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Characterisation of human mesenchymal stem cells and monitoring of long term cultivation via multicolour flow cytometry

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Introduction

Mesenchymal Stem Cells (MSCs) are believed to have a high potential for cell based therapies as well as for tissue engineering applications. The most common source for MSCs is currently the bone marrow (BM). However BM presents several disadvantages (low number of MSCs, variablilty due to the age of the donor, risk for the donor), so that alternative sources are needed. An increasing interest for human umbilical cord has been observed during the recent years. Human umbilical cord is an attractive source of MSCs for several reasons:

- ↑Easily accessible
- ↑Non controversial source
- ↑No risk for the donor
- ↑High frequency of MSCs
- ↑Young MSCs: isolated at birth
- ↑High expansion potential

The aim of our investigations is to develop efficient and reproducible methods to isolate and expand MSCs from the human umbilical cord. In this work we describe the characterisation of the isolated cells as well as the monitoring of the expansion cultures via multiparametric flow cytometry.

Human umbilical cord: source for MSCs.

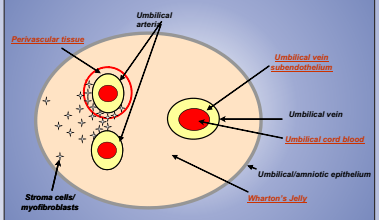


Figure 1: Cross section of an umbilical cord and localization of MSCs (in red).

Isolation

Figure 2 summarizes the isolation protocol:

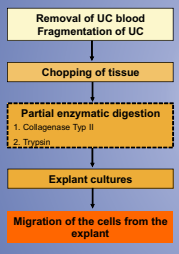
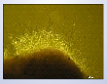
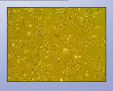


Figure 2: Isolation procedure

Day 1:
Fragmented umbilical cord. After removal of the umbilical cord blood the tissue was chopped and transfered to culture flasks containing α-MEM and 10 % human serum.



Day 10:
Cells migrating from the explants can be observed.



Day 21:
After removal of the explants a culture of plastic adehrent fibroblastic cells was obtained. More than 21x10^6 HUC (human umbilical cord)-cells were harvested and cryopreserved (P0).

Method

There is currently no unique marker allowing the identification of MSCs. The combination of several markers and criteria is necessary to identify MSCs. Thus multiparametric flow cytometry is an appropriate approach to characterise the isolated HUC-cells and evaluate the success of the procedure. We used a 3 color protocol combining the analysis of the three different surface antigens:

- ▪Cd90, Thy-1 membrane glycoprotein: positive MSC marker
- ▪Cd73, 5´-nucleotidase: positive MSC marker
- ▪Cd45, leukocyte common antigen: negative MSC marker

The Fluorochromes fluorescein isothiocyanate (FITC), phycoerythrin (PE) and the tandem dye PE-Cy5 (all obtained from BD Biosciences) were combined for the quantitative detection of the surface antigens. The emission spectra of the dyes and the filter set used can be seen in Figure 3.

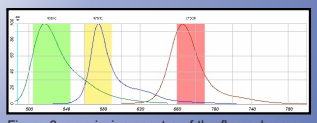
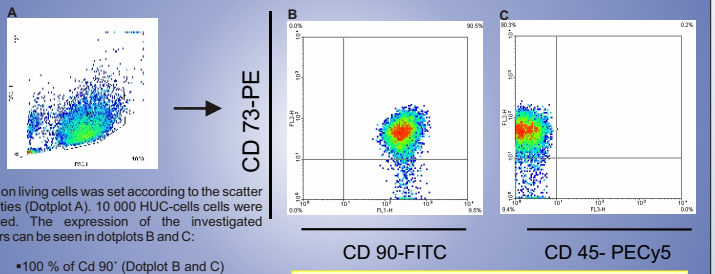


Figure 3: emmission spectra of the fluorochromes and filter set used.

Characterisation via multicolor flow cytometry



A gate on living cells was set according to the scatter properties (Dotplot A). 10 000 HUC-cells cells were analyzed. The expression of the investigated markers can be seen in dotplots B and C:

- ▪100 % of Cd 90$^+$ (Dotplot B and C)
- ▪90% of Cd 73$^+$ (Dotplot B)
- ▪100 % of Cd45$^-$ (Dotplot C)

> 90 % of the HUC-cells show the expected phenotype

Immunophenotype of HUC-cells

Figure 4 summurizes the immunophenotype of the isolated HUC-cells. Cells were found to be positive for the three stemm cell markers Cd90, Cd105, and Cd 73 as well as for Cd 44(marker related to cell adhesion). HUC-cells were also negative for Cd 34 and Cd45, which permits to exclude epithelial as well hematopoietic cells. The expression profile agrees with the reported MSC phenotype.

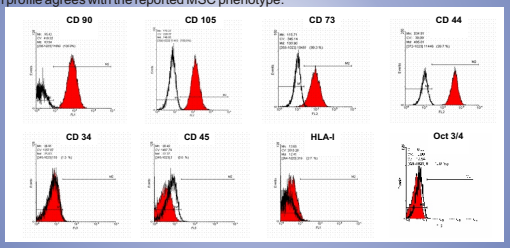


Figure 4: Immunophenotype of the isolated HUC-cells. 10 000 events are displayed. Only the fluorescence of living cells is considered.

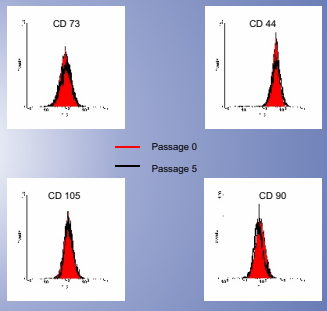
Flow cytometric monitoring over long term expansion

The phenotype of the cells was monitored over 5 passages. Cells were seeded out at 4000 cells/ cm² and cultured in α -MEM containing 10% Human serum.

The immunophenotype remains stable over all observed passages.

The isolated cells showed a high proliferating potential. We calculated a doubling time of 25,9 ± 1,29 hours (cells in passage 1, day 0-7)

Starting from 7x10^5 cells more than 4x10^9 cells could be theoretically obtained at passage 5.



Outlook

We describe a simple approach to isolate MSCs from the human umbilical cord.

The isolated HUC-cells are plastic adehrent, present a fibroblast morphology and show the expected MSC immunophenotype.

HUC-cells are highly proliferative and stable according to the suface antigen expression profile.

Presently, the multilineage differentiation potential of our HUC- cells is investigated.

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