Standardized Cryopreservation Protocol and Quality Control for Biobanking of Natural Killer (NK) Cells

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Abstract

Natural Killer (NK) cells play a key role in cell-based therapies due to their unique biological properties and therapeutic potential, particularly in cancer and infectious diseases. However, NK cell-based therapies rely heavily on effective long-term preservation that maintains cell viability and functional integrity. Therefore, the development of reliable cryopreservation protocols is essential. Advances in biotechnology have accelerated the progress of cell-based therapies, which depend on the quality and stability of stored cells. This study aims to develop and validate a standardized quality control protocol for the long-term cryopreservation of human NK cells stored in liquid nitrogen (−196 °C).

Peripheral blood samples from healthy volunteers (n = 4) were collected for the isolation of NK cells. The isolated cells were expanded under optimized culture conditions and subsequently cryopreserved for a five-year longitudinal study using various cryoprotective agents (CPAs), including dimethyl sulfoxide (DMSO) and propylene glycol (PG). The cryopreservation protocol was validated through comprehensive evaluations conducted at baseline and at six-month intervals (0, 6, and 12 months), assessing cell viability, stability which measured by population doubling time and functional potency. The functional potency was evaluated using NK cell cytotoxicity assays, with analysis performed via fluorescence microscopy.

NK cells cryopreserved with 10% DMSO, 10% PG, and 5% DMSO + 5% PG showed comparable baseline outcomes (viability 99%, PDT 79.1 h, cytotoxicity 37.82%). At 6 months, viability remained high in DMSO (96.25%) and DMSO+PG (97.25%) but declined in PG (85.25%), with a marked increase in PDT in the PG group (179.69 h) compared to DMSO (85.75 h) and DMSO+PG (104.58 h). At 12 months, viability further decreased to 92.5% (DMSO), 82.13% (PG), and 88.25% (DMSO+PG), while PDT remained stable in DMSO (82.37 h) but elevated in PG (186.66 h) and DMSO+PG (106.07 h). NK cytotoxic activity was maintained across all conditions throughout (35.98–37.98%). Overall, DMSO, alone or in combination with PG, demonstrated superior preservation of viability and proliferative capacity, while functional potency remained largely stable.

This study establishes a standardized biobanking protocol that ensures the long-term stability and functional preservation of stored human cells. The proposed approach provides a valuable framework for future biomedical research and personalized medicine and has the potential to support the advancement of biotechnology at the regional level.

Keywords: Biobank, Cryopreservation, Immune Cells, Natural Killer (NK), Quality Control, Immunotherapy

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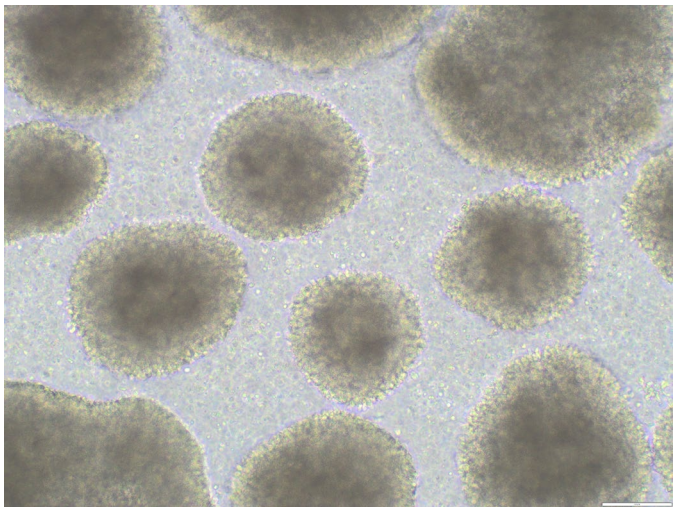

Figure 1. Morphological assessment of expanded NK cells at Day 14 before freezing at 0 month. Representative phase-contrast micrograph (10x magnification) illustrating robust cellular expansion. The culture exhibits characteristic multi-cell aggregates (clusters), indicating high metabolic activity and proliferation. Cells maintain a healthy, spherical morphology with diameters ranging from 4 to 10 micron, consistent with the phenotype of activated Large Granular Lymphocytes (LGLs).

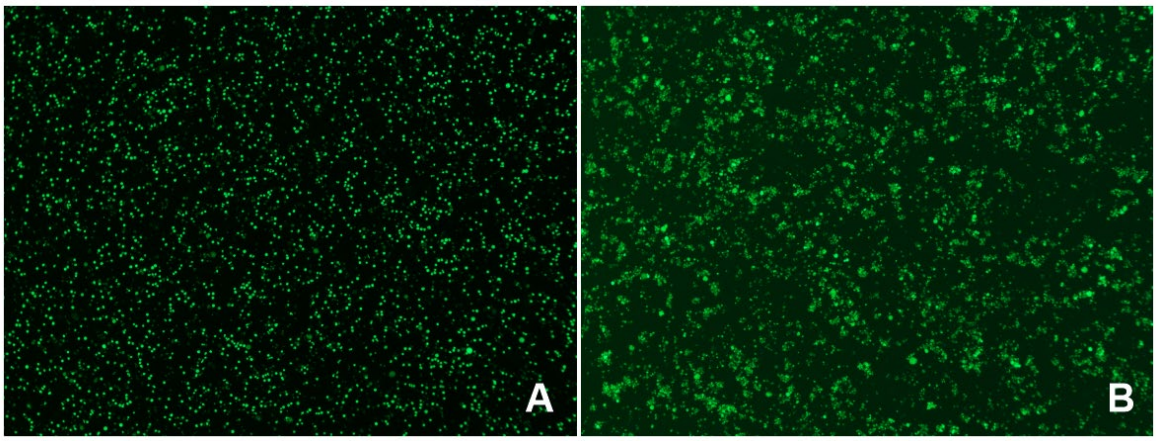

Figure 2. The functional potency of NK cells before cryopreservation was evaluated using a cytotoxicity assay and analyzed by fluorescence microscopy. (A) NK cells were co-cultured with CFSE-labeled K562 target cells. (B) NK cells after 4 hours of co-culture with CFSE-labeled K562 target cells, demonstrating normal cytotoxic activity.