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ISOLATION METHOD AND LONG-TERM IN VITRO EXPANSION OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

Adipose tissue is an accessible and abundant source of mesenchymal stem cells (MSCs) for regenerative medicine, yet cell yield and expansion potential depend strongly on the isolation method, while the useful expansion window is limited by replicative senescence. **The aim** is to compare mechanical (explant) and enzymatic isolation of human adipose-derived MSCs (AD-MSCs) and to characterise the growth kinetics of enzymatically isolated cells during serial expansion to passage 7. **Methods.** AD-MSCs were isolated from adipose tissue by two approaches, mechanical (explant) and enzymatic digestion, and selected by adherence to plastic. Cells were cultured in DMEM/F12 (1:1) supplemented with FBS and bFGF at 37 °C, 5% CO₂, in 25/75/175 cm² flasks, with medium changes and passaging at 70–90% confluence. Cell number and viability were measured at each passage with an automated counter (Countess, Invitrogen; trypan blue). Population doublings (PD = log₂(N_f/N_i)), cumulative PD (cPD) and doubling time (DT) were calculated per passage. Expanded cells were cryopreserved; sterility was verified by bacterial culture and PCR screening. **Results.** Mechanical explant isolation failed to yield a proliferating culture (single cells only, no growth). Enzymatic isolation produced adherent, spindle-shaped MSC-like cells that expanded steadily through seven passages over 52 days. cPD increased almost linearly, reaching 27.7 by P7. PD per passage rose from 2.4–3.0 (P1–P2) to a peak of 5.1–5.2 at P4–P5, then declined to 4.4 (P6) and 3.8 (P7); mean DT was 1.8 days (range 1.2–2.9). Viability remained high throughout (77–94%, mean ~87%). Sustained proliferation with preserved viability indicates active self-renewal, whereas the fall in per-passage PD at P6–P7 suggests early replicative slowing. Cells were cryopreserved successfully, and sterility with PCR screening confirmed the absence of microbial contamination. **Conclusions.** Enzymatic isolation markedly outperforms the mechanical explant method for AD-MSC recovery and enables reproducible long-term expansion with high viability and substantial cumulative doublings. Quantitative kinetic parameters (PD, cPD, DT) give an objective basis for defining the expansion window before senescence.

Keywords: adipose-derived mesenchymal stem cells, isolation method, enzymatic digestion, population doubling, growth kinetics, cryopreservation.

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