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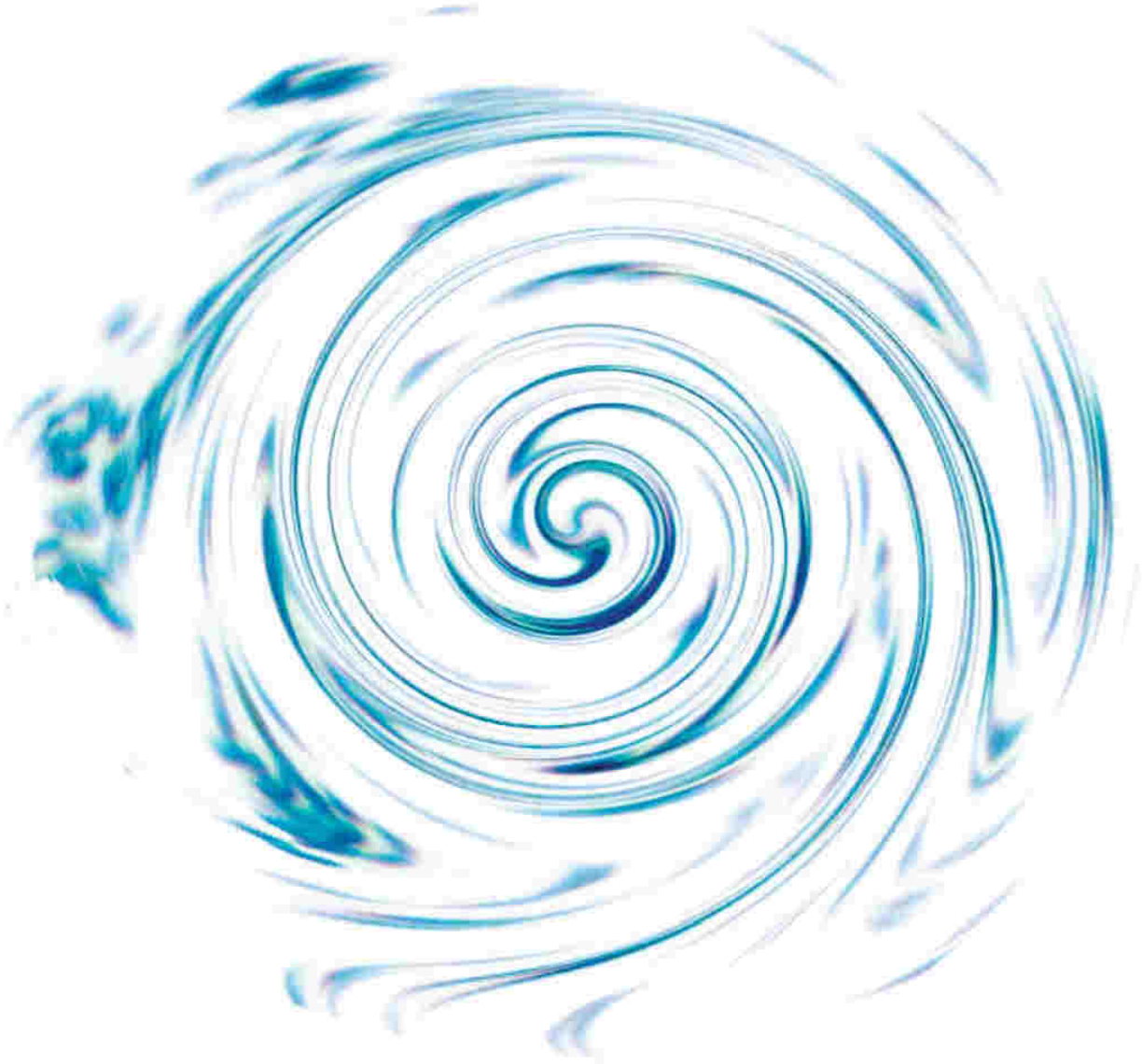
Zellwerk GmbH·HiPer-Gruppe



RP TECHNOLOGY

Mass Expansion of Mesenchymal Stem Cells from Human Bone Marrow

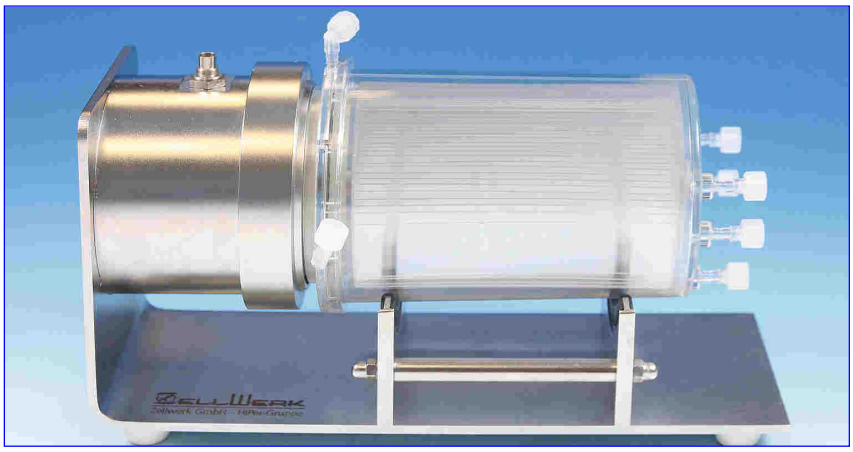
Bone Marrow Derived MSCs for Cell Therapy

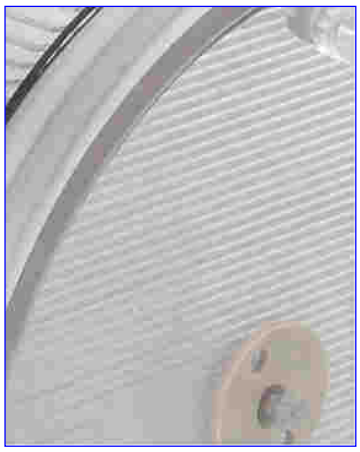
Multipotent Mesenchymal Stem Cells (MSCs) can be isolated from the mononuclear fraction of human bone marrow as adherent growing colonies. These cells are of high clinical interest in autologous as well as allogenic settings, they are able to differentiate into a variety of cell types. MSCs have shown efficiency in the treatment and prevention of Graft-versus-Host Disease (GvHD), in supporting organ tolerance after transplantation, in reducing ischemic damage, in regeneration of retinal epithelium, in the therapy of neuro-degenerative diseases and further diseases. These cells participate in the regeneration of tissues including muscle, bone, cartilage, tendon, cardiac, pancreatic ß cells and nerve tissues. Bone marrow MSCs are of particular value in bone tissue engineering since they have high osteogenic differentiation capacities. However, both, therapeutic cell transplantation and tissue engineering require high numbers of MSCs (10^9 and more).

ZRP Cell Cultivation System – Equipment

Expansion of bone marrow derived MSCs to amounts mentioned above is still difficult. Due to their strong contact inhibition, large cultivation areas are needed. The ZRP Cell Cultivation System enables mass expansion of MSCs under conditions suited to generate "Advanced Therapy Medicinal Products" (ATMPs) according to international standards.

Key elements of the ZRP Cell Cultivation System are the ZRP GMP Breeder, combining features of a laminar flow work-bench and a cell culture incubator, and the ZRP Control Unit for automatic regulation of the parameters of a cell culture process, e.g. pH, pO_2, temperature, medium perfusion and medium feeding rate. Different types of ZRP Bioreactors can be operated inside the ZRP GMP Breeder. The ZRP Bioreactors of the H series are of particular advantage when cells with strong contact inhibition have to be expanded (like bone marrow derived MSCs).

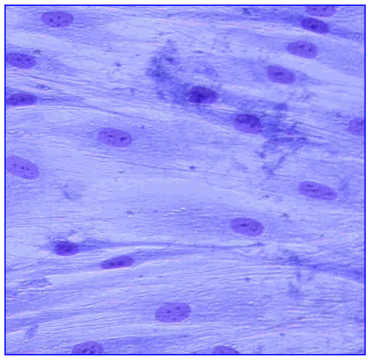


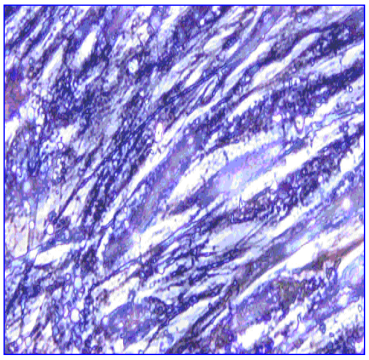


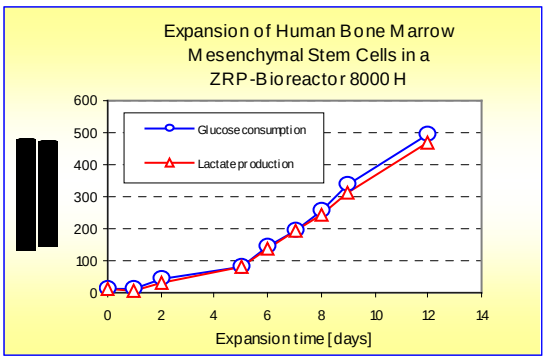
ZRP Bioreactors of the H series are equipped with a high number of stacked horizontal sheets thus generating an extremely large cultivation area in a handy bioreactor vessel. MSCs grow in a monolayer without change in differentiation status and in a fibroblast-like shape. Large amounts of adherent stem cells can thus be grown in a single, closed, easy to operate bioreactor run. This enables the mass production of bone marrow derived MSCs exhibiting similar characteristics in a standardized, GMP-compliant process. With respect to clinical use (ATMPs) the low consumption of media is a further advantage in raising cells to the high amounts required for cell therapy.

ZRP Cell Cultivation System – Outcome

MSCs from human bone marrow grow rapidly in H series ZRP Bioreactors at an exponential rate, showing fibroblast-like morphology. Only low amounts of MSCs are needed to start expansion (around 200 cells/cm² seeding area) and cultivation is easy to operate by the automatically working process routines (with pH, pO_2, bed rotation and feeding rate adjusted to cell growth). Neither passaging nor other manipulation steps are needed as in standard flask culture. During 20 to 30 days of cultivation expansion of 100- to 1.000-fold is achieved and cells have grown to a subconfluent monolayer. The diagram shows a typical cultivation of human bone marrow MSCs in a ZRP Bioreactor 8000 H. In this experiment, cell growth was assayed by glucose consumption and lactate production in medium samples on a daily basis.





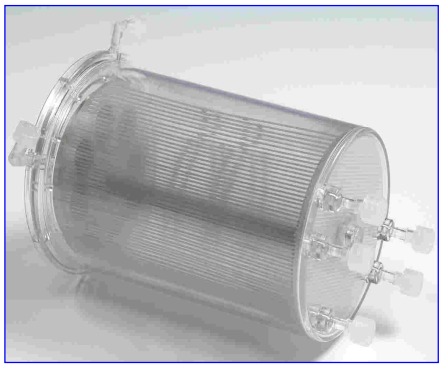


Cells grow homogenously distributed on the polymeric sheets a few days after inoculation of the bioreactor **(left)**. After three weeks, MSCs have grown to a confluent monolayer **(middle)**. These cells are valuable in cell therapy since they lack immunogenicity and are not recognized by allogenic T and NK cells, and they are identified after bioreactor cultivation due to expression of typical surface markers CD44, CD73, CD90 and CD105. The cell cultivation process is documented so that standard operating procedures (SOPs) for reproducible expansion of patient cells may be elaborated. The diagram **(right)** shows cell growth during cultivation for a representative experiment assayed by glucose consumption and lactate production in medium samples on a daily basis.

Bioreactor Types Available

The Zellwerk GmbH has developed different size versions of the ZRP Bioreactors of the H series. The Bioreactors 3000 H and 8000 H are available as presterilized types equipped with tubes, disposable sensor holders and Luer Lock connectors. These bioreactors are certified as Medical Devices of the group IIA. This makes it easy to start a cultivation run for production of MSCs as ATMPs to be used in cell therapies. We also offer re-usable R&D versions of our bioreactors, as shown in the example of the ZRP Bioreactor 20000 H in the figure below.

ZRP Bioreactor 3000 H Disposable
Around $3 \cdot 10^8$ cells can be achieved in one run



ZRP Bioreactor 8000 H Disposable
Around $8 \cdot 10^8$ cells can be achieved in one run

ZRP Bioreactor 20000 H R&D
$2 - 4 \cdot 10^9$ cells can be achieved in one run

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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