Expansion of mesenchymal stem cells from frozen UCB
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Abstract

Umbilical cord blood (UCB) has recently been the focus of clinical applications due to its relative accessibility, its high number of primitive progenitor cells and its relatively low frequency of graft-versus-host-disease. UCB contains two types of stem cells: hematopoietic stem cells (HSC) and mesenchymal stem cells (MSC). HSC have been used for treatment of leukemia since the 1960's. Clinical studies show that MSC can be used in regenerative medicine hereby treatment of cardiac diseases, diabetes and certain neurodegenerative diseases. However, due to the low number of MSC in UCB, ex vivo expansion is required. It has previously been demonstrated that MSCs can be isolated from fresh UCB, however limited data exists with isolation of MSC from frozen UCB samples. The joint project is to establish an optimal protocol for isolation of MSCs from frozen UCB, and characterize their MSC specific phenotype. The MSCs were derived from thawed UCBs by using a known thawing protocol and Ficoll separation of the mononuclear fraction. We used flow cytometry to analyze the expanded cells for MSC surface markers (CD73, CD90 and CD166) and the presence of non-specific MSC markers like CD34 and CD13. The protocol used in this study resulted in adherent cell populations with MSC morphology. CD90*, CD73*, CD166*, CD34* and CD13* cell populations were identified with flow cytometry analysis. In conclusion, this study demonstrated that the used freezing, thawing and MSC isolation protocol resulted in MSC populations that could be used for regenerative medicine.

Method

Processing

The collected umbilical cord blood (NCB) is processed in the laboratory in Copenhagen, Denmark. The overall aim of the process is to separate the UCB into plasma, red cells anduffy coat, in which the stem cells are contained. The total nucleated cell (TNC) viability and the number of CD34 positive cells are counted.

Freezing

Before freezing of theuffy coat, cryopreservation (Ficoll) procedure is added. Herein, theuffy coat is placed in a freezing media (DMSO, FBS) and frozen. Avena stems were used as a cryoprotectant at a concentration of minus 180°C. The frozen UCBS is stored in the freezer at a stringent bank.

Thawing

As devised by the Stemcare thawing media was used. The thawing media was run on a 3°C gradient under gentle agitation. After resuspending the sample in the thawing media, the sample was exposed to a temperature of minus 180°C. The frozen UCB was thawed in the upper phase of a stringent bank.

Ficoll

The ficoll gradient separation method has previously been applied for fresh UCB and MSC isolation. MSC isolation from the ficoll enriched cell population prepared for the ficoll gradient separation method showed comparable with MSC isolation from fresh UCB samples. The ficoll enriched cell population was isolated via Ficoll gradient separation method (Ficoll, 1070). In this process, the mononuclear fraction was isolated via ficoll gradient separation method. The ficoll gradient separation method can be used as a work flow well after clarification containing the mononuclear cell fraction from the plated and whole cord blood sample.

Cultivation/Expansion

Mononuclear cells were washed and seeded into the forming cell culture medium (FCS) enriched medium (20% FCS 80% DMEM/LG) and placed in a humidified incubator. Additional media was added to the cell culture, and the media was changed, and the final cell culture medium was added. At day 4-5, the cell culture medium was changed, and additional media was added to the final cell culture medium. At day 7-10, the cell culture medium was changed, and additional media was added on the cell culture medium. After day 10, the cell culture medium was changed, and additional media was added on the cell culture medium.

Analysis

The surface marker profile of UCs/cells were analyzed with flow (FACSAria II), ELISAs, genoscopy and other methods. The UCB derived MSCs were characterized using Q-PCR, flow cytometry, and other methods. The qPCR and flow cytometry were performed with ThermoFisher, Bioneer, stemcare and other methods.

Results

Expansion

We were able to establish adherent MSC-like colonies that continued to divide and eventually establish a confluent monolayer. At day 10-12, the MSC colonies were treated with FBS to obtain a confluent monolayer. At day 10-12, the MSC colonies were treated with FBS to obtain a confluent monolayer.

Flow cytometry analysis

Flow cytometry analysis (BD FACSArray) was done using a gating strategy, in which a cell population (FSC)/SSC<50%/50% and positive for CD34 was gated and analyzed further in histogram-setting with the FlowJo software. As illustrated in the figure, the gated population was negative for CD34 (hematopoietic marker) and CD13 (monoblast/monocyte marker) and positive for the classical MSC markers CD73, CD90 (endothelial marker) and positive for the classical MSC markers CD73, CD90 (endothelial marker) and negative for CD13 (monoblast/monocyte marker). The gated population was negative for CD34 (hematopoietic marker) and CD13 (monoblast/monocyte marker) and positive for the classical MSC markers CD73, CD90 (endothelial marker) and positive for the classical MSC markers CD73, CD90 (endothelial marker) and negative for CD13 (monoblast/monocyte marker). The gated population was negative for CD34 (hematopoietic marker) and CD13 (monoblast/monocyte marker) and positive for the classical MSC markers CD73, CD90 (endothelial marker) and positive for the classical MSC markers CD73, CD90 (endothelial marker) and negative for CD13 (monoblast/monocyte marker). The gated population was negative for CD34 (hematopoietic marker) and CD13 (monoblast/monocyte marker) and positive for the classical MSC markers CD73, CD90 (endothelial marker) and positive for the classical MSC markers CD73, CD90 (endothelial marker) and negative for CD13 (monoblast/monocyte marker). The gated population was negative for CD34 (hematopoietic marker) and CD13 (monoblast/monocyte marker) and positive for the classical MSC markers CD73, CD90 (endothelial marker) and positive for the classical MSC markers CD73, CD90 (endothelial marker) and negative for CD13 (monoblast/monocyte marker). The gated population was negative for CD34 (hematopoietic marker) and CD13 (monoblast/monocyte marker) and positive for the classical MSC markers CD73, CD90 (endothelial marker) and positive for the classical MSC markers CD73, CD90 (endothelial marker) and negative for CD13 (monoblast/monocyte marker). The gated population was negative for CD34 (hematopoietic marker) and CD13 (monoblast/monocyte marker) and positive for the classical MSC markers CD73, CD90 (endothelial marker) and positive for the classical MSC markers CD73, CD90 (endothelial marker) and negative for CD13 (monoblast/monocyte marker). The gated population was negative for CD34 (hematopoietic marker) and CD13 (monoblast/monocyte marker) and positive for the classical MSC markers CD73, CD90 (endothelial marker) and positive for the classical MSC markers CD73, CD90 (endothelial marker) and negative for CD13 (monoblast/monocyte marker). The gated population was negative for CD34 (hematopoietic marker) and CD13 (monoblast/monocyte marker) and positive for the classical MSC markers CD73, CD90 (endothelial marker) and positive for the classic...