Manufacture and preparation of human placenta-derived mesenchymal stromal cells for local tissue delivery.

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Public Summary:

INTRODUCTION: Mesenchymal stromal cells (MSCs) are a type of - stem cell that can be isolated from several tissue sources including bone marrow, adipose, umbilical cord, and placenta. These cells are of interest to researchers for their potential application in the treatment of a wide range of diseases. Our lab has previously demonstrated that placenta-derived mesenchymal stromal cells (PMSCs) are readily expandable and secrete a myriad of angiogenic and immunomodulatory factors that are thought to elicit paracrine effects in vivo, modulating the body's endogenous wound healing responses. We also found that these cells are compatible with a wide-range of delivery vehicles and matrices which can aid in their local delivery to the site of injury. In this study, we describe the development of a Current Good Manufacturing Practice (CGMP)-compliant process to isolate, expand and bank PMSCs for use as stem cell therapy. Our process includes steps taken to screen these cells for purity and sterility, all of which can be rapidly adapted to CGMP cell production in a future clinical trial. Furthermore, we characterize the compatibility of PMSCs with an FDA-approved extracellular matrix derived from porcine small intestine submucosa (SIS-ECM, Cook Biotech) that can be used as a vehicle for precise local cell delivery. METHODS. PMSCs from three donors were isolated from placental chorionic villus tissue using an explant culture methodology. Cells were isolated and expanded, and cryopreserved in master and working cell banks. PMSCs were screened for sterility, absence of contaminating Mycoplasma species, and low endotoxin levels. The cell phenotype was qualified using flow cytometry to examine expression of well-established MSC surface markers and expression of markers of pluripotency. The multipotency of the PMSCs was demonstrated using tri-lineage differentiation assays, and the cells were determined to have normal karyotypes. Optimal loading density and viability of PMSCs on SIS-ECM were determined using MTS cell proliferation and fluorescent live/dead assays, respectively. Secretion of angiogenic and neuroprotective growth factors was analyzed using enzyme-linked immunosorbent assays (ELISA). RESULTS. PMSCs were rapidly expanded and banked. Viable master and working cell banks were stable with minimal decrease in viability at 6 months. All PMSCs were sterile, free from mycoplasma species, karyotypically normal and had low endotoxin levels. PMSCs were homogeneous by immunophenotyping and expressed little to no pluripotency markers. Optimal loading density on SIS-ECM was 3–5×105 cells/cm2, and seeded cells were >95% viable. Angiogenic and neurotrophic factor secretion was detectable from PMSCs seeded on plastic and SIS-ECM with variability between donor lots. DISCUSSION. PMSCs from placental tissues can be rapidly expanded and banked in stable, viable cell banks that are free from contaminating agents, are genetically normal, and phenotypically pure. Local delivery of PMSCs can be achieved using SIS-ECM, which was demonstrated here to maintain cell viability and protein secretion. While our work here builds a solid foundation in the development of clinically-oriented cell isolation and manufacturing procedures, future work in vivo is necessary to further optimize cell seeding and transplantation to maximize therapeutic capabilities.

Scientific Abstract:

BACKGROUND: In this study we describe the development of a Current Good Manufacturing Practice (CGMP)-compliant process to isolate, expand and bank placenta-derived mesenchymal stromal cells (PMSCs) for use as stem cell therapy. We characterize the viability, proliferation and neuroprotective secretory profile of PMSCs seeded on clinical-grade porcine small intestine submucosa extracellular matrix (SIS-ECM; Cook Biotech). METHODS: PMSCs were isolated from early gestation placenta chorionic villus tissue via explant culture. Cells were expanded, banked and screened. Purity and expression of markers of pluripotency were determined using flow cytometry. Optimal loading density and viability of PMSCs on SIS-ECM were determined using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetr azolium (MTS) cell proliferation and fluorescent live/dead assays, respectively. Growth factors secretion was analyzed using enzyme-linked immunosorbent assays (ELISA). RESULTS: PMSCs were rapidly expanded and banked. Viable Master and Working Cell Banks were stable with minimal decrease in viability at 6 months. All PMSCs were sterile, free from Mycoplasma species, karyotypically normal and had low endotoxin levels. PMSCs were homogeneous by immunophenotyping

and expressed little to no pluripotency markers. Optimal loading density on SIS-ECM was 3-5 x 105 cells/cm2, and seeded cells were >95% viable. Neurotrophic factor secretion was detectable from PMSCs seeded on plastic and SIS-ECM with variability between donor lots. DISCUSSION: PMSCs from early gestation placental tissues can be rapidly expanded and banked in stable, viable cell banks that are free from contaminating agents, genetically normal and pure. PMSC delivery can be accomplished by using SIS-ECM, which maintains cell viability and protein secretion. Future work in vivo is necessary to optimize cell seeding and transplantation to maximize therapeutic capabilities.

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