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Optimization and monitoring of long-term cultivation of Human Umbilical Cord-derived Mesenchymal Stem Cells

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Introduction: *The increasing demand for human mesenchymal stem cells (hMSCs) for cell-based therapies and tissue engineering leads to tremendous research effort to find novel sources as well as derivation methods for MSCs. Due to its easy availability and, compared with the bone marrow, an approx. 30 times higher frequency of hMSCs, the human umbilical cord presents a promising alternative source for these cells. Since the medium is a key parameter for a successful cultivation, we first focused on the optimization of the medium composition. Therefore, we tested different sera with regard to the proliferative and differentiative potential of human umbilical cord-derived MSCs. To this date, in vitro expansion of hMSCs has commonly been achieved in media containing fetal calf serum (FCS). With respect to clinical applications, the use of human serum (HS) instead of FCS is considered as favorable, because it reduces the risk of viral or bacterial contaminations.*

Materials and methods: *Human Umbilical Cord Cells (HUC-cells), isolated from whole umbilical cord tissue, were seeded at a density of 4000 cells/cm² in T-25 culture flasks and were cultivated in alpha-MEM, supplemented with four different FCS and one human serum respectively. Each medium was additionally supplemented with 50 µg/ml gentamicin. At 80- 90% confluence the cells were passaged and the proliferative potential was monitored by calculating cumulative cell doublings based on obtained cell numbers over a period of eight passages. The size distribution of cells in a population was monitored using the CASY®1 DT by Innovatis. The comparative analysis of cell surface epitopes (CD90, CD105, CD73, CD44, CD34, CD45 and HLA-I) were performed by flow cytometric analysis.*

Table 1: *Used sera (all 10%)*

FCS 1	*standard quality*
FCS 2	*gold quality*
FCS 3	*pre-tested for amnion cells*
FCS 4	*heat inactivated*
HS	*human serum (pooled without regard to blood type and rhesus factor)*

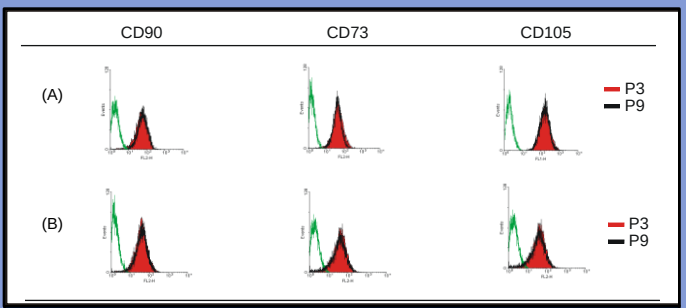

Figure 1: *Immunophenotypic characteristics of HUC-cells grown in HS* (A) *and FCS "pre-tested for amnion cells* (B).

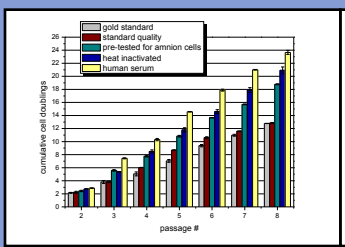

Figure 2: *Influence of different sera on the proliferative potential of HUC-cells, shown by cumulative cell doublings for a period of eight passages.*

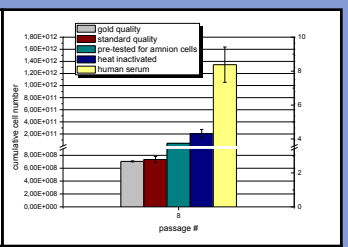

Figure 3: *Influence of different sera on the proliferative potential of HUC-cells, shown by the obtained number of cells for a period of eight passages.*

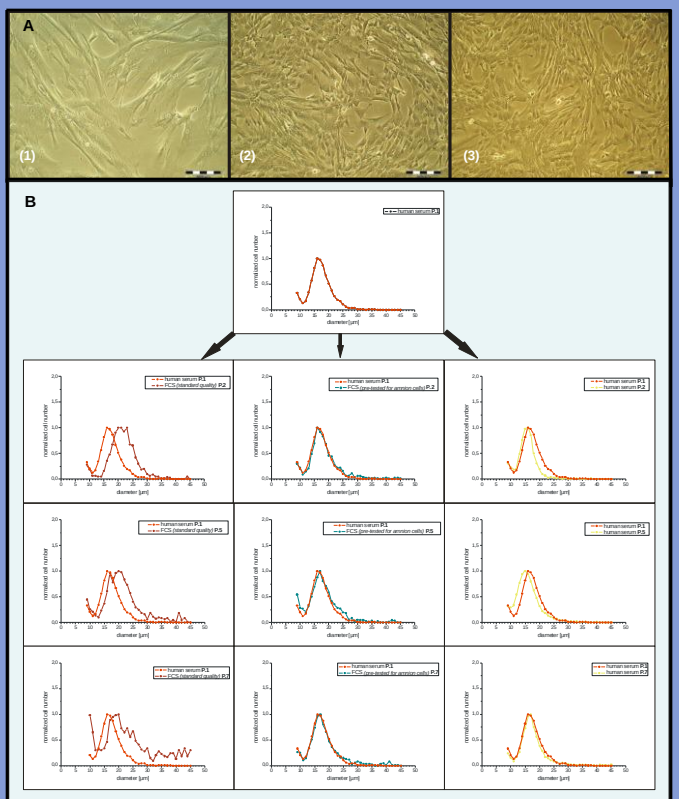

Figure 4: (A) *Varieties in morphology of HUC-cells cultured in different sera* ((1) *FCS "standard quality"*; (2) *FCS "pre-tested for amnion cells"*; (3) *HS; all 10x magnified)* (B) *Influence of different sera on the size distribution of cells in a population. Almost confluent grown cells were enzymatically detached from the culture flask surface and then analyzed using the CASY®1 DT cell counter. The results (number of counts per increment of 1µm) were normalized by dividing y-values by the highest y-value.*

Results: *Cells expressed typical mesenchymal stem cell markers independently of the chosen serum such as CD73, CD90 and CD105 (Fig. 1) and lack expression of CD45 and CD34 (data not shown) throughout all examined passages. However, differences in the proliferation potential of HUC-cells were observed when cultivated in presence of different sera. Cells cultivated in HS displayed the highest proliferation potential compared to all tested fetal calf sera as shown by calculated cumulative cell doublings and cumulative cell numbers. The use of human serum lead to a total number of 24 cumulative cell doublings over a period of eight passages, which was the highest number of doublings compared to all other tested FCS and even 1.8 times higher compared to the cumulative doublings of cells cultivated in FCS "gold quality" (Fig. 2). Same tendencies could be observed with respect to the total number of cells that were obtained for the duration of cultivation. Here, the use of FCS "gold quality" and FCS "standard quality" lead to approx. 7x10⁸ cells which were 285 times less cells than obtained in medium supplemented with FCS "heat inactivated" and even 1857 times less cells obtained in HS-supplemented medium (Fig. 3).*

The use of different sera lead to wide varieties in cell morphologies (Fig. 4 (A)). Cells cultivated in FCS "standard quality" and FCS "gold quality" (data not shown) tended to drastically enlarge in cell size compared to cells grown in media supplemented with FCS "pre-tested for amnion cells", FCS "heat inactivated" (data not shown) and HS. Furthermore, "standard" and "gold quality" FCS lead to a more heterogenic size distribution of cells in a population, whereas the size distribution of cells grown in every other tested serum was almost consistent (Fig. 4 (B)).

Conclusion: *Our results indicate, that a fast and efficient expansion of HUC-cells is carried out best when the culture medium is supplemented with human serum. The decreased supportive properties of some of the tested FCS on the cell proliferative potential have to be investigated in detail. In this context, additional experiments on cell cycle analysis and senescence of the cells should be performed.*

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