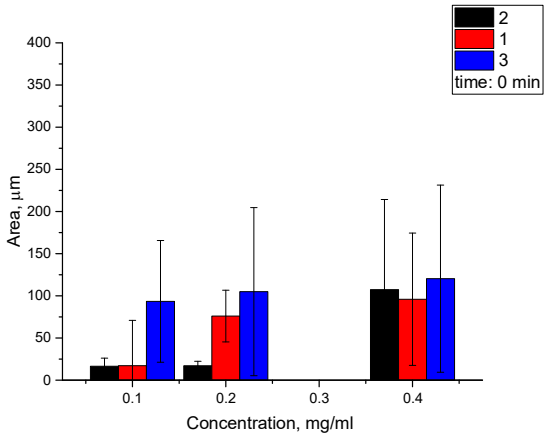
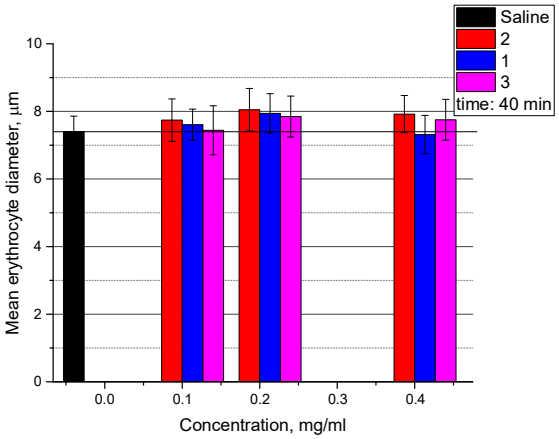
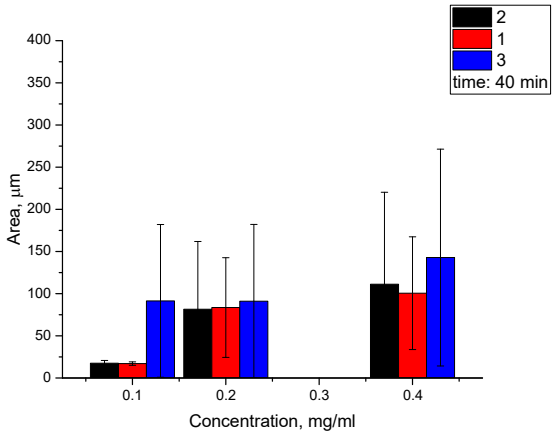


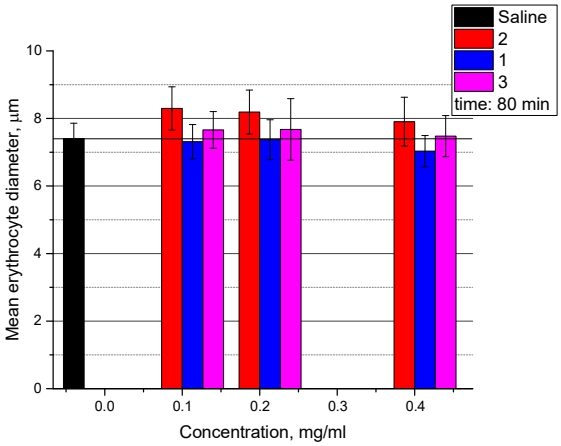
Figure 3S3-1-2. Images obtained from 0.25% erythrocyte suspension in solution $NaYF_4$ type 3. 0.1 mg/ml: an increase in the number of aggregates can be seen during incubation time; 0.2 mg/ml: increasing size and number of aggregates are observed as incubation progresses; 0.4 mg/ml: an increase in the number of aggregates can be seen during incubation time.

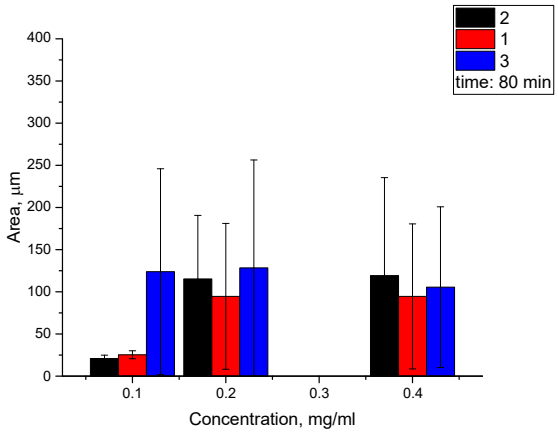

Saline
2
1
3
time: 0 min
Mean erythrocyte diameter, µm
Concentration, mg/ml

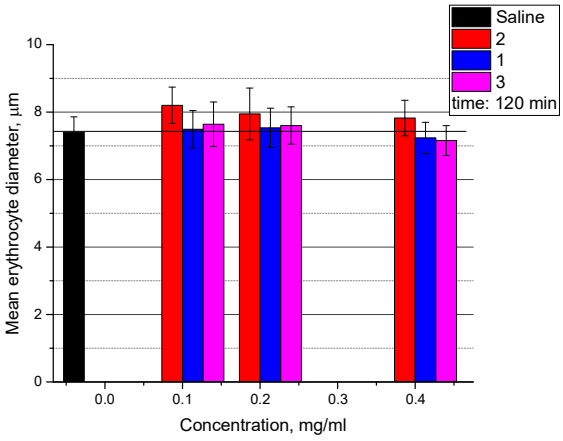

2
1
3
time: 0 min
Area, µm
Concentration, mg/ml

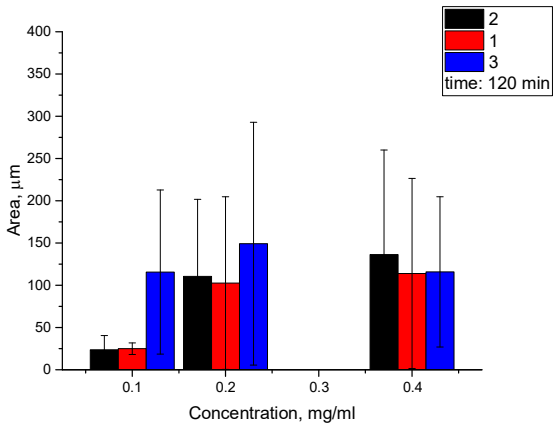

Saline
2
1
3
time: 40 min
Mean erythrocyte diameter, µm
Concentration, mg/ml


2
1
3
time: 40 min
Area, µm
Concentration, mg/ml


Saline
2
1
3
time: 80 min
Mean erythrocyte diameter, µm
Concentration, mg/ml


2
1
3
time: 80 min
Area, µm
Concentration, mg/ml


Saline
2
1
3
time: 120 min
Mean erythrocyte diameter, µm
Concentration, mg/ml


2
1
3
time: 120 min
Area, µm
Concentration, mg/ml

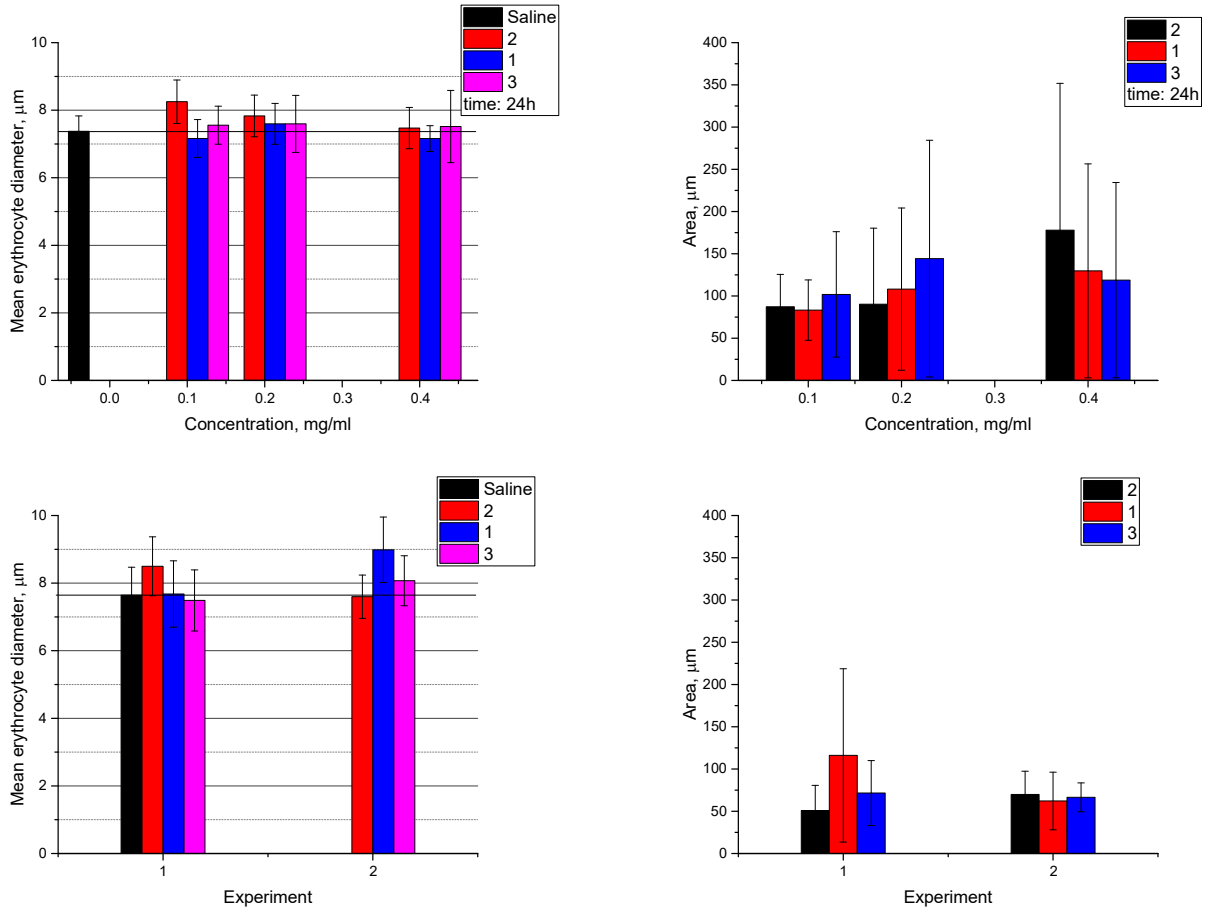


Figure S3-2. Size distribution of erythrocytes and the area of their aggregates. Designations in the figures: 1 - type 1, 2 - type 2, 3 - type 3. 0 min: with increasing concentrations, a significant increase in the average area of erythrocytes is observed, in all samples except erythrocytes with particles of type 2 at concentrations of 0.1 and 0.2 it changed by 0.2 µm2, the diameter of erythrocytes relative to the control: with particles of type 2 did not change, with particles of type 1 it increased, with particles of type 3 decreased, 40 min: with increasing concentration, an increase in the average area of erythrocytes in all samples is observed, the diameter of erythrocytes in all samples decreased after 40 min of incubation, but at a concentration of 0.4 mg/ml nanoparticles of type 1, the diameter decreased with particles of type 2 and the diameter increased with particles of type 3, 80 min: with increasing concentrations, an increase in the average area of erythrocytes is observed in all samples, except for the sample with a concentration of 0.4 mg/ml, where a decrease is observed. The diameter of erythrocytes after 80 minutes of incubation: for particles of type 2 it decreases with increasing concentration, for particles of type 1 it became smaller than the control group, for particles of type 3 it decreased with respect to the previous measurement, 120 min: with increasing concentrations, an increase in the average area of erythrocytes is observed in all samples, except for the sample with a concentration of 0.4 mg/ml, where a decrease is observed. The diameter of erythrocytes after 120 minutes of incubation: in particles of type 2 it decreases with increasing concentration, in particles of type 1 it became smaller than the control group, in particles of type 3 it decreased with respect to the previous measurement, 24 hour: with increasing concentrations, an increase in the average area of erythrocytes is observed in all samples, except for the sample with a concentration of 0.4 mg/ml, where a decrease is observed. The diameter of erythrocytes after 80 minutes of incubation: for particles of type 2 it decreases with increasing concentration, for particles of type 1 it became smaller than the control group, for particles of type 3 it decreased with respect to the previous measurement.

Experiment 1 - rat without irradiation, experiment 2 - rat after irradiation (after irradiation, the average diameter of erythrocytes increased in particles of type 1 and type 3, and decreased in particles of type 2, while the area remained practically unchanged.).