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Isolation and expansion of GMP-grade adipose-derived stem cells for clinical application in urology

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Introduction & Objectives: Human adipose-derived stem cells (hADSCs) are a very promising tool for regeneration of urinary tract with tissue engineering approach. However, setting and optimization of procedures compliant with Good Manufacturing Practice (GMP) is required for enabling future clinical trials with use of hADSCs. The goal of this study was to find and evaluate the most optimal culture conditions for hADSCs compliant with GMP.

Materials & Methods: Subcutaneous adipose tissue harvested from 10 patients was used for hADSCs isolation. Adipose tissue was digested with Collagenase NB6. Stromal Vascular Fraction (SVF) cells were seeded at a density of 5 x 10^4 cells/cm^2. Four different serum-free media were compared: **MI:** DMEM/F12 (1:1) + 10% Human platelet lysate; **MII:** MEM alpha + 10% Human platelet lysate; **MIII:** CTS™ StemPro™ MSC SFM; **MIV:** DMEM + 10% Human platelet lysate. Cells were cultured for up to 10 passages. Cell number and viability were measured with use of the trypan blue exclusion test after every passage. Immunophenotype, cell cycle and apoptosis were analyzed by flow cytometry.

Results: hADSCs cultures were successfully established in all four media. Decrease of proliferation rate as well as number of cells harvested from 1 cm^2, was observed for all media since passage 2. The highest amount of harvested cells was observed in MIII, alongside similar numbers in MII and MIV, and the worst for MI. Flow cytometry analysis of immunophenotype revealed a significant decrease of CD105 expression in prolonged culture in all four media, with the fasted drop in MI. Cell cycle analysis indicated greater size of G2/M cell population in MI. The highest number of apoptotic cells was observed in MIII.

Conclusions: MI occurred as the worst medium as the culture was maintained for no more than 6 passages. MIII presents best results, but necessity of covering the growth area with additional layer of substrate, not intended for direct administration into humans, disqualifies possibilities of using it for creation of tissue engineered grafts. MII and MIV display similar, promising results, with more controllable growth rate in favour of MIV. Results indicate that the best of the tested media for hADSCs culture, compliant with GMP, are MII and MIV, but additional research evaluating differentiation potential of hADSCs cultured in these media are required.

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