Membrane-separated cocultivation of cord blood hematopoietic stem cells with stromal cell lines

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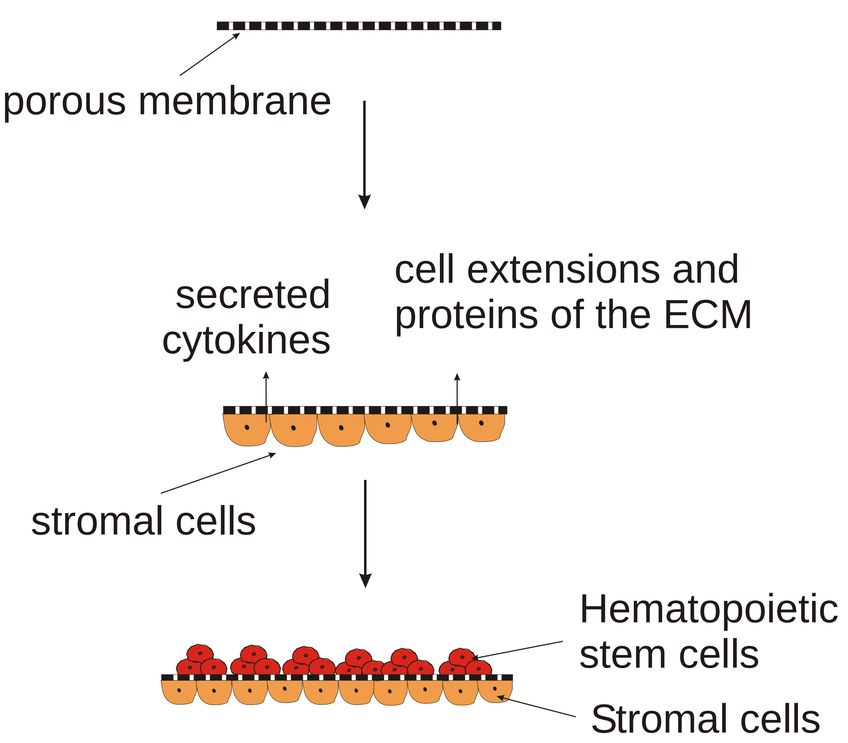
Cord blood hematopoietic stem and progenitor cells (HSPC) represent an important cell source for transplantation of cancer patients after high dose chemotherapy if autologous cells are not suitable (e.g. patients with hematopoietic malignancies) and no adult allogeneic donor is available.

The main disadvantage of cord blood is the low number of cells obtained due to the small volume of blood collectable from umbilical cords. Without an expansion of the HSPC a transplantation is mostly limited to juvenile patients.

We developed a novel small scale membrane bioreactor for parallelized membrane-separated cocultivation of HSPC with stromal cells. This system imitates the natural hematopoietic environment with stromal growth factors, ECM components and direct cell-cell contact between HSPC and stromal cells while maintaining a physical separation of the cells allowing an easy cell harvest.

Membrane-separated Coculture - the principle -

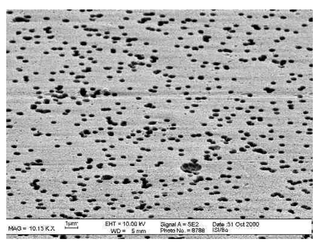


Materials and Methods

Cells:	cord blood $CD34^+$, $5*10^3$/mL Stromal cell lines Sl/Sl, M2-10B4 mod. and MS-5
Volume:	1 mL for suspension control (well plate) 3,6 mL for mini-membrane bioreactor
Culture Medium:	X-Vivo 10, serum-free
Cytokines:	TPO (20ng/mL), SCF (50 ng/mL), IL-3 (10 ng/mL), FL (50 ng/mL)
Culture Time:	7-12 days
Readout:	cell count, CFC and CAFC assays
Animal Experiment:	irradiated NOD/SCID mice were transplanted with cells and after 6 weeks the percentage of human cells in the blood and bone marrow was determined by FACS analysis.

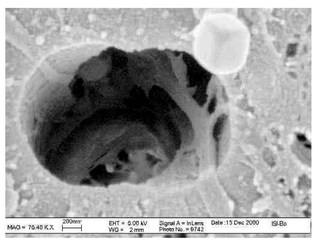
Membrane-separated Coculture - the system -

Comparison of different pore sizes



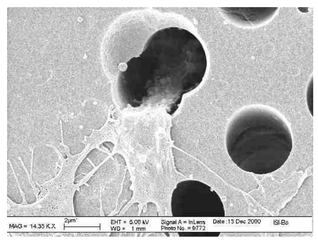
0.45 µm

A pore size of 0.45 µm is too small to allow stromal cell extensions or extracellular matrix proteins to pass the membrane.



1 µm

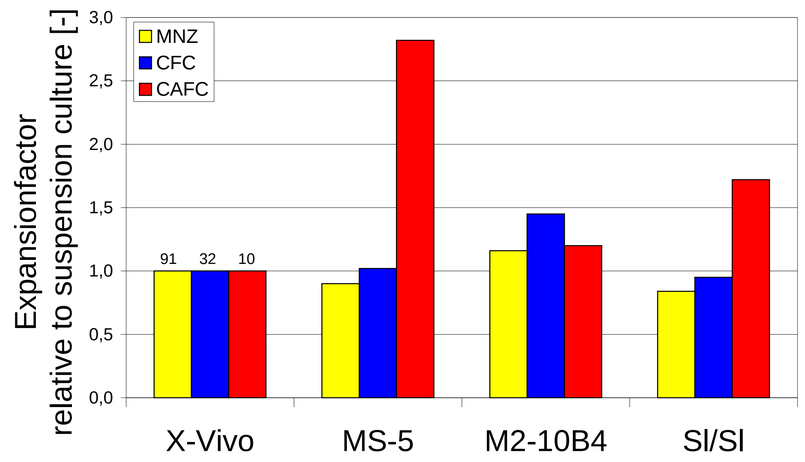
A pore size of 1 µm enables direct cell-cell contact through the membrane while effectivly retaining the cells.



3 µm

A pore size of 3 µm bears the risk of stromal cells passing the membrane especially if pore overlapping occurs.

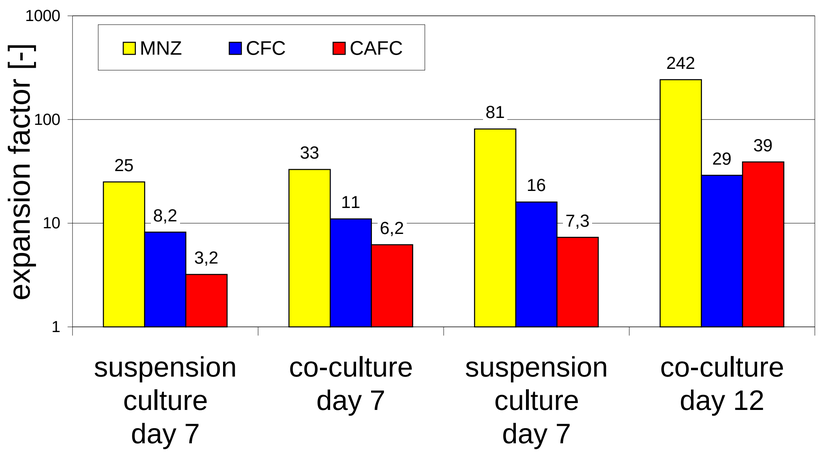
Comparison of different stromal cell lines



Three different stromal cell lines were compared for their supportive effect on hematopoietic stem cell expansion in the membrane-separated co-culture. Expansion factors are given relative to a standard suspension culture.

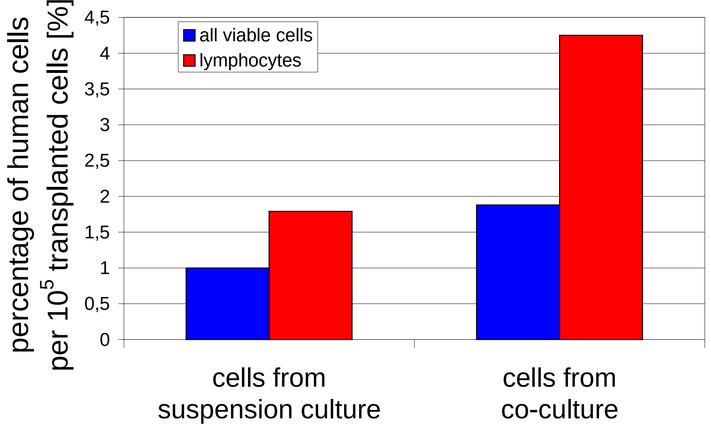
While the expansion of the total cell number and of the CFC is mainly unaffected, the expansion of the CAFC is strongly increased for the MS-5 cell line, which was used for all subsequent experiments.

Prolonged cultivation time



Membrane-separated cocultivation allows prolonged cultivation compared to suspension culture, resulting in strongly increased expansion factors.

Transplantation to NOD/SCID mice



CD34+ cells were cultivated for 7 days either in standard suspension culture or in the membrane-separated coculture system using the MS-5 stroma cell line. Afterwards cells were transplanted to irradiated NOD/SCID mice.

30% of the BM cells and 6% of the PB cells of the mice transplanted with cells from the co-culture were of human origin.

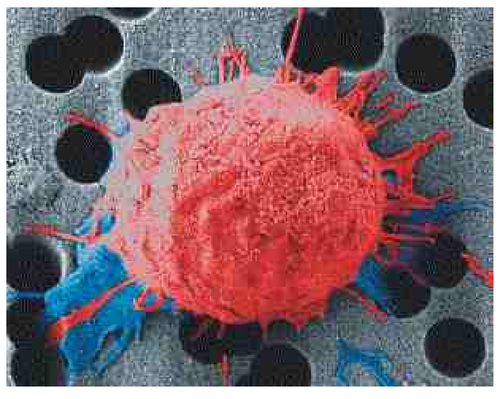
Cells from co-culture showed a higher engraftment potential than cells from suspension culture.

Summary

The membrane-separated bioreactor allows efficient and prolonged ex vivo expansion of cord blood hematopoietic stem cells, while maintaining a physical separation from the stromal cells.

Expansion factors are superior to common suspension culture.

Expanded cells successfully engrafted irradiated NOD/SCID mice better than cells cells from suspension culture.



Hematopoietic stem cell in close contact with stromal cell extensions reaching through the pores of the membrane.