

Expansion of Human Natural Killer Cells for Cancer Therapy

Natural Killer Cells

Natural Killer Cells (NK Cells) are of utmost therapeutical significance. NK cells emerge as a very promising tool in fighting cancer of the haematopoetic system and many solid malignancies, as well as some severe autoimmune diseases and viral infections. In former therapeutic approaches, *in vivo* generation of NK cells, enhancing of their cytotoxicity and immune competence was triggered by application of interleukines and other immune system activating compounds, and this approach did show some clinical success in patients. More effective results were obtained when NK cells were isolated, expanded *in vitro* and subsequently injected into patients, and in some of them, disease regression could be observed. However, the use of autologous NK cells without knowing cytotoxic potential and abnormalities occurring in NK cells from cancer patients might be responsible for unexplainable results which occurred. Thus, allogenic NK cells, particularly those from haploidentical donors, are now preferred in treating haematological and other frequent occurring malignancies. *In vitro* cultured NK cells of this origin exhibit high cytotoxicity against a lot of cancer diseases and problems with GVHD are rare. However, there are still some obstacles in NK cell therapy. It is still difficult and costly to obtain high numbers of NK cells in high purity, which is mandatory for successful NK cell therapy. Out of this reason, clinical studies using qualified, ATMP grade NK cells with sufficient cell numbers for high dose treatments and repeated doses are missing.

Process Technology for Mass Expansion of NK Cells

Zellwerk has developed a process for mass expansion of purified NK cells. The unique process was developed with Uharek *et al.*, Charité Berlin. It can be incorporated into a GMP environment. This mass expansion cannot be achieved by any other method to culture immune cells described thus far, without the use of feeder cells and animal derived medium supplements. This is possible with our novel Laminar Flow Bioreactor types, which are operated in our ZRP Cell Cultivation System.



The ZRP Cell Cultivation System comprises the ZRP GMP Breeder combining laminar flow workbench with cell culture incubator functions. Our system provides all features to operate the novel Laminar Flow Bioreactor types: Circulation of medium in the bioreactor, perfusion with fresh medium, overlay perfusion with gases and continuous measurement of pH and pO₂ values. The process is controlled by the ZRP Control Unit, which is easily operated via a touch screen and process data are evaluated using a software which is supplied together with the ZRP System.

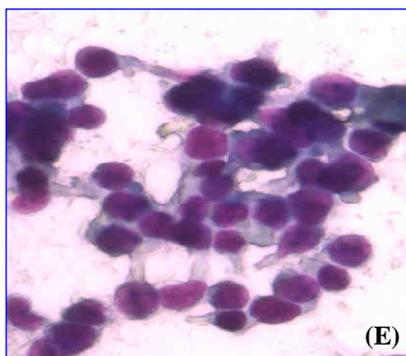
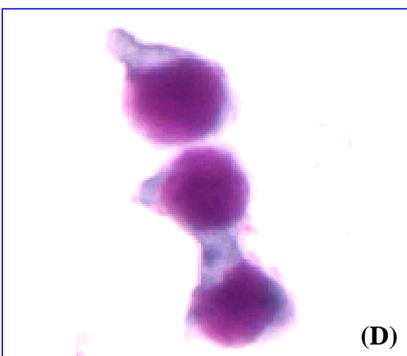
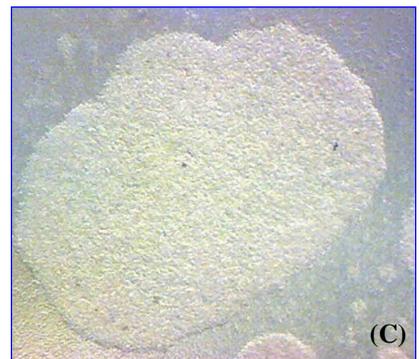
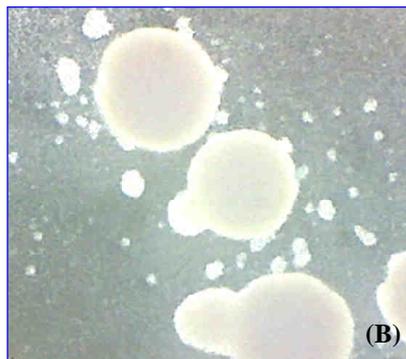
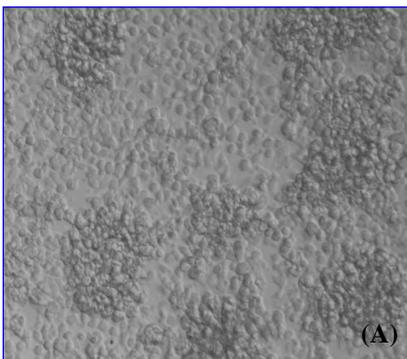
Disposable Laminar Flow Bioreactors

Disposable Laminar Flow Bioreactors with 50 ml and 500 ml volumes are available for immune cell culture. The ZRP Bioreactor 50 M and its larger version, the ZRP Bioreactor 500 M will be delivered together with tube connections and sensor holders for pH and pO₂ as sterile packaged units.



NK cells can be grown in these bioreactors for therapeutic and scientific use. Purified CD3⁻ CD56⁺ NK cells obtained from peripheral blood mononuclear cells of donors or patients can be expanded in sequential bioreactor runs 1.000-fold to 5x 10.000-fold without changing of characteristic surface markers. Expanded cells show high lytic activity in standard assays. Thus, high doses of cells become available for high and repeated dosage treatments with NK cells. In addition, the ZRP Cell Cultivation System is as well suited for mass expansion of T lymphocytes, whole mononuclear cell fractions and other haematopoietic cells.

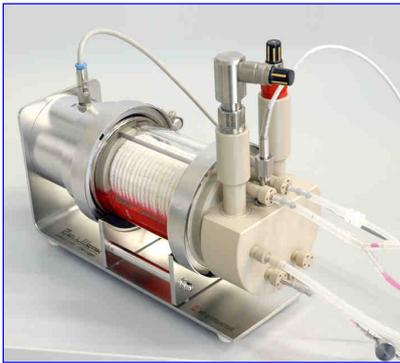
NK Cell Expansion in a ZRP Bioreactor 50 M



Microscopic images of NK cells sedimented to the bottom of the bioreactor vessel. **(A) - (C)** Early phase of expansion with NK cells. **(A)** Cells are covering the bottom of the bioreactor after inoculation of the bioreactor and **(B)** start to form cellular 3D agglomerates after several days. **(C)** Larger aggregate of NK cells in a later phase of expansion. **(D) + (E)** Giemsa stained NK cells showing some extracellular matrix formed by the NK cells.

Other Bioreactor Types

Several types of ZRP Bioreactors have been developed by Zellwerk GmbH, in addition to the ZRP Bioreactors 50 M and 500 M for NK and immune cell expansion. As examples, the ZRP Bioreactor 500 for 3D cell culture and tissue engineering and the ZRP Bioreactors of the H series for expansion of mesenchymal stem cells and different primary cells for cell therapy are shown.



ZRP Bioreactor 500

Over 10^{10} Cells
Tissue Engineering, Cell Lines,
Protein Expression



ZRP Disposable Bioreactor 8000 H

5 - $10 \cdot 10^8$ Cells
Mesenchymal Stem Cells,
Primary Cells

GMP and Regulatory Documentation Package

Zellwerk offers a Regulatory Documentation Package for Integration of the ZRP System into a GMP environment:

- Documents with regulatory requirements according to EU, FDA, and ICH standards
- Technical description and documentation of the validation of the system
- Documents for qualification according to DQ, IQ, OQ and PQ
- Documents for quality risk management
- Standard operating procedures (SOPs)



Zellwerk is located in the Berlin area,

Zellwerk GmbH
Ziegeleistr. 7
D-16727 Oberkraemer
Germany

Fon: +49 3304 3826811
Email: info@zellwerk.biz
Web: www.zellwerk.biz