Ruprecht-Karls-Universität Heidelberg

Medizinische Fakultät Mannheim

Dissertations-Kurzfassung

Standardized Isolation and Expansion of Mesenchymal Stem Cells for Clinical Application

Autor: Asli Serife Kocaömer
Institut / Klinik: Institut für Transfusionsmedizin und Immunologie
Doktorvater: Prof. Dr. H. Klüter

Mesenchymal stem cells (MSC) are currently in focus regarding their clinical potential in cell therapy and tissue engineering. At the moment, however, most isolation and expansion protocols for clinical-scale production of MSC use culture media supplemented with Fetal Calf Serum (FCS) which poses a potential risk for infections as well as immunological reactions. My goal was to develop a standardized isolation and expansion protocol for MSC which does not depend on FCS as a supplement, therefore enabling the safe GMP-conform use of MSC in clinical settings. Pooled human AB-Serum (AB-HS) and thrombin-activated Platelet-Rich-Plasma (tPRP) were chosen as suitable alternatives to FCS and their effect on adipose tissue-derived MSC was studied. MSC isolated from Adipose Tissue (AT) of 10 donors were cultured under three different conditions: 1) 10% FCS, 2) 10% AB-HS and 3) 10% tPRP. Colony-forming units (CFU) were counted and cumulative doubling rates determined while MSC were maximally expanded. The differentiation capacity toward the adipogenic and osteogenic lineages was assessed both at passages 1 and 7. Cell surface markers were analyzed with flow cytometry at an early passage as well as a late passage.

MSC isolation was successful for all donors and culture conditions. However, both AB-HS and tPRP induced a 20-23% higher growth compared to FCS, attaining a cumulative population doubling rate of 40 at passage 8, compared to 31 with FCS ($p<0.001$). Growth of AB-HS and tPRP cells leveled off after 40-50 population doublings (P8-P10). FCS growth lagged behind, only reaching the same population doublings after 13-14 passages. Colony numbers were comparable (1 CFU per 1000 cells), whereas colony sizes were much larger for AB-HS and tPRP. Cells of all culture conditions consistently differentiated towards both adipogenic and osteogenic lineages at passages 1 and 7. Cell surface marker expression was characteristic for MSC and comparable for all culture conditions with the exception of a distinct CD14/CD45+ side population for AB-HS and tPRP cells ranging from 5-30% depending on the donor tissue. A semi quantitative cytokine profile of AB-HS and tPRP did not reveal any major differences in the presence of growth factors between the two human supplements. Even though MSC cultured with alternative supplements exhibit higher proliferation and retain MSC qualities, they differ in their morphology, growth pattern and adhesion, suggesting differences in metabolism and protein expression. Therefore, further investigations will be needed to characterize these cells regarding their protein and gene expression. In conclusion, I showed that pooled human AB-Serum and thrombin-activated Platelet-Rich-Plasma are viable alternatives to FCS for the isolation and expansion of AT-MSC. These human sources are better characterized regarding potential infectious threats, while providing a higher proliferation rate and retaining differentiation capacity and mesenchymal stem cell marker expression throughout long-term culture. I propose AB-HS as first choice in replacing FCS in GMP-compliant manufacturing processes based on the ease of preparation. The use of animal-free serum supplements will facilitate adherence to GMP regulations and pave the way for clinical application of MSC.