# Abstracts of the 49th annual conference of young scientists "Cold in Biology and Medicine. Current Issue in Cryobiology, Transplantology and Biotechnology" May 14–15, 2025, Kharkiv, Ukraine

Probl Cryobiol Cryomed 2025; 35(4):231
https://doi.org/10.15407/cryo35.04.231

## Biocryoimmunology — a new scientific revelation in bioscience and medical practice

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This study explores the ineractions between pathological cells — particularly malignant tumors — as autonomous micro-systems and the host immune macro-system. Based on novel experimental findings, the concept of a "cryo-immunologic time bomb" (Korpan, 2024) is proposed to describe immune activation triggered by ultra-low temperature exposure. Cryogenic treatment of tumor cells initiates a cascade of events: cell and protein fragmentation, immune recognition of modified self-tissues, and the generation of biocryoantigens. These changes stimulate both local and systemic immune responses, culminating in what is termed the "biobomb" reaction. *In vitro* models using normal and malignant blood cells exposed to –180 °C were studied. Immune profiling (CD3, CD4, CD8, CD19, CD16/56, HLA-DR, CD57) was conducted via BD FACSLyric™ flow cytometry in 19 patients. Notable findings included post-treatment elevation of $CD56^+$, $CD45^+$, $CD69^+$ lymphocytes following pokeweed mitogen stimulation.

Cryomodified proteins and cryocell particles are central to this immune mechanism. The discovery of BioCryoAntigens introduces a new class of therapeutic and diagnostic targets. Clinical data demonstrate long-lasting systemic immune modulation after cryosurgical tumor ablation. This work highlights biocryoimmunology as a frontier for cancer therapy and immunomodulation strategies.

Probl Cryobiol Cryomed 2025; 35(4):231
https://doi.org/10.15407/cryo35.04.231a

## Wharton's jelly tissue cryopreservation for efficient mesenchymal stromal/stem cell retrieval

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Preservation of Wharton's jelly (WJ) tissue through cryobiological methods presents a promising alternative to conventional mesenchymal stromal/stem cell (MSC) banking strategies. Long-term *in vitro* expansion of WJ-derived MSCs (WJ-MSCs) can lead to undesirable phenotypic drift and reduced functional properties. This study focuses on the development of a cryopreservation protocol for WJ tissue, aiming to ensure the retrieval of functionally competent MSCs without extensive *in vitro* manipulation.

Human umbilical cords were collected post-cesarean section with informed maternal consent. WJ segments were cryopreserved in a 10% DMSO solution prepared with either standard culture medium or autologous cord blood serum. A two-step controlled-rate freezing protocol was applied. For cell isolation, a modified explant method was employed: tissue pieces were cultured on gelatin-coated surfaces with periodic gentle trypsinization to collect migrating cells.

The impact of cryopreservation on MSC yield, viability, colony-forming unit (CFU) potential, and differentiation capacity was assessed in comparison to freshly processed tissue. Although cryopreserved samples showed a ~50% reduction in total cell yield, the isolated MSCs retained high viability, CFU activity, and differentiation potential, comparable to cells from fresh tissue.

These results substantiate the feasibility of WJ cryopreservation as a reliable strategy for long-term MSC storage. By minimizing prolonged *in vitro* culture, this approach supports the maintenance of native cell properties, enhancing the safety and efficacy of cell-based therapies. The established cryobiological protocol contributes to the advancement of standardized MSC biobanking for future regenerative applications.