Technical Physics Letters, 2025, Vol. 51, No. 9

The effect of electron beam on erythrocytes in pathology

© *T.E. Gogoleva*[1], *S.N. Mamaeva*[1], *M.M. Rychkov*[2], *G.V. Maksimov*[3]

[1] North-Eastern Federal University, Yakutsk, Russia
[2] Tomsk Polytechnic University, Tomsk, Russia
[3] Moscow State University, Moscow, Russia
E-mail: gogoltakuo98@mail.ru

Received April 29, 2025
Revised June 11, 2025
Accepted June 11, 2025

Venous erythrocytes of patients diagnosed with breast cancer were examined using a scanning electron microscope (SEM). The samples are dry smears on slides previously irradiated with electron beam (50, 100, 150 Gy) on a CMB-8 betatron. SEM images were obtained, cell sizes and shapes were analyzed, the percentage of morphological types was determined, histograms of the size distribution were constructed, and nanoscale structures on the surface were detected. Significant morphological changes have been identified. Nanoscale structures persist after irradiation, which may indicate their viral nature.

Keywords: erythrocytes, ionizing radiation, electron beam, betatron, intraoperative radiation therapy, breast cancer, nanoscale structures.

DOI: 10.61011/TPL.2025.09.61824.7917

Despite the rapid development of diagnostic and therapeutic methods, the number of oncological diseases continues to grow worldwide. Specifically, this is true of breast cancer (BC), which remains the most common of all malignant neoplasms. According to the World Health Organization, BC is at the top of the structure of tumors of the female reproductive system [1]. In Russia, BC ranks first in the distributions of both malignant neoplasm morbidity (22.5 %) and mortality (15.9 %) among the female population [2]. A multidisciplinary approach to the study of risk factors for BC development is needed to understand the causes of the disease and improve prevention and treatment methods [3]. Despite all this, reliable data on the mechanisms of relapse and metastasis of cancerous tumors are currently lacking. Research efforts contributing to the emergence of new concepts regarding the development of pathology and the body's response to therapy are required in order to develop more efficient methods of therapy and its monitoring. Intraoperative radiation therapy (IORT) with high-energy electron beams is one of the modern methods of BC therapy [4]. With the proper choice of energy, electron beam is harmless to healthy tissues located behind a tumor, since the range of electrons in the irradiated medium is limited [5]. It is known that a large number of microvessels form in the region of tumor localization [6], which raises the probability of exposure of erythrocytes to ionizing radiation (IR). An in-depth study of changes in the morphology of erythrocytes induced by IR may help identify possible negative effects and determine the ways to correct them.

The influence of ionizing radiation on the hematopoietic function of bone marrow leads to anemia. However, changes in the oxygen transport function of erythrocytes caused by IR at the molecular level remain understudied [7–10]. IR induces the formation of free radicals in blood cells, which may cause damage to cell membranes, proteins, and DNA (in particular, reticulocytes). In erythrocytes, this results in damage to membrane structures, disrupting normal metabolism and causing osmotic cell instability, which may lead to lysis [9].

The aim of the present study is to investigate morphological changes in erythrocytes of patients with breast cancer under electron irradiation in model conditions using scanning electron microscopy (SEM).

Venous blood samples (1 ml in volume in test tubes) of seven BC patients aged 37–78 years of the Yakutsk Republican Oncology Dispensary were examined. These samples in test tubes were irradiated with an electron beam produced by a small-sized KMB-8 betatron (Tomsk Polytechnic University, Russia). The total dose delivered in three 50 Gy fractions was 150 Gy. Smears were prepared immediately after each stage of irradiation; i. e., four smears were prepared for each blood sample in test tubes before and after irradiation. The test tubes were secured on a special table and introduced into a tube (replaceable applicator) of the betatron. The smears of irradiated samples were applied to a glass slide, dried in air at room temperature (23 °C), and examined using an electron microscope (JSM-7800F JEOL, Japan) at low-kilovolt accelerating voltages (1 and 2 kV) with a voltage of 8−10 V applied to the object under study. The JMicroVision v1.2.7 software toolbox was used to determine the number and linear dimensions of erythrocytes within the field of view in SEM images under a magnification of 1000×. Histograms of linear dimensions of erythrocytes before and after irradiation were plotted in Origin Pro based on the obtained data.

SEM images of erythrocytes obtained as a result of experiments revealed that irradiated blood contains a large number of dysmorphic cells (Fig. 1).

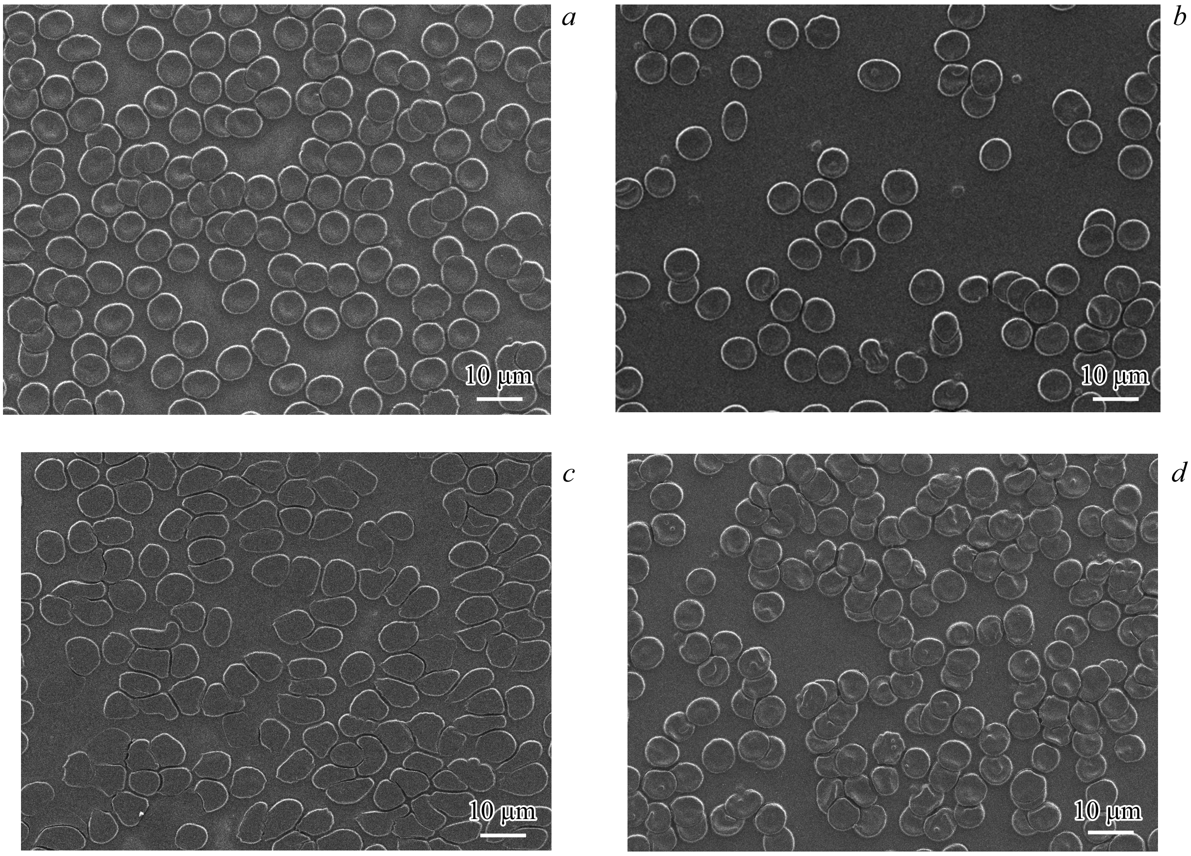

Figure 1. Example SEM images of erythrocytes of patients diagnosed with breast cancer. *a* — prior to irradiation, *b* — first stage of electron irradiation (50 Gy), *c* — second stage of electron irradiation (100 Gy), and *d* — third stage of electron irradiation (150 Gy).

Percentage of erythrocytes of different shapes

Dose, Gy	Discocyte, %	Ovalocyte, %	Spherocyte, %	Schistocyte, %	Plastocyte, %	Acanthocyte, %	Stomatocyte, %	Echinocyte, %
0 (prior to irradiation)	29.9	28.6	4.2	11	16.7	9.6	–	–
50	26.6	19.4	6.5	4.8	20	10.6	3.2	8.9
100	–	17.3	11.5	28.8	–	42.3	–	–
150	–	5.4	19.8	14.4	26.2	18.9	11.7	3.6

The number of dysmorphic erythrocytes increases with each stage of irradiation. While discocytes and ovalocytes, which are normal erythrocytes, are prevalent prior to irradiation and at the first stage of irradiation (50 Gy), the number of different types of dysmorphic erythrocytes (schistocyte, spherocyte, plastocyte, acanthocyte, stomacocyte, echinocyte) and their percentage increase (see the table) at each subsequent stage.

Figure 2 presents the linear size distributions of erythrocytes obtained prior to irradiation and after the first, second, and third stages of irradiation.

Comparing the sizes of erythrocytes determined in the process of irradiation, one sees that the average linear sizes of erythrocytes decrease after the first two stages of irradiation (50 and 100 Gy) and increase after the third stage (150 Gy; Fig. 3). This may be attributed to an increase in the number of spherocytes (with linear sizes exceeding the linear dimensions of normal erythrocytes) and echinocytes. Erythrocytes assume such irreversible forms (spherocytes) before hemolysis. The increase in average size of erythrocytes in the course of this experiment is consistent with the results of a study of the effect of IR on the blood of mammals, which revealed an increase in hematocrit [11].

In addition, according to SEM data, nanoscale structures (NSSs), which may be viral particles or exosomes, are found on the surface of erythrocytes of patients diagnosed with breast cancer (Fig. 4).

SEM images of erythrocytes demonstrate that these particles do not vanish after exposure to IR. This suggests that an irradiation dose of 150 Gy is insufficient to destroy them.

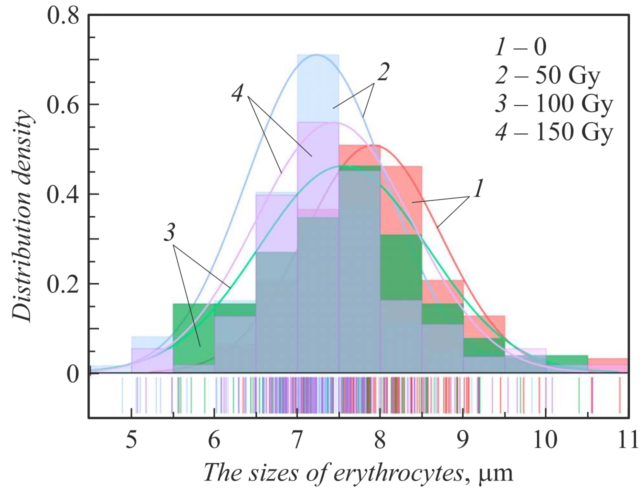

Figure 2. Size distributions of erythrocytes of BC patients before irradiation and after the first, second, and third stages of irradiation. A color version of the figure is provided in the online version of the paper.

It was noted by Bliznyuk [12] and Sadraeian [13] that doses ranging from 10 to 50 kGy are used for inactivation of viruses *in vitro* in radiation sterilization of biological materials. Thus, this phenomenon may indicate that the observed NSSs are likely to be viral particles, which are characterized as microorganisms with high resistance to radiation.

The resistance of NSSs to radiation is verified by the results of other studies, where their number remained unchanged even after radiation therapy [14,15]. This suggests that these structures may be viral particles.

The study showed that NSSs on the surface of erythrocytes are not destroyed at high doses and their number remains unchanged. This indicates that the particles in question are viral particles [14]. NSSs emerging in blood plasma during radiation therapy may be regarded as exosomes that arise as a result of tumor irradiation [14]. Therefore, it is fair to assume that the increase in the number of NSSs on the surface of erythrocytes and in blood plasma after IORT may be indicative of secretion of exosomes by cancer cells of the surface layer („tumor bed“); this may later cause a relapse or metastases and indicate that a large number of cancer cells remain in surrounding tissues after resection of the neoplasm. Thus, the studied variations of shape and size of erythrocytes in bulk, the presence of NSSs on their surface, and the constancy of their number under IORT may be regarded as data characteristic of the effects of radiation during IORT. In addition, such experiments may contribute to the study of the dose homogeneity index (a measure of uniformity of the dose distribution within the target volume) during IORT; i. e., an increase in homogeneity of the dose distribution may be associated with a lack of exosomes in blood. An increase in the number of NSSs in blood samples suggests a possible unfavorable prognosis after IORT, which may be associated with the fact that the required radiation dose homogeneity was not achieved. Owing to non-uniform irradiation, insufficient doses are delivered to certain parts of the target, which, in turn, may facilitate tumor recurrence. The examination of NSSs forming in blood under the influence of radiation may contribute to the development of more efficient IORT procedures providing dose homogeneity (e. g., research into the design of various betatron applicators and tissue-equivalent boluses for them).

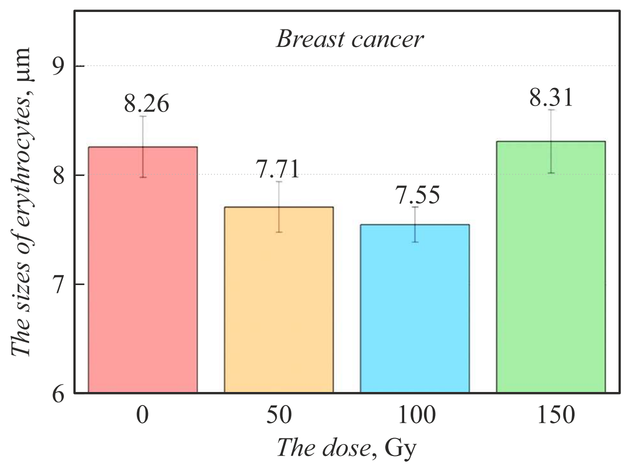

Figure 3. Average sizes of prior to irradiation and after the first, second, and third stages of electron irradiation of blood samples of BC patients.

Thus, such studies of the effect of electron irradiation on erythrocytes are of great importance for the development of new methods of protection against IR and for refining the radiotherapy technique (i. e., increasing the efficiency of IORT).

Funding

This study was carried out as part of state assignment No. FSRG-2024-0002 with financial support from the Endowment Fund of the Ammosov North-Eastern Federal University.

Compliance with ethical standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed voluntary consent was obtained from each study participant.

Conflict of interest

The authors declare that they have no conflict of interest.

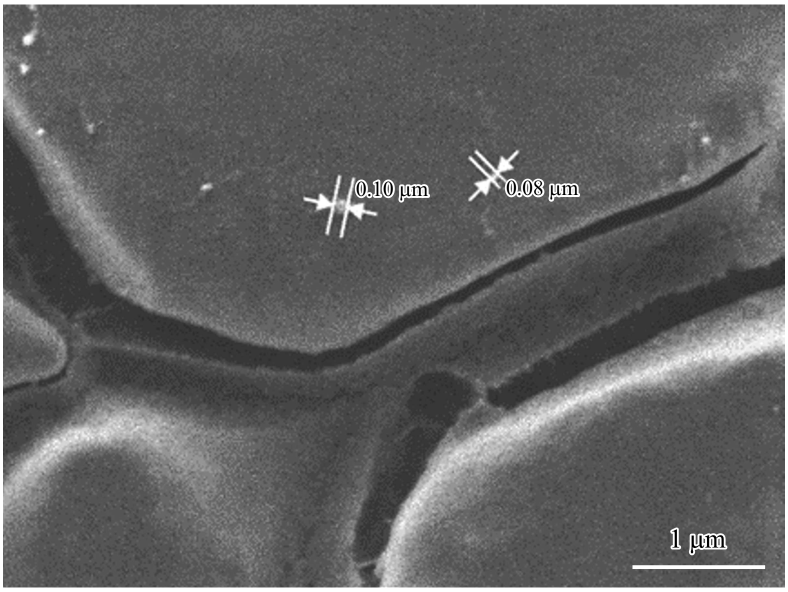

Figure 4. Nanoscale structures on the surface of erythrocytes of patients diagnosed with breast cancer.

References

[1] *International agency for research on cancer* [Electronic source]. https://gco.iarc.who.int/today/en/dataviz/pie?mode=cancer&types=0&sexes=2&populations=643

[2] *Zlokachestvennye novoobrazovaniya v Rossii v 2023 godu (zabolevaemost' i smertnost')*, Ed. by A.D. Kaprin, V.V. Starinskii, A.O. Shakhzadova (Mosk. Nauchno-Issled. Onkol. Inst., M., 2024) (in Russian). https://oncology-association.ru/wp-content/uploads/2024/08/zis-2023-elektronnaya-versiya.pdf

[3] I.A. Akulova, *Klinicheskoe znachenie vysokodoznoi brakhiterapii pri provedenii dopolnitel'nogo oblucheniya lozha udalennoi opukholi pri rake molochnoi zhelezy*, Candidate's Dissertation in Medical Sciences (Nats. Med. Issled. Tsentr Onkol., SPb., 2023) (in Russian).

[4] F.A. Calvo, R.M. Meirino, R. Orecchia, Crit. Rev. Oncol. Hematol., **59** (2), 106 (2006). DOI: 10.1016/j.critrevonc.2005.11.004

[5] V.B. Sorokin, Izv. Tomsk. Politekh. Univ., **312** (S2), 361 (2008) (in Russian). https://cyberleninka.ru/article/n/formirovanie-elektronnogo-terapevticheskogo-puchka-meditsinskogo-betatrona-kmb-10-1

[6] A.N. Remizov, *Meditsinskaya i biologicheskaya fizika* (Vyssh. Shk., M., 1999) (in Russian).

[7] Yu.B. Kudryashov, B.S. Berenfel'd, *Osnovy radiatsionnoi biofiziki* (Izd. Mosk. Gos. Univ., 1982) (in Russian).

[8] O.G. Luneva, S.V. Sidorenko, G.V. Maksimov, R. Grygorczyk, S.N. Orlov, Biochem. Moscow Suppl. A, **9** (3), 161 (2015). DOI: 10.1134/S1990747815040078

[9] F.D. Shiffman, *Patofiziologiya krovi* (Binom–Nevskii Dialekt, M.–SPb., 2000) (in Russian). https://studfile.net/preview/21491608/

[10] B.I. Polivoda, V.V. Konev, G.A. Popov, *Biofizicheskie aspekty radiatsionnogo porazheniya biomembran* (Energoatomizdat, M., 1990) (in Russian).

[11] S.N. Mamaeva, S.M. Ivanova, V.V. Shutova, G.V. Maksimov, Vestn. Mosk. Gos. Tekh. Univ. im. N. E. Baumana. Ser. Estestv. Nauki, No. 5 (104), 86 (2022) (in Russian). https://cyberleninka.ru/article/n/issledovanie-izmeneniy-sostoyaniya-eritrotsitov-i-plazmy-krovi-obezyan-pri-vozdeystvii-ioniziruyuschego-izlucheniya

[12] U.A. Bliznyuk, *Novye podkhody k razvitiyu metodov radiatsionnoi obrabotki biologicheskikh ob"ektov*, Doctoral Dissertation in Mathematics and Physics (Mosk. Gos. Univ., M., 2024) (in Russian).

[13] M. Sadraeian, L. Zhang, F. Aavani, E. Biazar, D. Jin, eLight, **2**, 18 (2022). DOI: 10.1186/s43593-022-00029-9

[14] S.N. Mamaeva, I.V. Kononova, V.A. Alekseev, N.A. Nikolaeva, A.N. Pavlov, M.N. Semenova, G.V. Maksimov, Int. J. Biomed., **11** (1), 32 (2021). DOI: 10.21103/Article11(1)_OA6

[15] S.N. Mamaeva, V.A. Alekseev, N.A. Nikolaeva, T.A. Krylova, A.A. Gabysheva, A.N. Pavlov, I.V. Kononova, G.V. Maksimov, Int. J. Biomed., **13** (1), 69 (2023). DOI: 10.21103/Article13(1)_OA8

Translated by D.Safin