

Expansion of Peripheral Blood Mononuclear Cells
(PBMCs) for Immune Therapy

Peripheral Blood Mononuclear Cells

Peripheral Blood Mononuclear Cells (PBMCs) in immune therapy. The PBMCs are used as a whole in fighting diseases (leukemia, cancer, infectious diseases), and they are also important as starting material of subsets of single types of these cells (T lymphocytes, NK cells, monocytes and dendritic cells). Stem cells from the PBMC fraction may also provide a substitute for aspirated bone marrow cells in immune system regeneration. It has been described that autologous PBMCs stimulated with tumor peptides mediate tumor regression in patients. High amounts of PBMCs, in particular of the T lymphocyte and NK cell populations, are mandatory for an efficient immunotherapy approach. Thus, there is need for making available therapeutic amounts of autologous, haploidentical or allogenic PBMCs in a standardized, GMP conform process without using feeder cells.

Expansion of PBMCs with the ZRP System

Efficient mass expansion of PBMCs. We have now developed two novel bioreactor types for the cultivation of immune cells with our ZRP Cell Cultivation System, consisting of a ZRP GMP Breeder and a ZRP Control Unit (left). PBMCs can be grown to over 10^9 cells in the **ZRP Bioreactor 50 M** (upper right) or even more than 10^{10} cells using the **ZRP Bioreactor 500 M** (lower right) for high dosage and multiple cell transplantations.



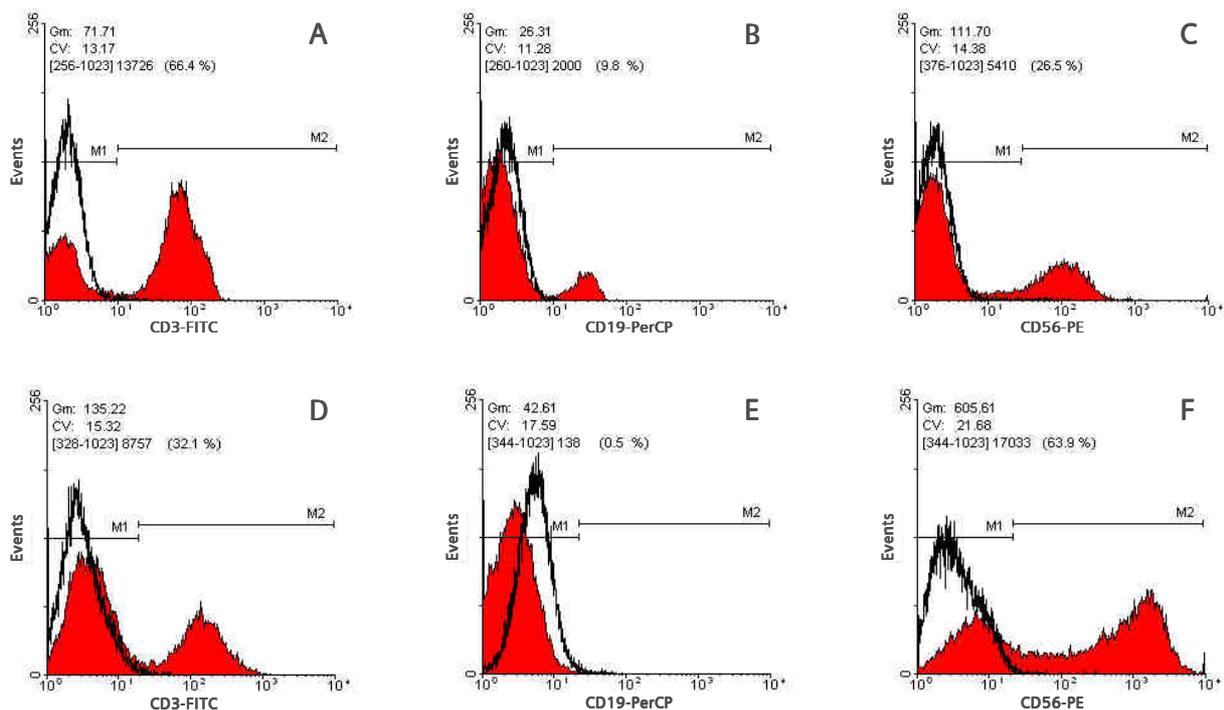
Zellwerk GmbH developed a unique cultivation process for PBMCs and isolated immune cells. After seeding the cells into the bioreactor, a perfusion stream, which is continuous during the expansion, is initiated and running through the labyrinthine network, dividing it into a resting lower phase, in which the cells are in touch with each other and underly no shear stress, and a laminar streaming upper fluid layer. Thus, the cells are supplied with fresh medium and nutrients during the whole cultivation process, and cell culture parameters (pH, pO₂, pCO₂ and temperature) are regulated by the ZRP Control Unit which is comfortably operated via an integrated touch screen and a sophisticated software. The process can be standardized and formulated as a standard operating procedure (SOP), yielding a highly reproducible cultivation of PBMCs for tansplantation into patients.

PBMCs as source of highly specific T lymphocytes

Antigen specific T lymphocytes can be expanded from a population of PBMCs by stimulation with viral or tumor peptides. Aigner *et al.* (2012) demonstrated that treatment with a specific tumor antigen resulted in an enrichment of specific CD3⁺ CD8⁺ T lymphocytes, which was more efficient with the ZRP Cell Cultivation System than in cell culture bags. We consider the unique cultivation method in which the PBMCs rest in contact with each other in the bottom fluid phase of the bioreactor to be responsible for enhancing the effect of peptide stimulation. After expansion of specific T cells from PBMCs, they can be isolated and expanded in a second bioreactor run to high cell numbers.

Composition of PBMCs

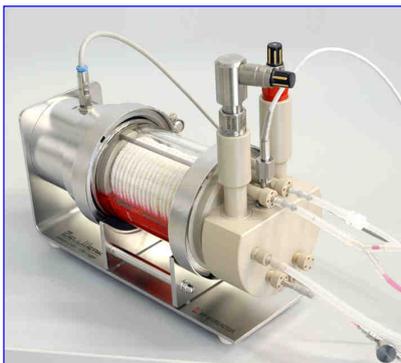
PBMCs offer a mixture of effective immune cells. Expansion of PBMCs in our ZRP Cell Cultivation System using the ZRP Bioreactor 500 M enables not only the mass expansion to 10¹⁰ PBMCs in one cultivation run. Using different media and supplements, single cell types in the PBMC population, i. e. T lymphocytes or NK cells, can be enriched selectively. The table and FACS diagrams below depict the distribution of these cell types in the PBMC fraction prior to and after cultivation in a ZRP Bioreactor 50 M. The relative amount of NK cells increased during cultivation.



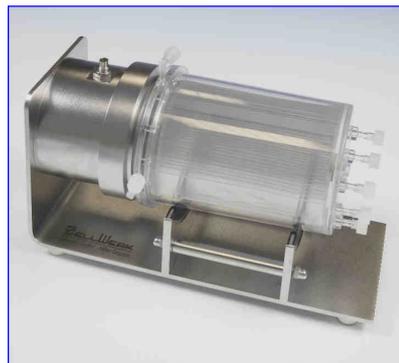
Cell Type	Marker	A-C: PBMC fraction prior to expansion [% positive]	D-F: PBMC fraction after expansion [% positive]
T lymphocytes	CD3 ⁺	66,4	32,1
B lymphocytes	CD19 ⁺	9,8	0,5
NK cells	CD56 ⁺	26,5	63,9

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 offers the growth of tumor tissue from patient specific tumor cells, which then can be tested *in vitro* against specific immune cell preparations in order to determine which proves most effective for individualized cancer therapy. The ZRP Bioreactors of the H series were developed for expansion of mesenchymal stem cells and different primary cells for cell therapy. In particular, MSCs are a valuable tool for tissue/organ regeneration, and for attenuated Graft-versus-Host Disease in the course of bone marrow/hematopoietic stem cell or T lymphocyte transplantation.



ZRP Bioreactor 500
Over 10^{10} Cells
Tissue Engineering, Cell Lines,
Recombinant Protein Production



ZRP Disposable Bioreactor 8000 H
5 - $10 \cdot 10^8$ Cells
Mesenchymal Stem Cells,
Primary Cells

GMP and Regulatory Documentation Package

The Zellwerk GmbH 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)



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