

Recapitulating the Hematopoietic Response to Ionizing Radiation in a Human Bone-Marrow-on-a-Chip

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4-Defense Biotechnology, Biomanufacturing and Bioprinting Center



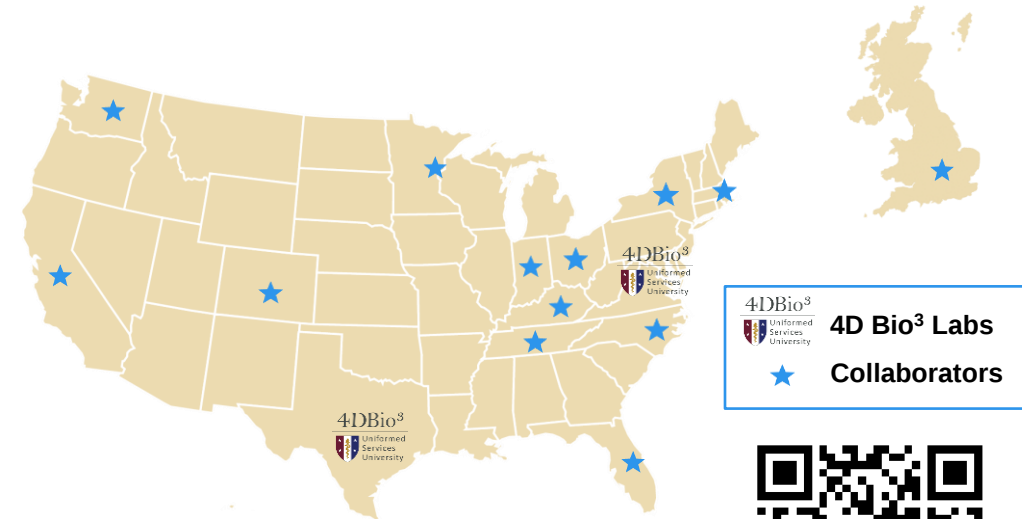
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The collage consists of six images arranged in a 2x3 grid. The top-left image shows two military personnel in camouflage uniforms attending to a patient in a medical setting. The top-middle image shows a laboratory setup with a blue motor and glass vials. The top-right image shows a white, curved, translucent object. The bottom-left image shows a man in a military uniform standing next to a presentation board. The bottom-middle image shows a man in a dark jacket looking at a large black cylindrical object. The bottom-right image shows a complex mechanical device with yellow components.

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- **Readiness** (organ-on-chip, CBRNE biomarkers and countermeasures, brain health)
- **Point-of-Need Devices** (austere manufacturing, novel biologics, biosurveillance, technology proving ground)
- **On-Demand Blood** (cell therapy, blood products, austere bioreactor)

4D Bio³ co-authors & collaborators



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USU collaborators:

- John E. Slaven, MS
- Regina M. Day, PhD

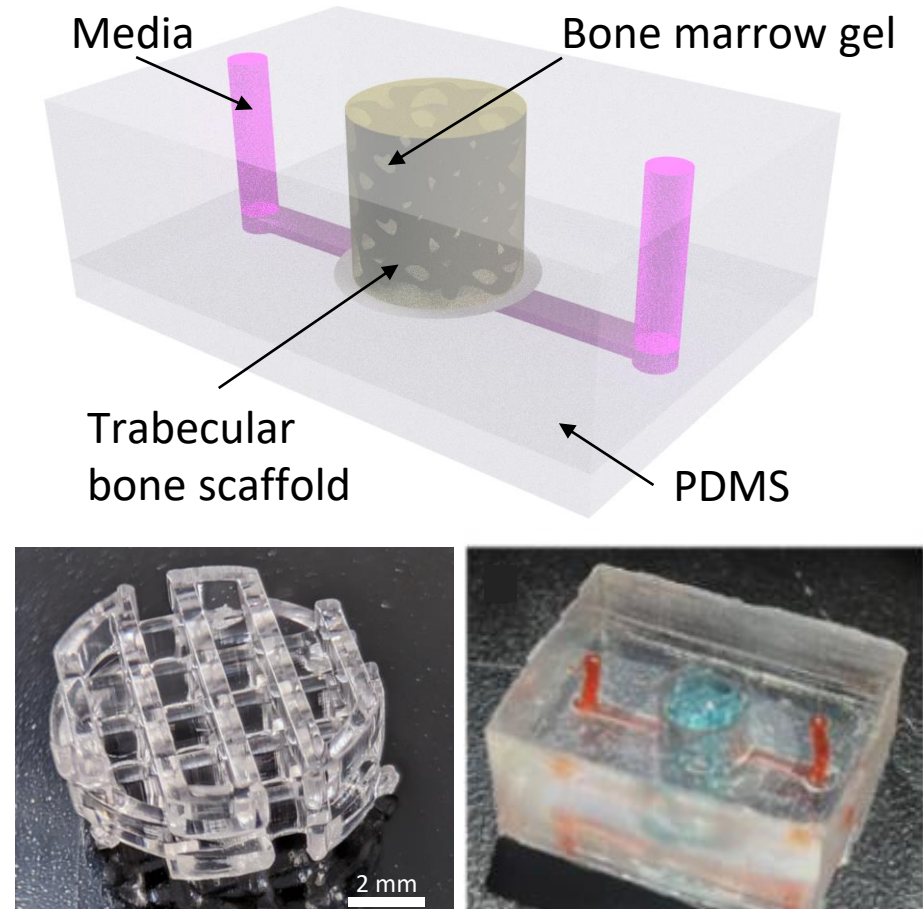
The need for new radiation models

- Effects observed in animal models are not necessarily representative of those seen in humans
- Animal models are expensive, time consuming, and have ethical concerns
- Histological sectioning of bone marrow requires decalcification of the tissue, a process which destroys important epitope markers

Goal: develop an *in vitro* model of bone marrow that can be used to discover and evaluate potential radiation countermeasures

Bone Marrow on Chip (BMoC) model

- 3D model containing important aspects of the bone marrow
- Primary human cells
 - CD34⁺ hematopoietic stem cells (HSCs)
 - Bone marrow stromal cells (BMSC)
 - Umbilical vein endothelial cells (HUVECs)
- Cells encapsulated in a hydrogel, which is cast around a porous 3D printed scaffold

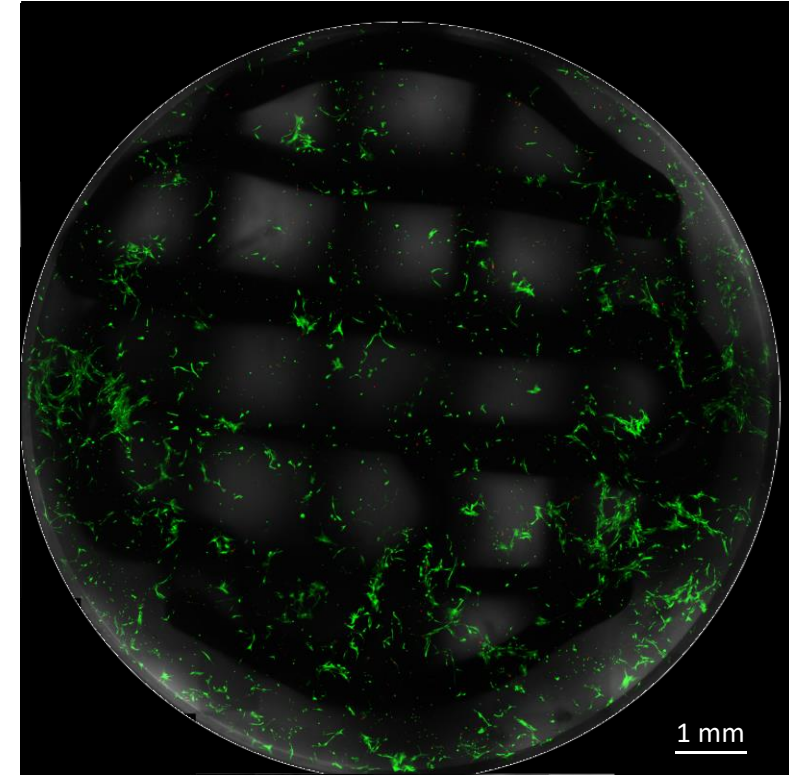
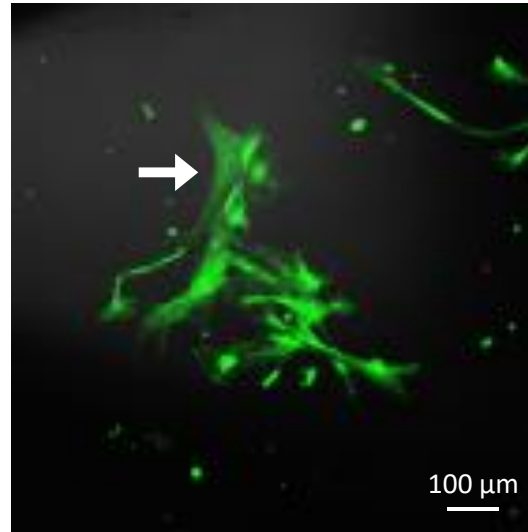
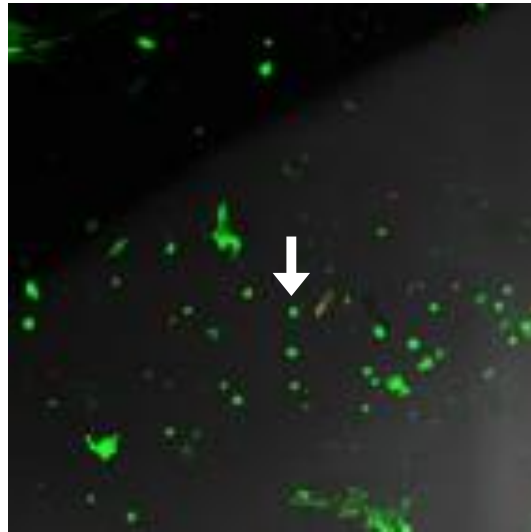
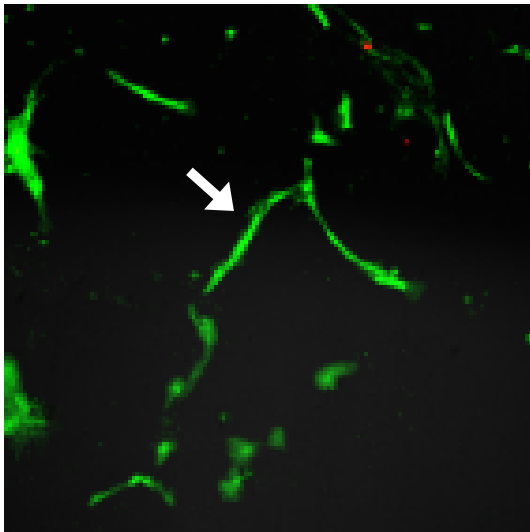


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BMoC supports cell growth and viability

- High viability of the triculture was observed after 10 days of culture in the hydrogel and 3D printed scaffold
- Various cell morphologies observed with Live/Dead assay



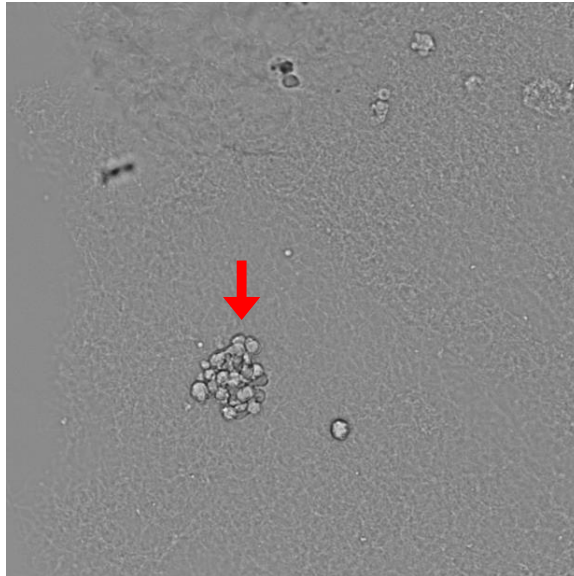
Substantial HSC proliferation in BMoC

- HSCs were expanded in culture for 9 days and placed in the BMOc model with supporting cells ('Day 9')
- BMOc were cultured for an additional 10 days

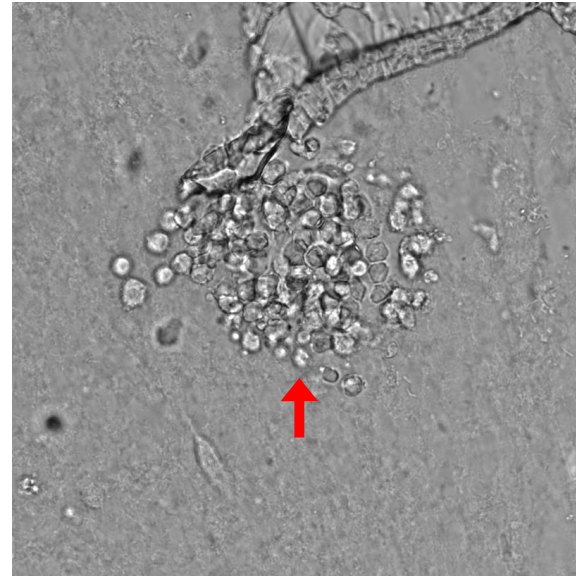
Day 10



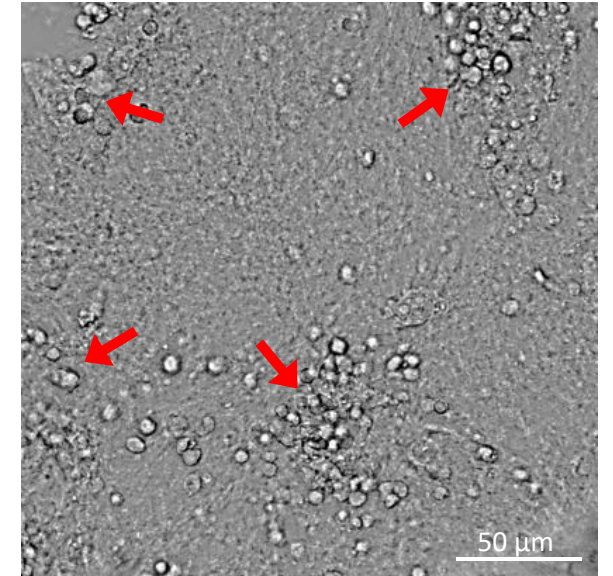
Day 14



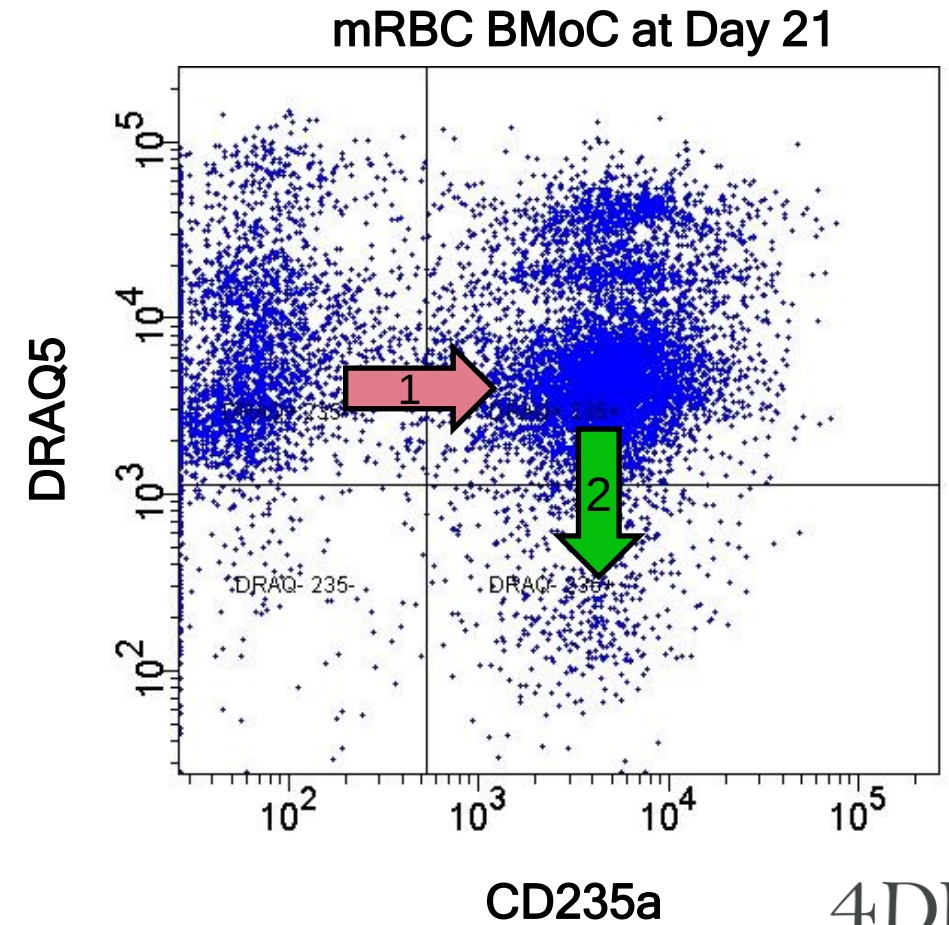
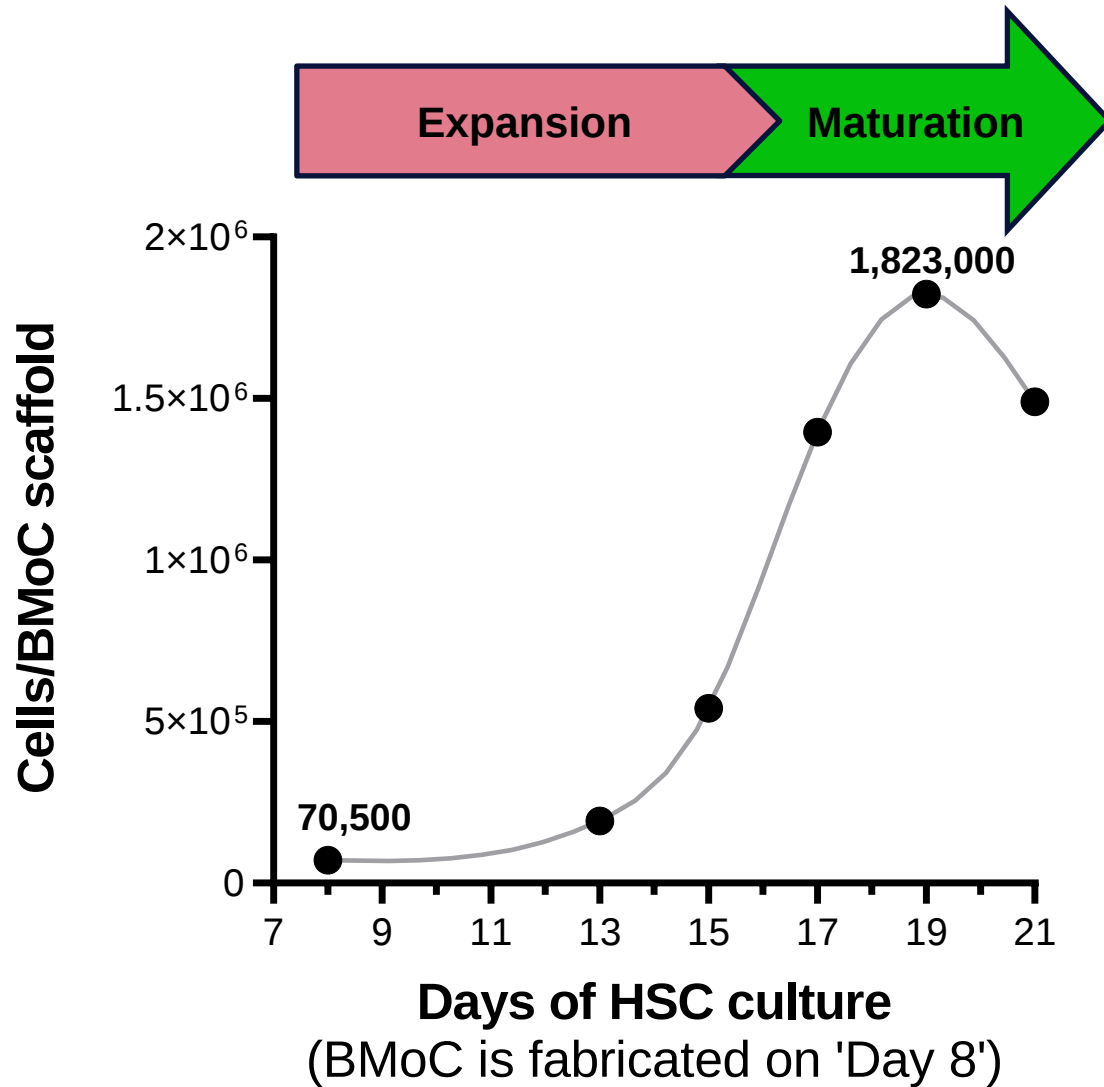
Day 17



Day 19



Proliferation & erythropoiesis in BMoC

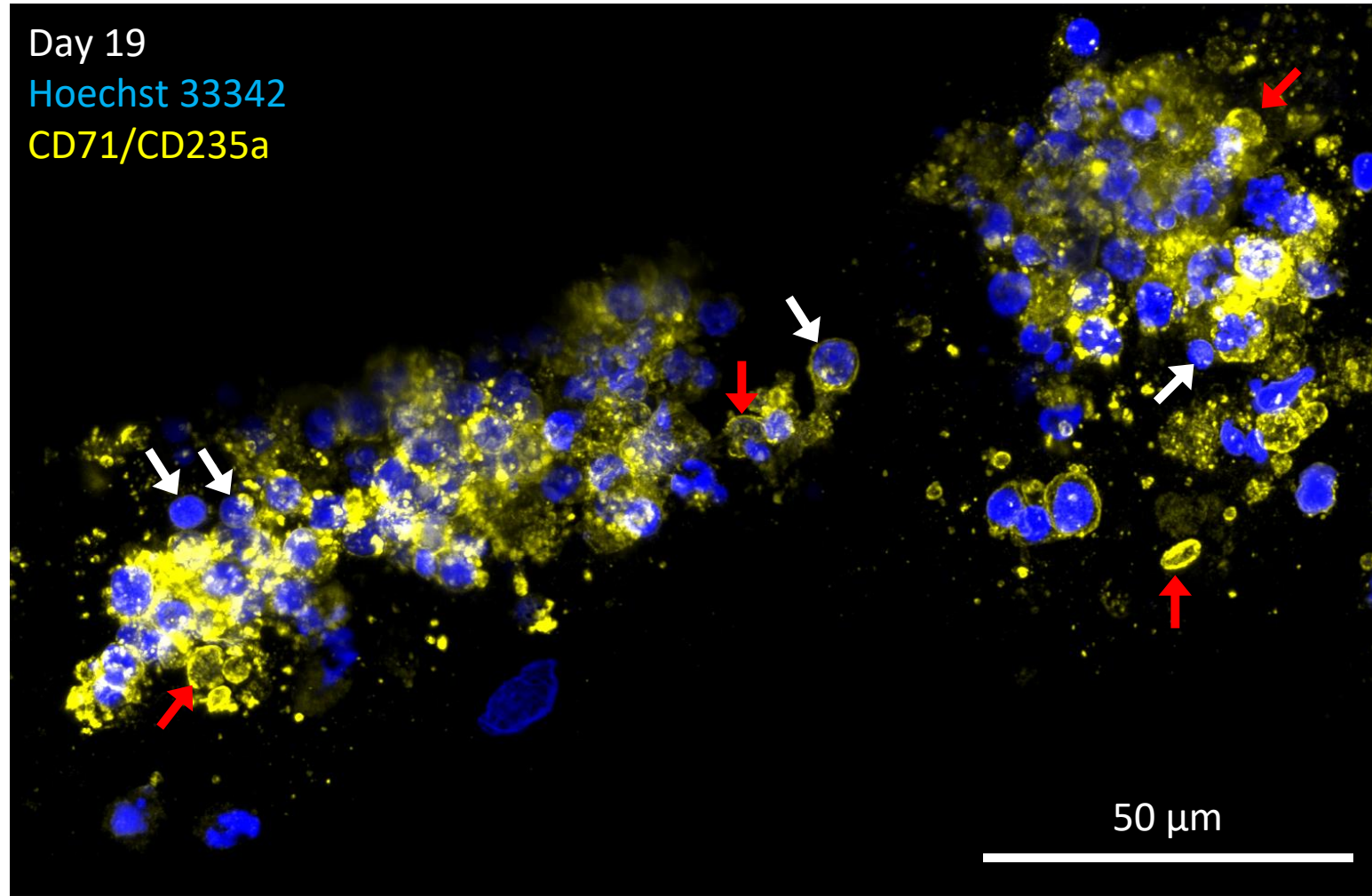
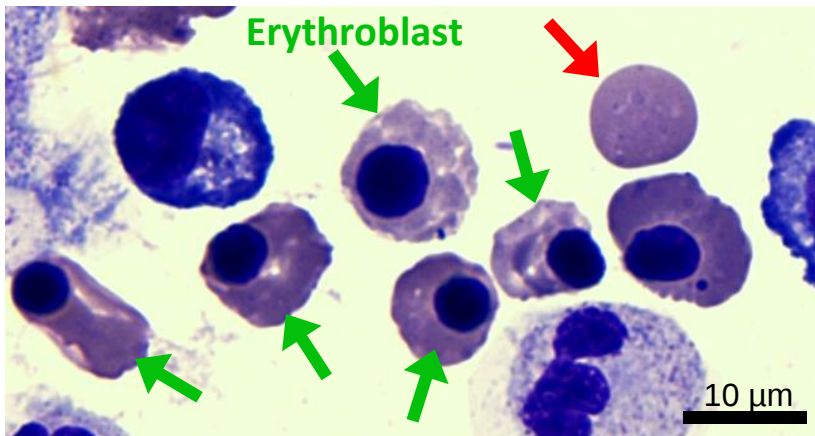


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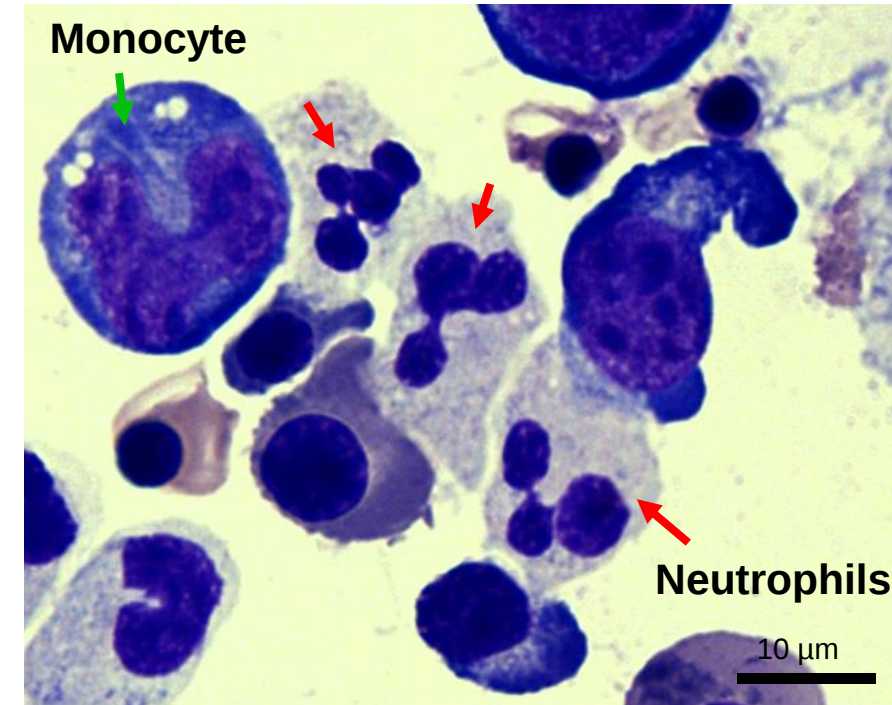
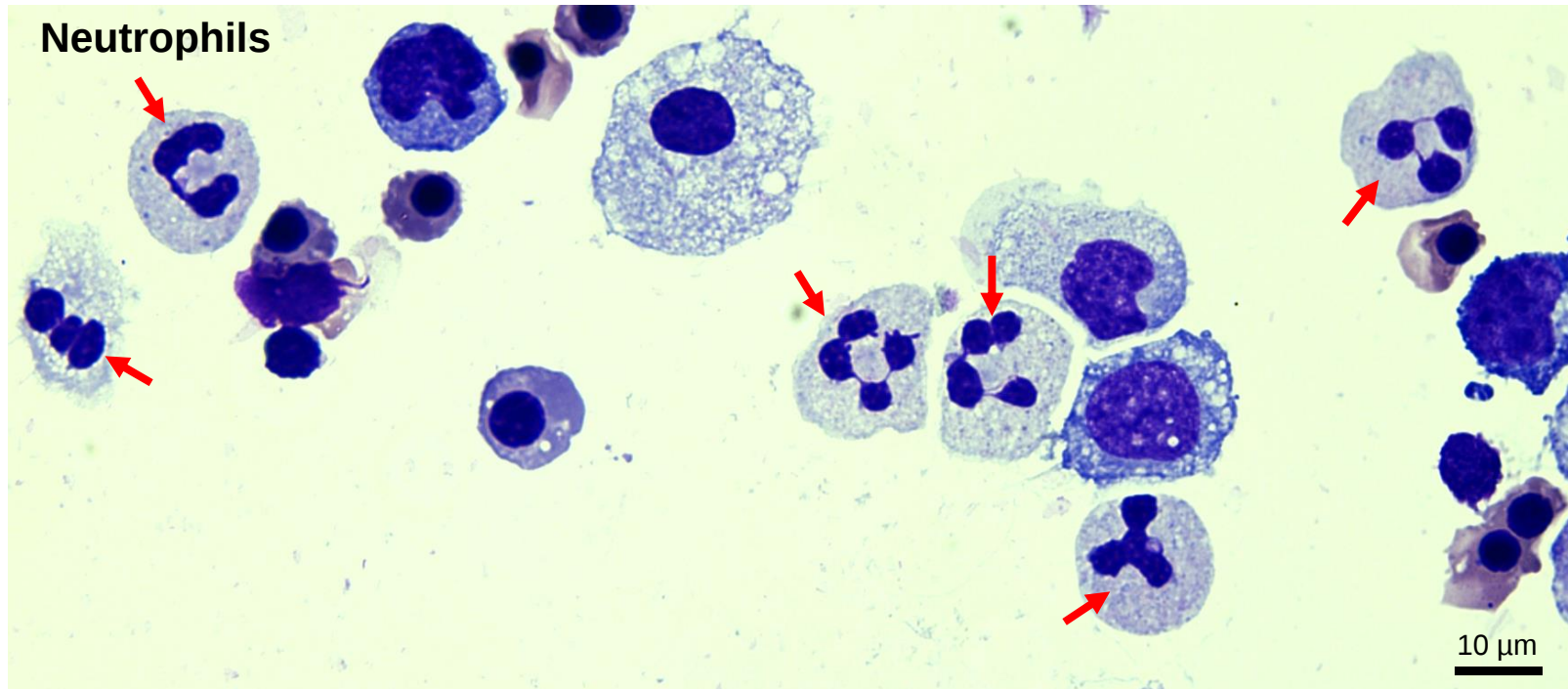
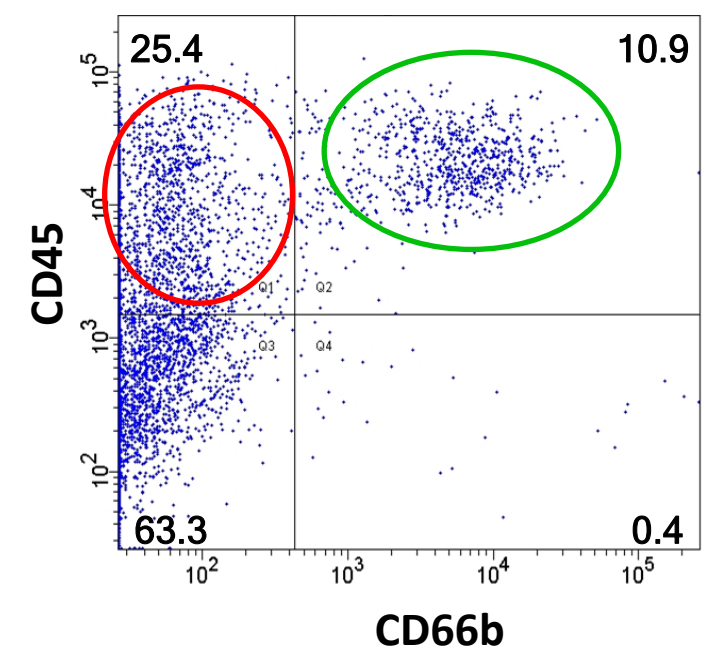
BMoC supports erythropoiesis

- • Clumps of cells contained enucleated reticulocytes/erythrocytes (RBC) measuring 5-8 μm in diameter (D19)
- ⇒ • Nuclei ejected from enucleating RBC (pyrenocytes) were also found



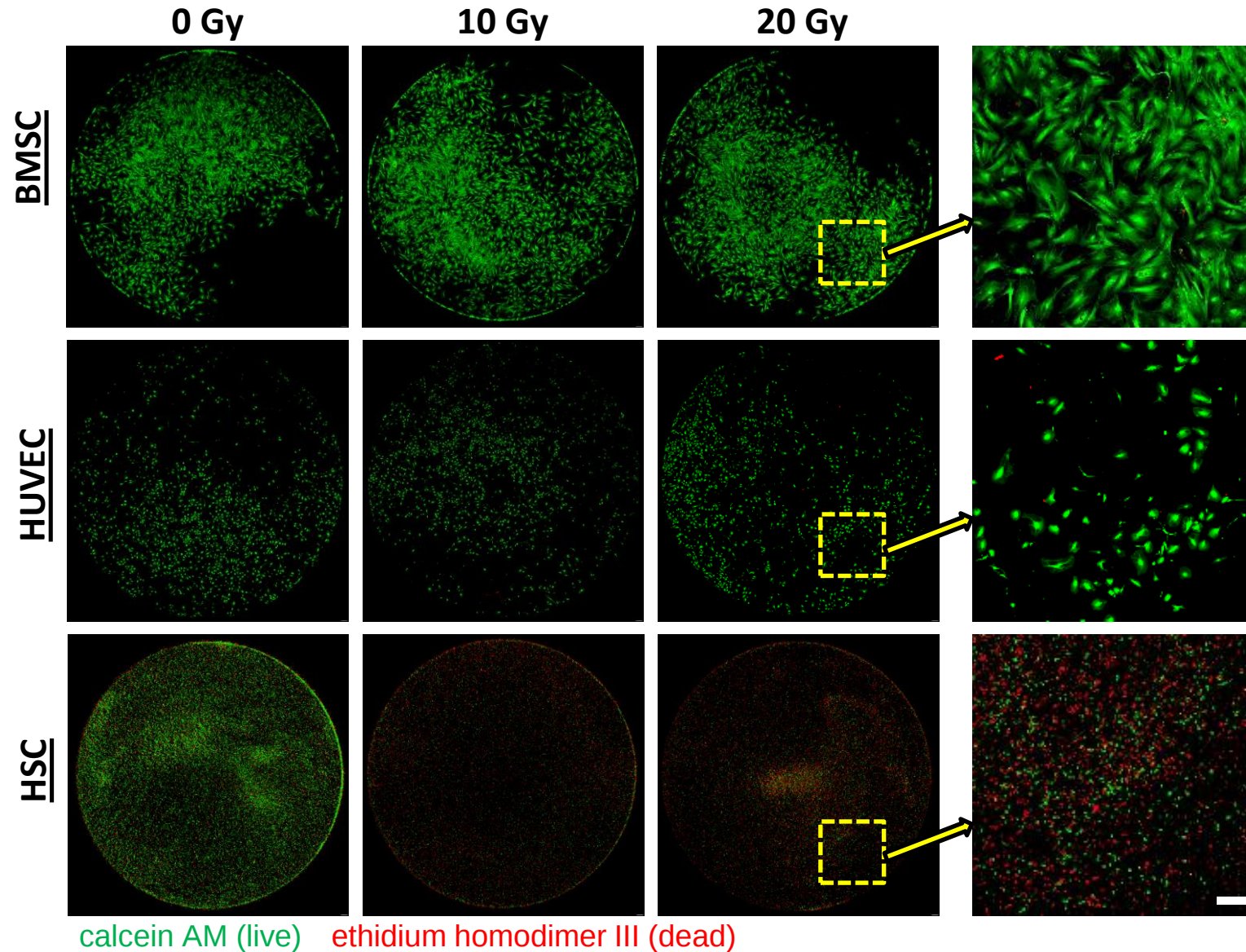
BMoC supports differentiation of myeloid lineage cells

- Cytospins were conducted to identify cell types in the BMoC gels and in expended media



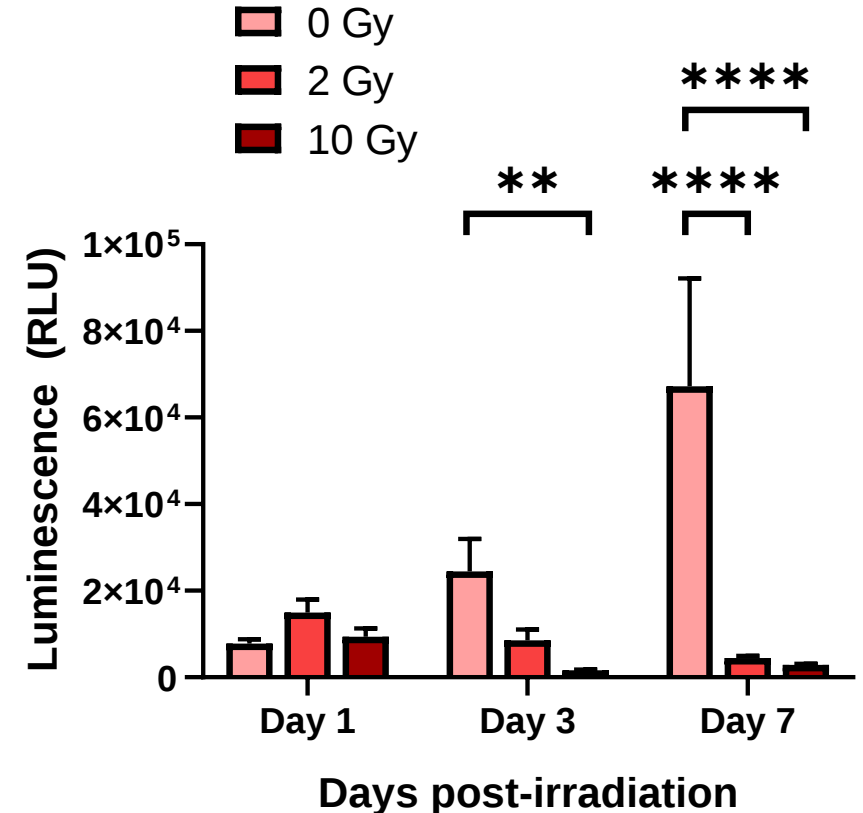
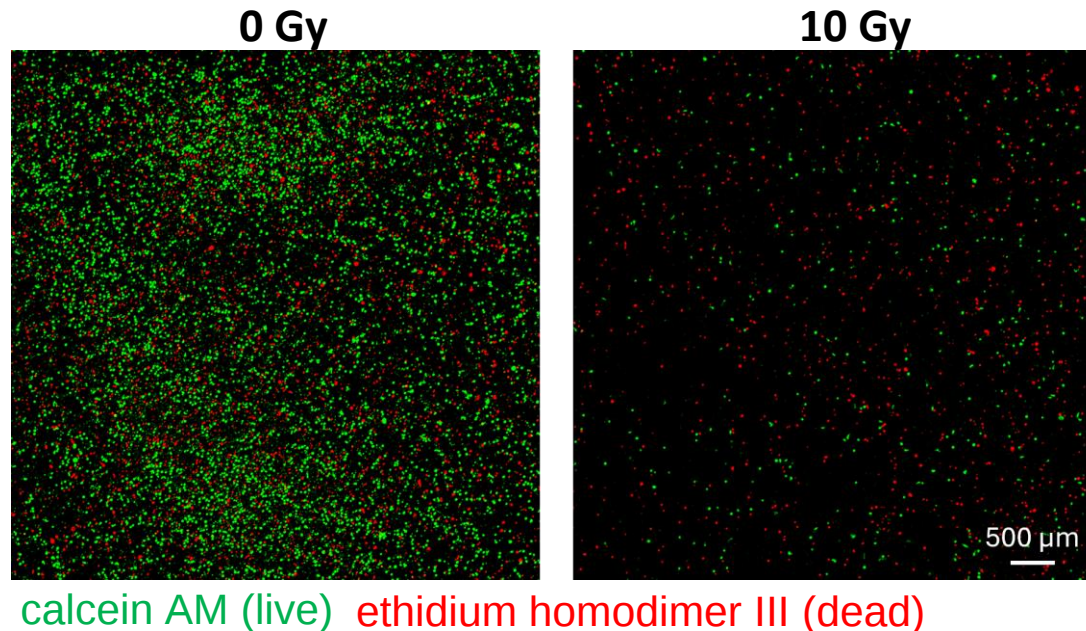
Radiation response differs by cell type

- Monocultures of cells in well plates were irradiated and assessed with **Live/Dead** 72 hours later
- RS2000 x-ray radiation source (1.16 Gy/min, 160 kV, 25 mA)
- 10 and 20 Gy had minimal impact on BMSC and HUVEC viability, but a profound effect on HSCs



CD34⁺ HSC are particularly radiosensitive

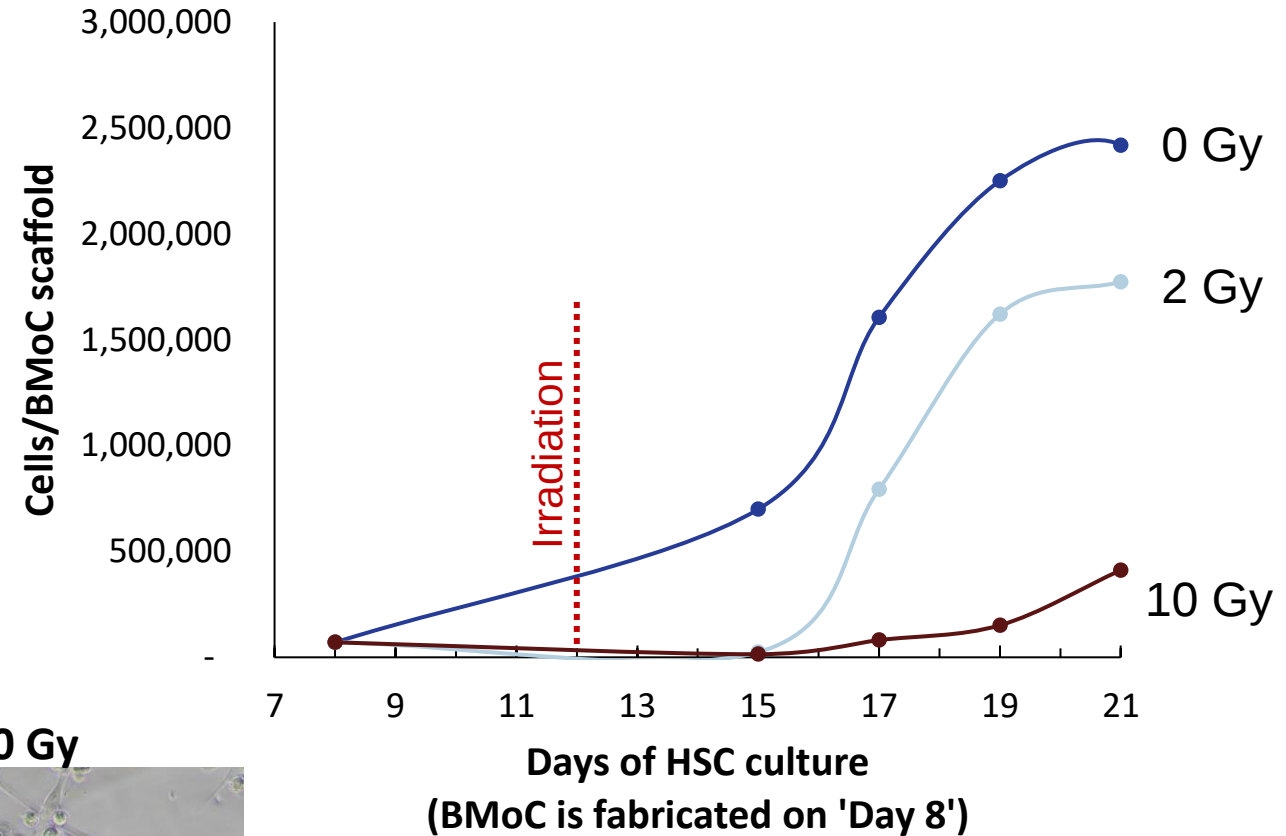
- ATP quantification showed a substantial increase in metabolic rate in non-irradiated controls over the culture period
- Significant decreases were seen at 2 and 10 Gy



** p < 0.01
**** p < 0.0001

Cell proliferation in irradiated BMoC

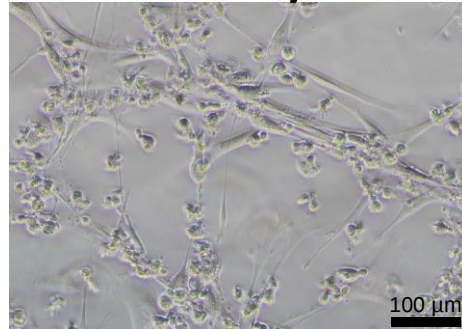
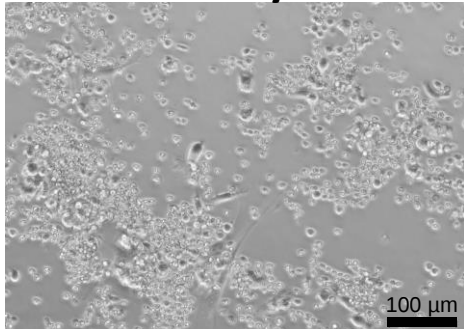
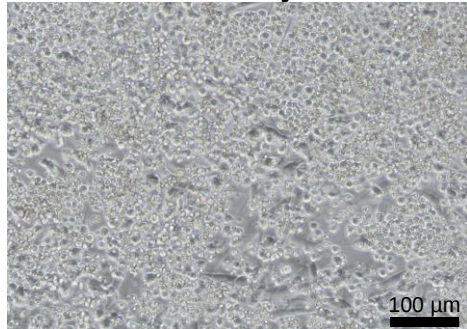
- Delayed expansion occurred in BMoC irradiated with 2 Gy while those irradiated with 10 Gy did not recover
- Hypertrophy observed and support cells (HUVEC, bMSC) represented a much larger share of total cell count



0 Gy

2 Gy

10 Gy



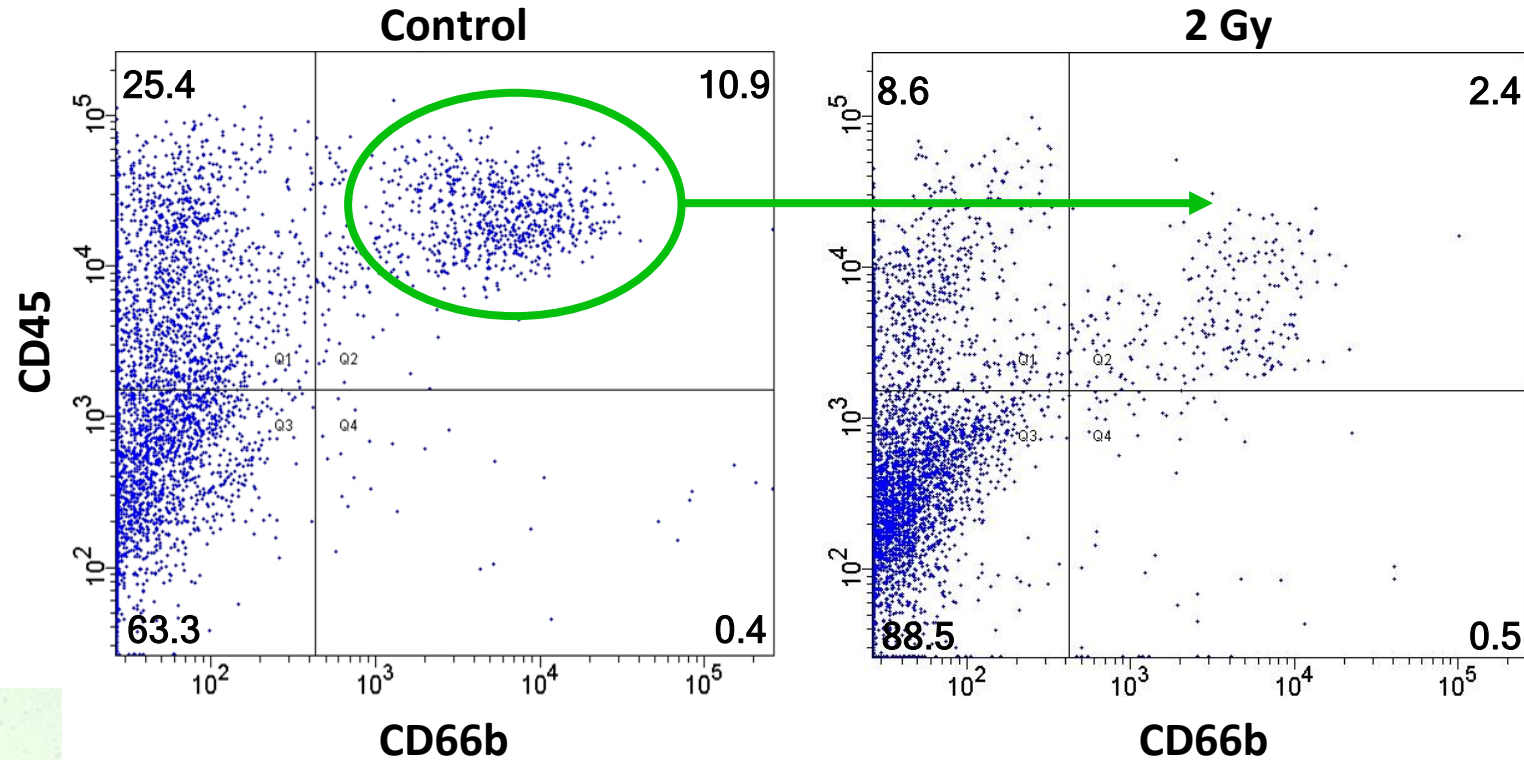
Day 21

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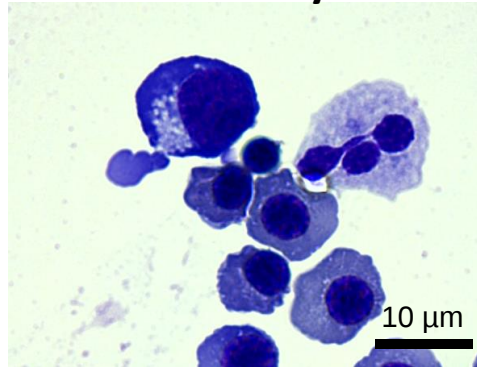
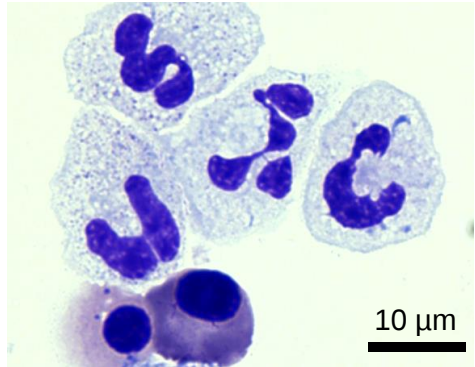
Neutrophil production is impacted at 2 Gy

- Even at 2 Gy, neutrophil production is markedly reduced
- Pan-leukocyte marker CD45 is also drastically curtailed at Day 21 of culture



Control

2 Gy



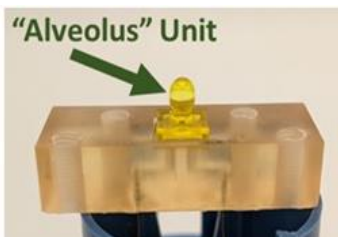
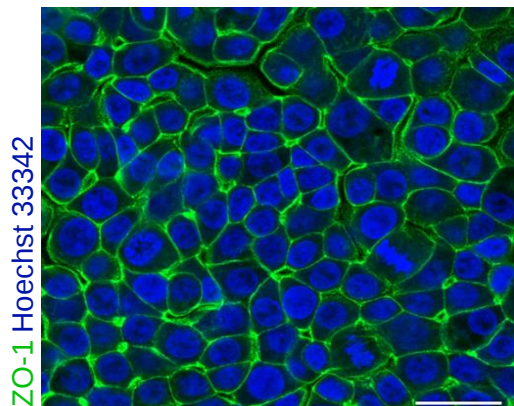
Day 21

Summary

- Demonstrated differentiation of various blood cell types in a BMoC model
- Recapitulated some of the known effects of radiation on hematopoiesis (*e.g.*, neutropenia)
- Future work will include integrating the BMoC model into a perfusion bioreactor system and test known countermeasures on hematopoiesis
- This BMoC model will enable studies of how radiation affects hematopoiesis using primary human cells and will offer a means to evaluate new countermeasures

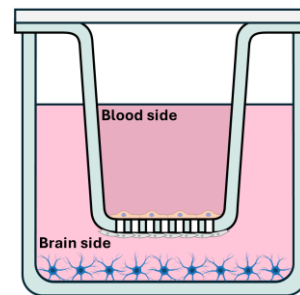
4D Bio³ & UMN Organ-on-Chip models

Lung

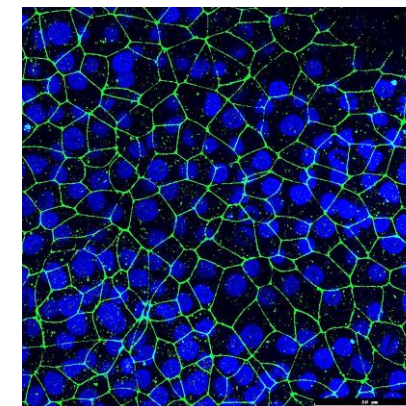


Angela Panoskaltis-Mortari, PhD (UMN)

Blood Brain Barrier



Samira Azarin, PhD (UMN)

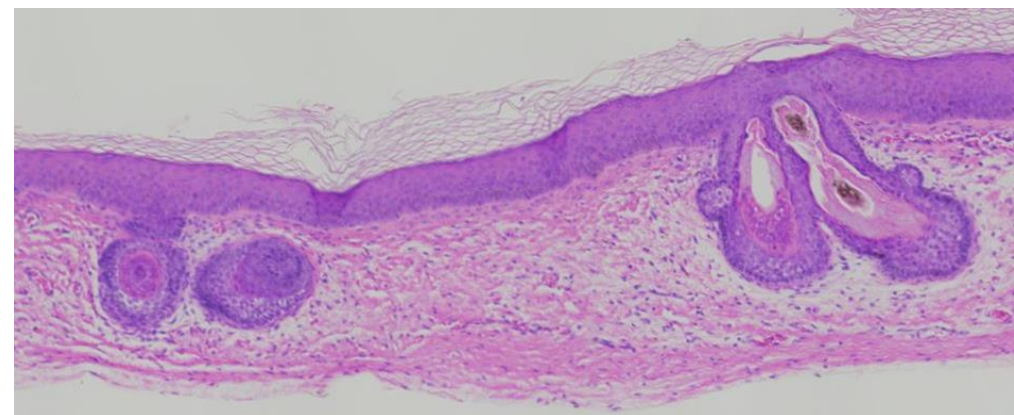


Cardiac



Brenda Ogle, PhD (UMN)

Skin



Tom Darling MD, PhD (USU)

Thank you for your attention

Acknowledgements:

- Armando Estrada, PhD
- Shweta Singh, PhD
- Shrayana Ghosh, MS
- John E. Slaven, MS
- Regina M. Day, PhD
- Vincent B. Ho, MD, MBA

Funding:

This research was funded by the Defense Health Program through USUHS Grant # HU00012020011 and administered by The Geneva Foundation.



USU & AFRRI:

- George Klarmann, PhD
- Tom Darling, MD PhD
- Alexandra Miller, PhD
- Gregory Holmes-Hampton, PhD
- Sanchita Ghosh, PhD



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