Joint Institute for Nuclear Research (JINR)
Institute of Theoretical and Experimental Biophysics
of the Russian Academy of Sciences (ITEB RAS)

Investigation of the Effects of Xenon Ion Beam Irradiation on EMT6/P 3D Tumor Spheroids within the NICA Project at JINR

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Report by: N. N. Chukavin

Aim and methods of the study

To investigate the effect of xenon ion beam irradiation on 3D tumor spheroids formed from murine EMT6/P breast carcinoma cells

3D spheroids formed from EMT6/P cell line, size 350 µm

Xenon ion beam Irradiation (0.5 to 2 Gy) (NICA facility) (2 experiments)

Gamma irradiation (0.5 to 2 Gy) (Cs-137 source, JINR) (1 experiment)

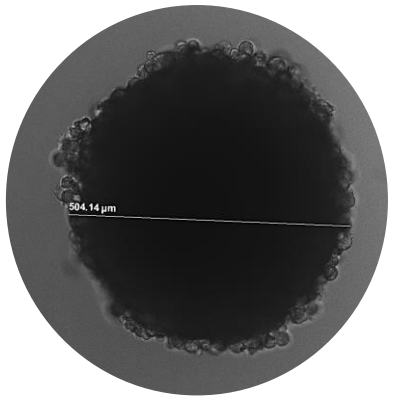
Analysis methods:

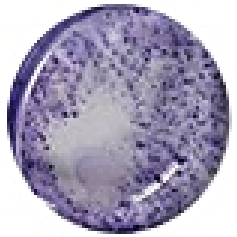
Clonogenic assay

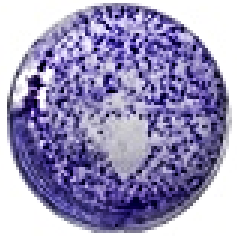
Cell migration analysis

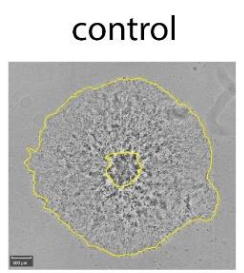
Cell cycle (flow cytometry)

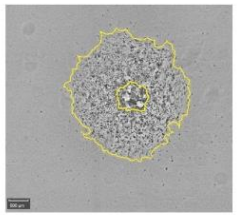
Cell death (flow cytometry)

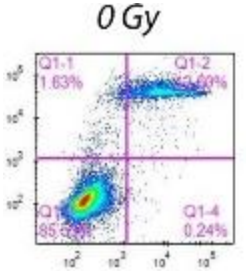


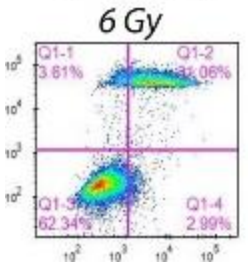


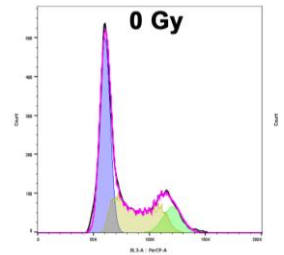


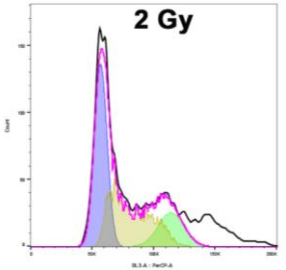






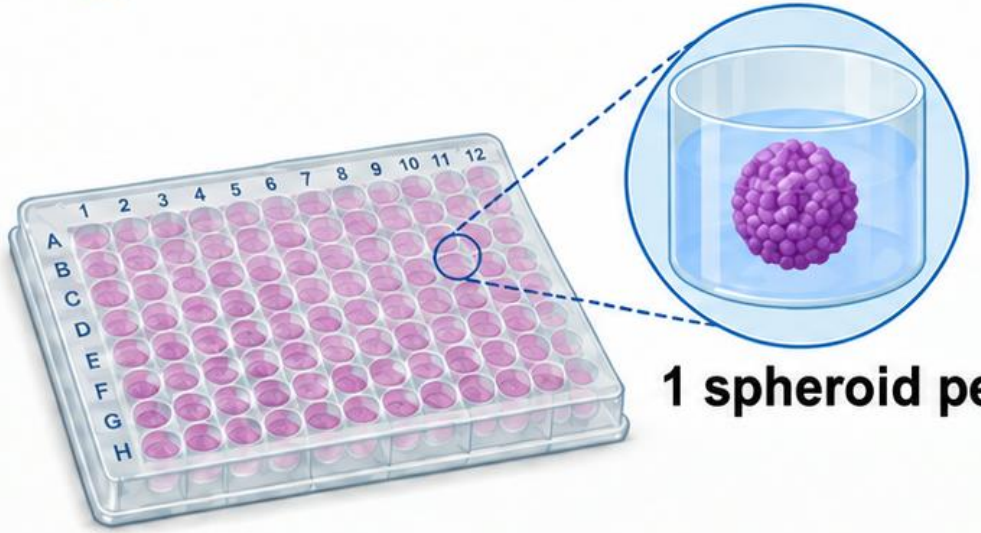






Experimental design

1 Seeding spheroids on 3-5 96-well plates



1 spheroid per well

2 Transportation from Pushchino to Dubna

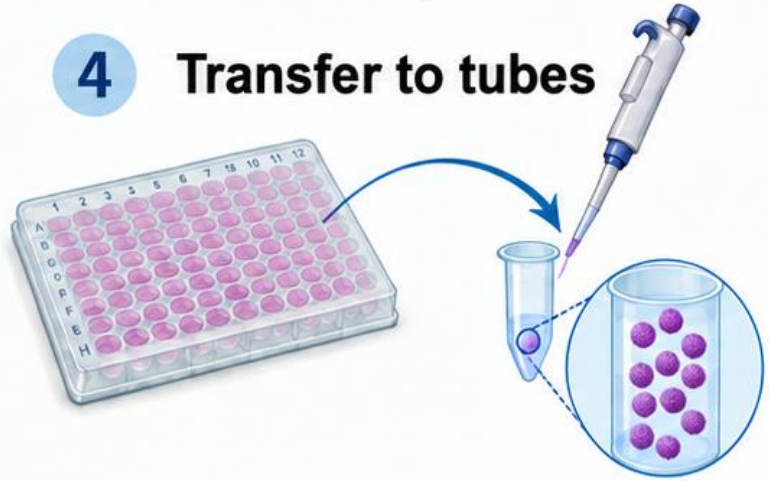
4-5 hours



3 Arrival at JINR



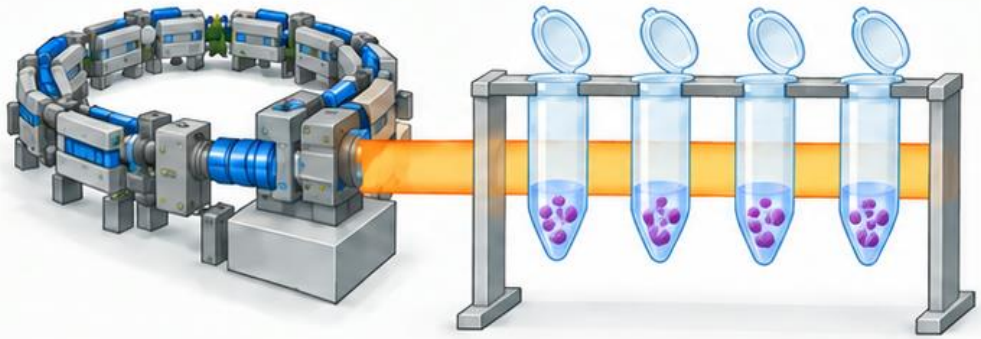
4 Transfer to tubes



10 spheroids per tube

5 Irradiation at NICA facility

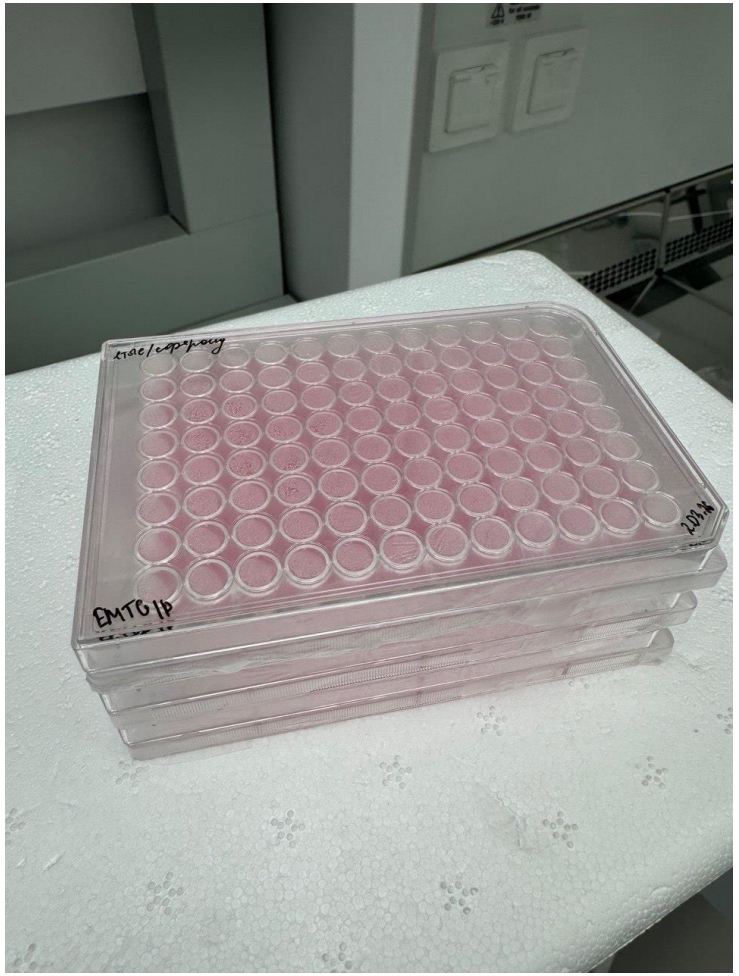
24 hours



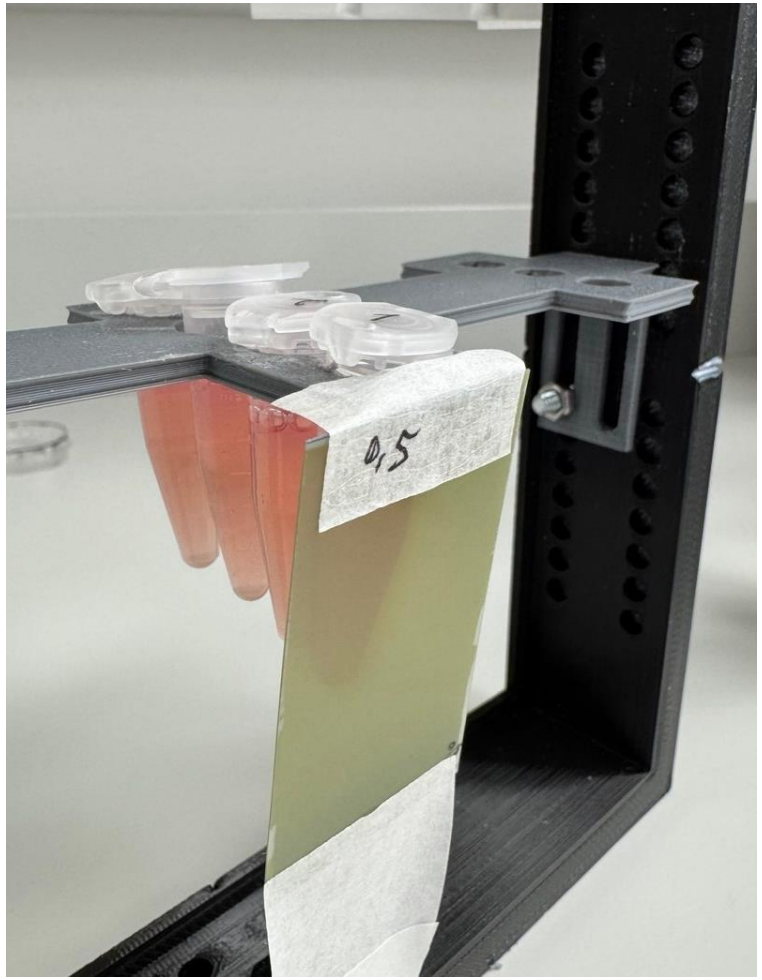
6 Transportation back to Pushchino



Sample preparation at the NICA facility upon arrival

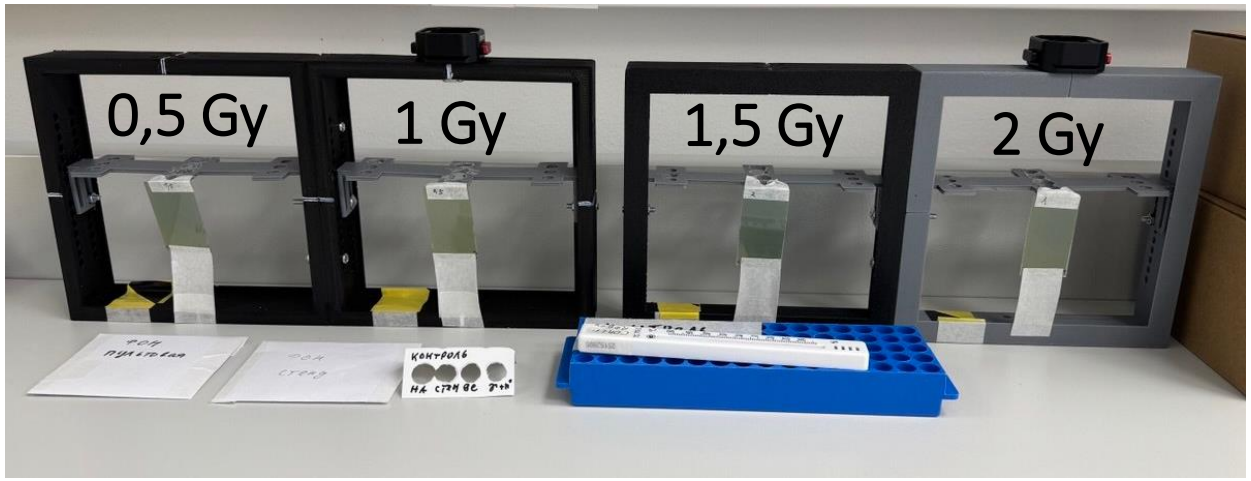


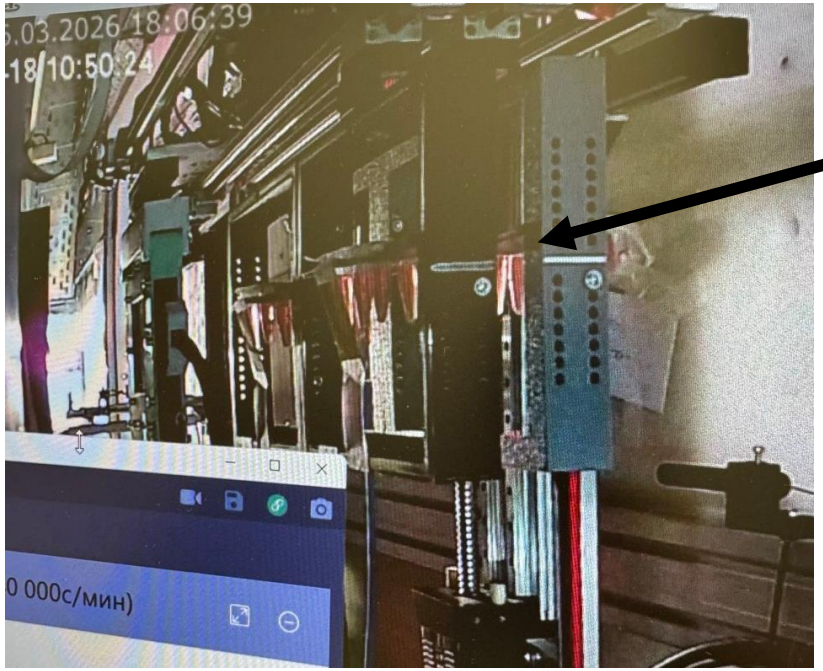




Upon arrival, the plates were placed in an incubator pending the signal to begin preparations for irradiation. Once the signal was given, the spheroids were transferred into tubes. Four tubes (1.5 ml) per frame constituted a single dose. The photograph shows the dosimetry film positioned at the front. In total, there were six groups: four experimental and two control groups (one in the irradiation room to measure background radiation, and one outside the irradiation zone

Irradiation at the NICA facility





Experiment No. 1: Energy 1.67 GeV/nucleon, pulsed beam (35/42 s).

Doses: 2 Gy (actual mean 1.83 Gy), 1.5 Gy (1.42 Gy), 1 Gy (0.91 Gy), 0.5 Gy (0.46 Gy), accounting for beam movement from the 1st to the 4th Eppendorf tube.

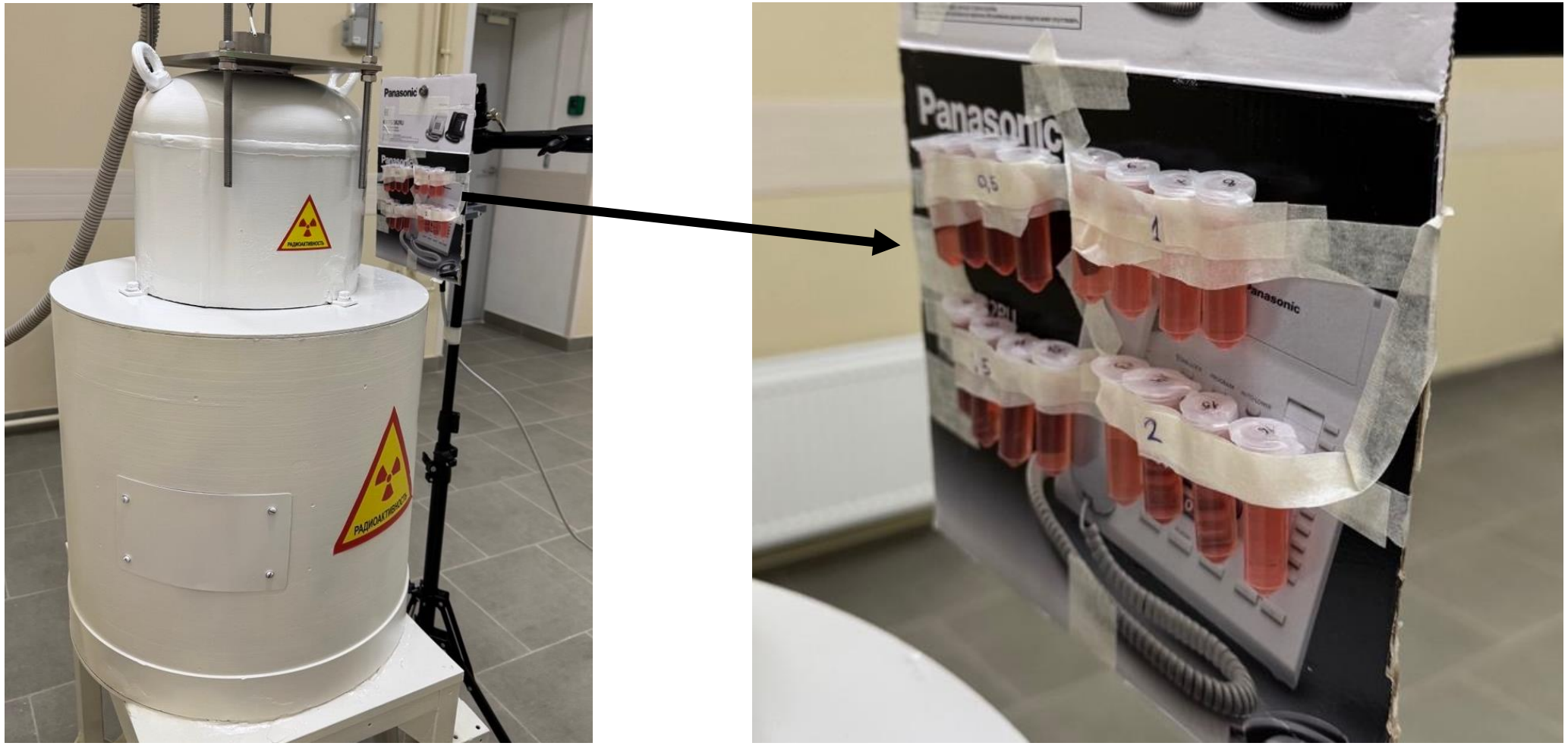
Prior to irradiation, the samples were positioned 40 cm from the center of the beam - that is, offset to the side. A control for background radiation was placed 40 cm away from the beam.

Experiment No. 2. Energy: 3 GeV/nucleon; pulsed beam (18/22 s).
Doses: approximately the same as in Exp. No. 1

The samples were suspended 30 cm from the beamline axis, awaiting irradiation. An average distance of 30–40 cm can be cited.

Temperature around was 27–29°C

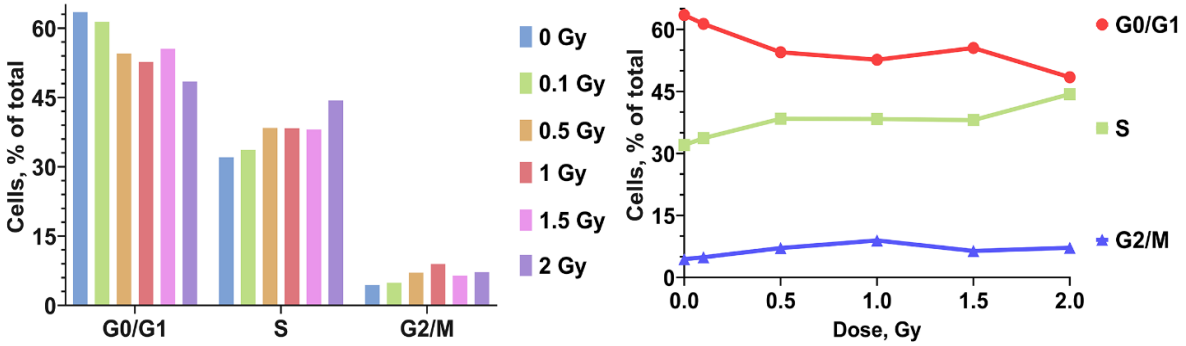
Gamma irradiation as a reference



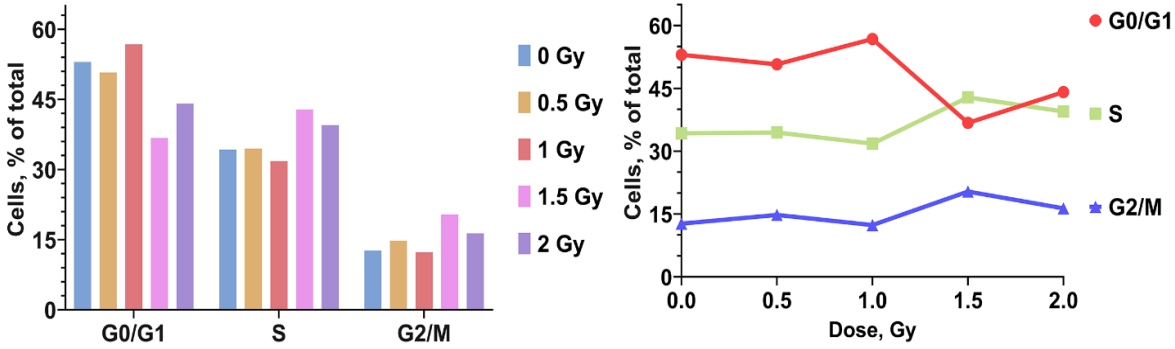
The 3D cell spheroids were immediately secured in the tubes and irradiated (in a single session, given the unrestricted access). Irradiation was performed using a Cs-137 gamma source (UPGD-2M/GOBO-60/IGTs-7-2 unit; activity up to 1.85×10^{12} Bq; source date: Nov 21, 2025; dose rate: approx. 1 Gy/h).
The focal distance was 25 cm. The dose was controlled by adjusting the exposure time at the source.

Cell cycle analysis *via* flow cytometry

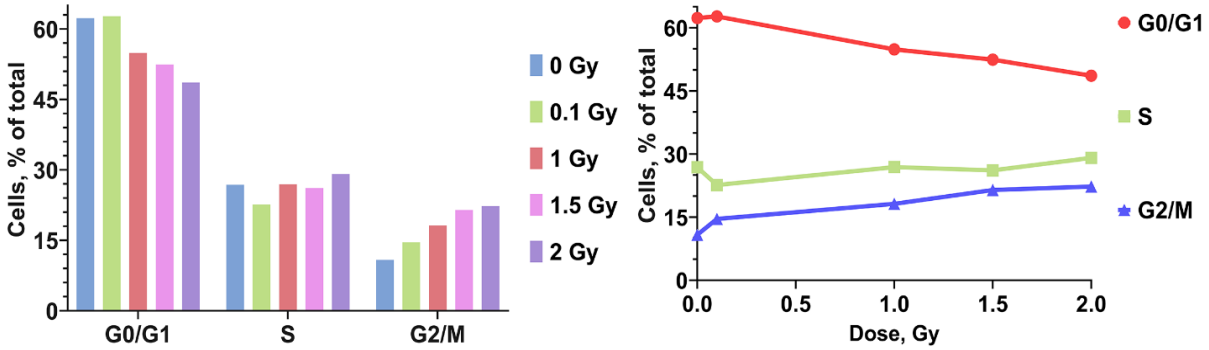
Xenon № 1 **1.67 GeV/nucleon, pulse 35/42 sec**



Gamma **Cs-137, dose rate of 1 Gy/h**



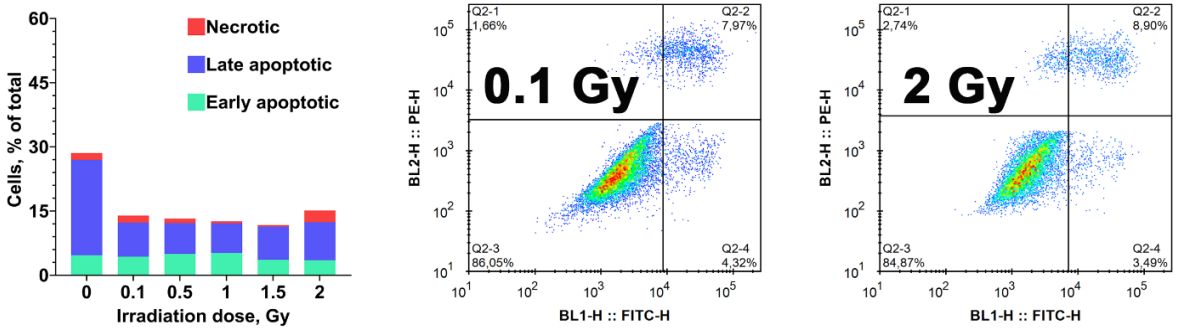
Xenon № 2 **3 GeV/nucleon, pulse 18/22 sec**



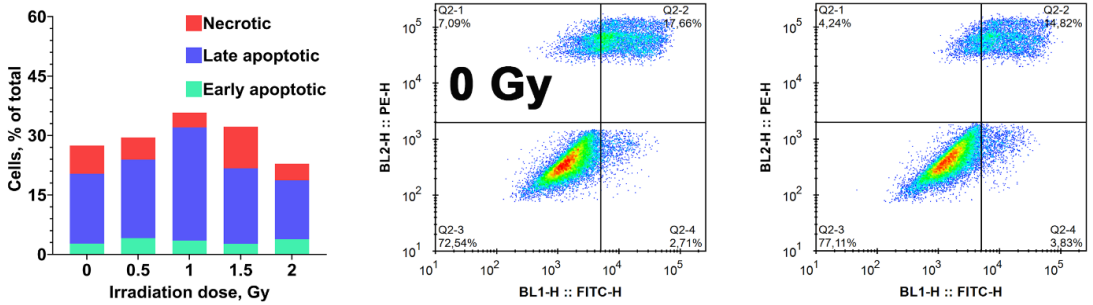
Irradiation was accompanied by a decrease in the proportion of G0/G1-phase cells and a redistribution of cells into the S and/or G2/M phases.

Cell death analysis *via* flow cytometry

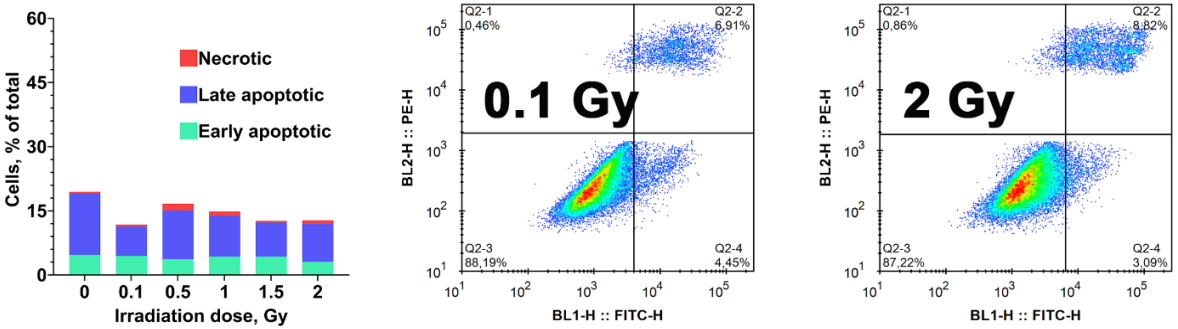
Xenon № 1 **1.67 GeV/nucleon, pulse 35/42 sec**



Gamma **Cs-137, dose rate of 1 Gy/h**



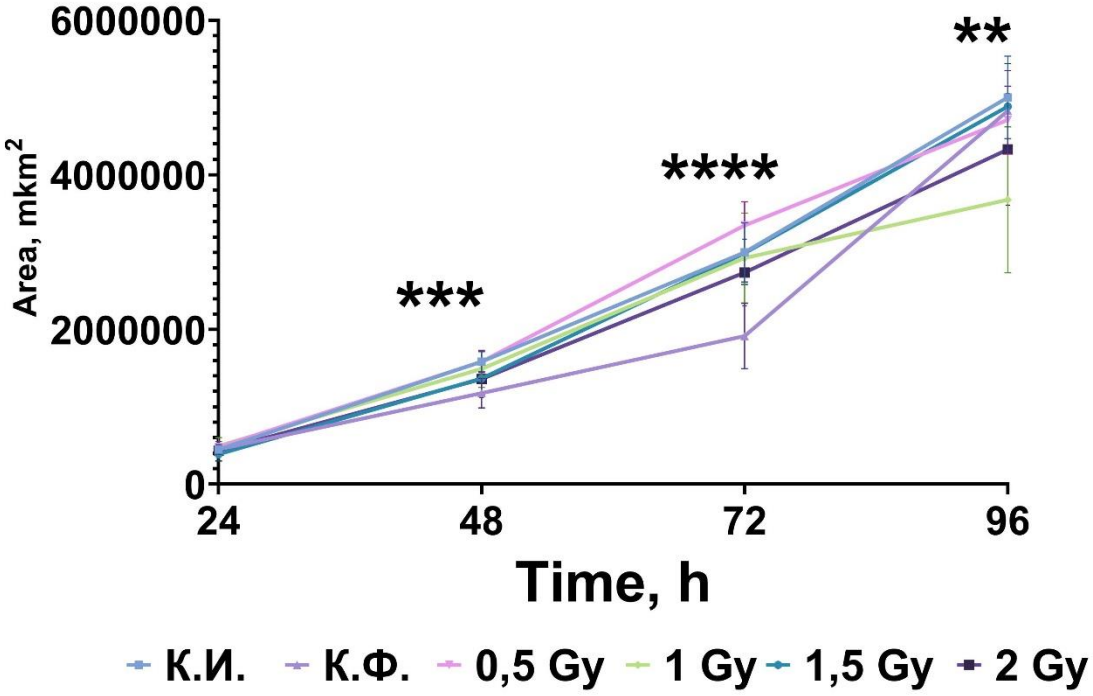
Xenon № 2 **3 GeV/nucleon, pulse 18/22 sec**



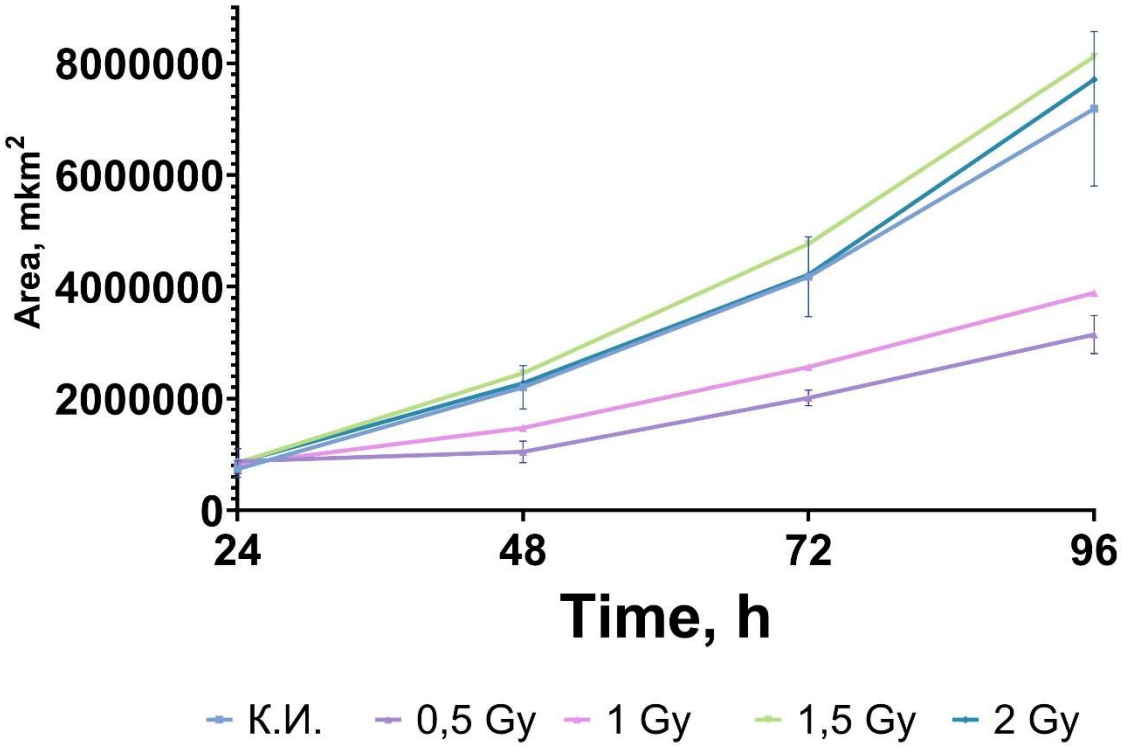
No consistent dose-dependent increase in apoptosis or inhibition of spheroid spreading was observed at the time point analyzed.

Cell migration analysis *via* microscopy

Xenon № 2



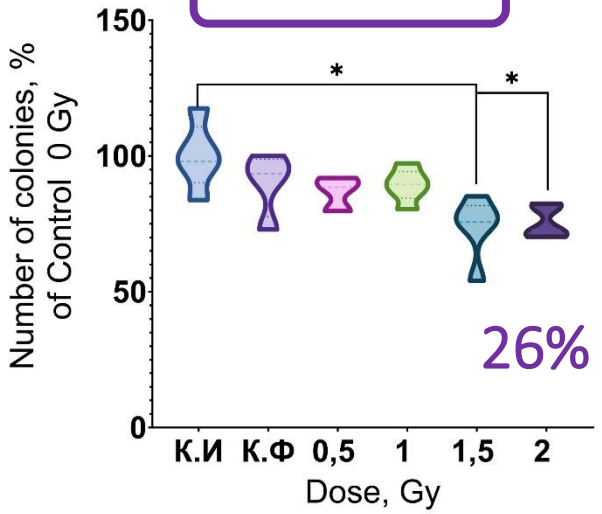
Gamma



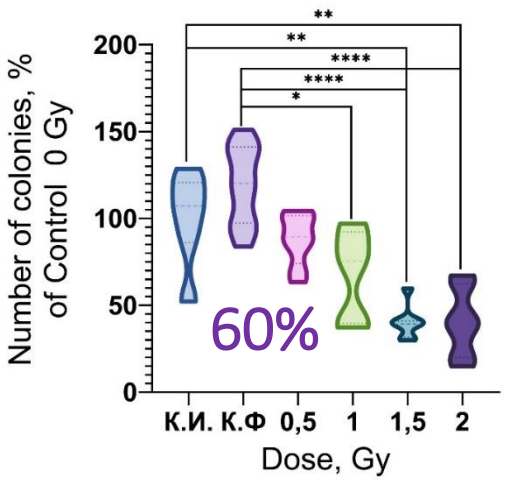
No decrease in the migratory activity of spheroid cells is observed with increasing radiation dose. Cell migration in the other groups remains at the control level. Thus, the results regarding migratory activity do not correlate with the results regarding spheroid cell survival following irradiation.

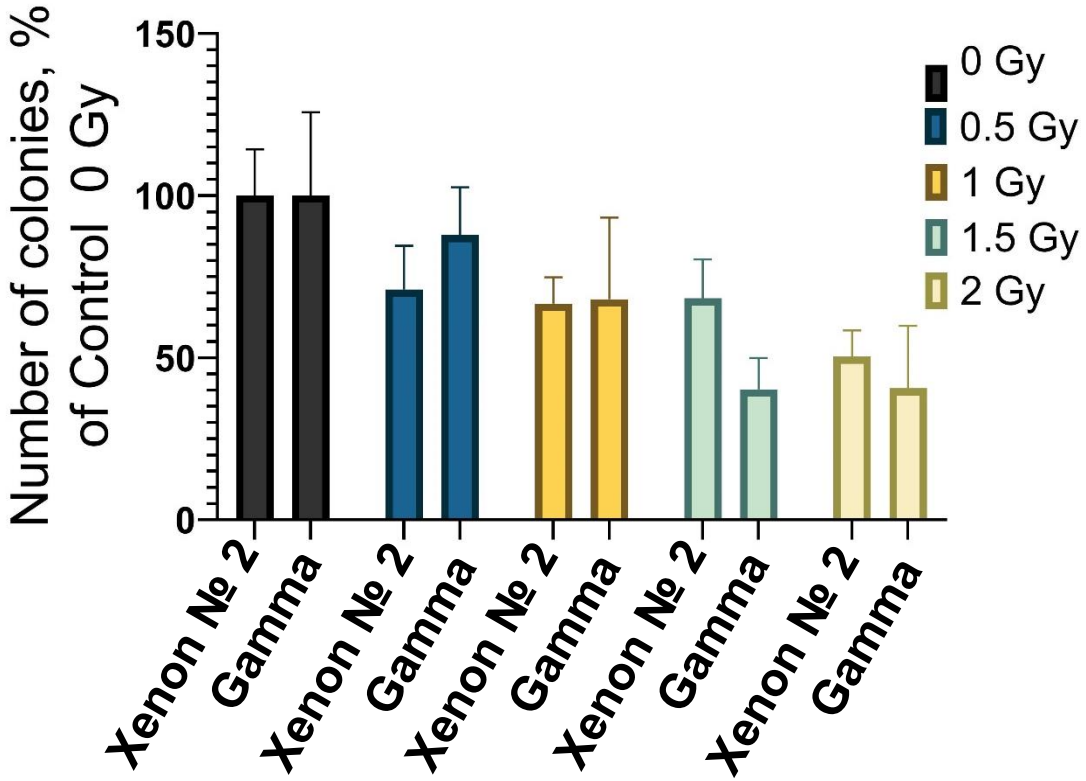
Clonogenic activity analysis *via* colony assay

Xenon № 1

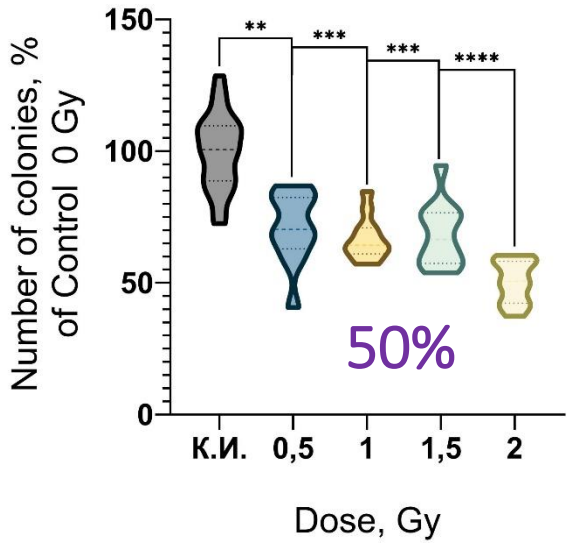


Xenon № 2





Gamma



The most pronounced decrease in clonogenic activity following xenon exposure was observed in the second series at nominal doses of 1.5–2 Gy.

Conclusions

The first radiation biophysics experiment at the NICA facility!

- 3D spheroids proved to be a suitable and relevant model for this study.
- Xenon irradiation was accompanied by a decrease in the proportion of G0/G1-phase cells and a redistribution of cells into the S and/or G2/M phases.
- No consistent dose-dependent increase in apoptosis or inhibition of spheroid spreading was observed at the time point analyzed.
- Analysis of cell migration activity is not considered a viable method for future analysis due to low sensitivity at these radiation doses.
- Three experimental series revealed a reduction in the colony-forming ability of EMT6/P spheroid cells following exposure to xenon ions and gamma radiation at 1.5–2 Gy.
- The clonogenic assay is the most accurate and reproducible method.
- The data obtained do not demonstrate an advantage of xenon over gamma radiation, nor do they indicate an independent effect of ion energy on the magnitude of the response.

Thank you for your attention!

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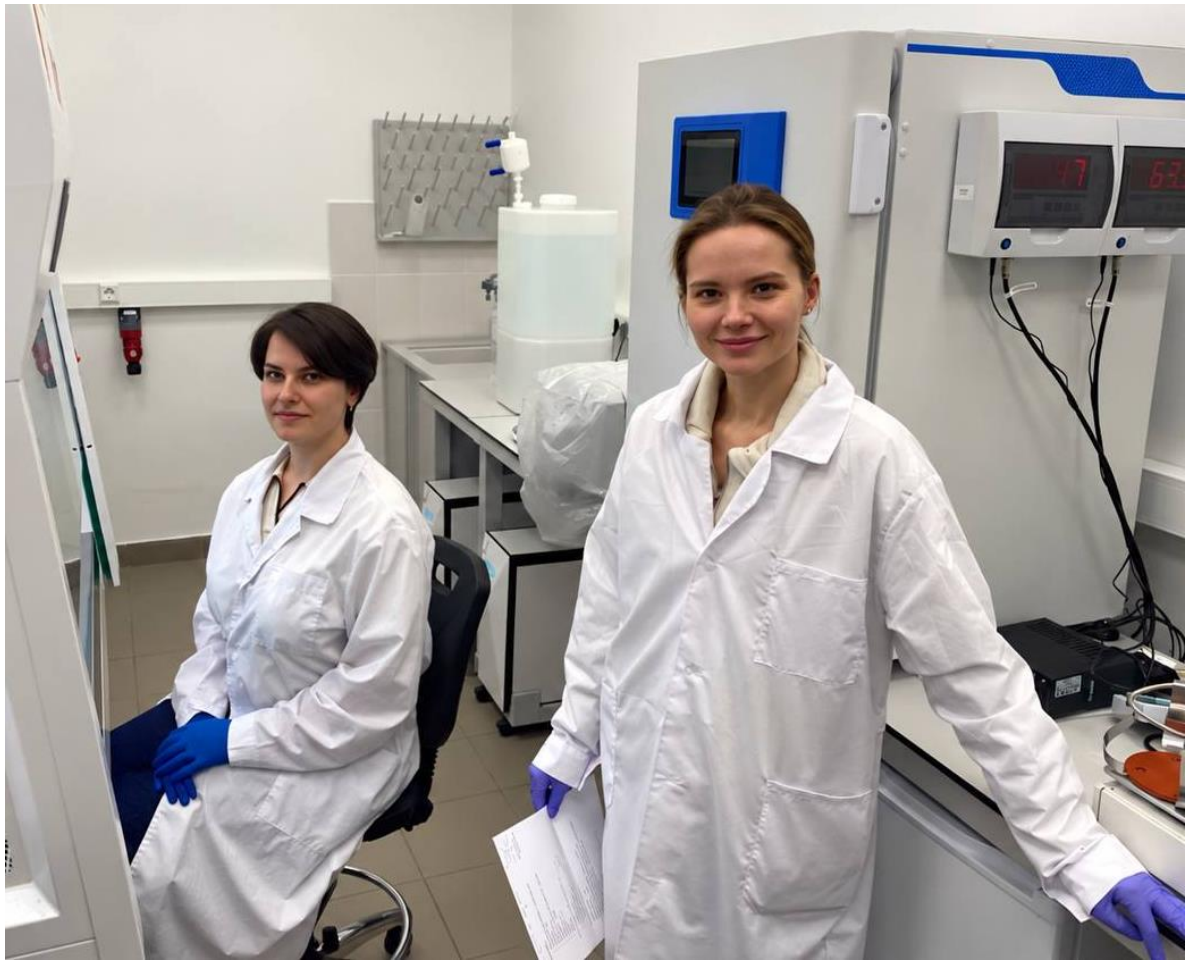



Technical conclusions following Experiments No. 1 and 2

1. Experiments No. 1 288 spheroids.
2. Experiments No. 2 480 spheroids
3. Optimal size of 3D cell spheroid is 350 µm.
4. Transport multiple spheroids (12–20 psc) directly in tubes without them fusing together.
5. Transportation conditions are optimal and do not affect the viability of the spheroids.
6. Requirement for matching radiation doses
7. Requirement additional experiments for statistical replicates

Publication Report

2026

- N.R. Popova, A.I. Kornienko, N.N. Chukavin, D.D. Kolmanovich, B.A. Bokl, I.V. Savintseva, A.L. Popov, O.V. Belov, **INVESTIGATION OF THE EFFECTS OF XENON ION BEAM IRRADIATION ON EMT6/P 3D TUMOR SPHEROIDS WITHIN THE NICA PROJECT AT JINR**, Тезисы на 30-я Международная научная конференция AYSS-2026 (Ташкент, Узбекистан).

- Сорокина С.С., Громова Д.С., Белов О.В., Попова Н.Р. **РОЛЬ МИКРОГЛИИ В РАДИАЦИОННО-ИНДУЦИРОВАННЫХ ИЗМЕНЕНИЯХ В МОЗГЕ: ОТ НЕЙРОПРОТЕКЦИИ ДО НЕЙРОВОСПАЛЕНИЯ**. Радиация и риск. №3, 2026

- Svetlana S Sorokina, Anton E. Malkov, Elizaveta A Zamyatina, A.E.Shemyakov, Oleg V. Belov, Nelli R Popova. **Impact of Helium Ions on Mouse Behavior, Memory, Microglia, and Blood Metabolome**, Biology, Q1 2026 (принята)

What's next? Plans.

1. Study of the radiobiological effects of irradiation in a high-dose mode (flash mode) under conditions of tumor hypoxia using three-dimensional spheroids of glioblastoma and pancreatic cancer.
2. Study of early and long-term neurobiological consequences of local cranial irradiation with protons and heavy ions.
3. Study of the sequential and combined effects of protons and heavy ions on tumor and normal biological objects in vitro.
4. Development of organoid-specific biosensor systems to study the molecular mechanisms of radiation-induced damage.

We are awaiting the technical capability to work with animals.