Cytotherapy, 2017; 19: 680–688

International Society for Cellular Therapy
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Manufacture and preparation of human placenta-derived mesenchymal stromal cells for local tissue delivery

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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-tetrazolium (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 $3–5 \times 10^5$ cells/cm^2, 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.

Key Words: *cell delivery, cell manufacturing, Current Good Manufacturing Practice, mesenchymal stromal cells, placenta, small intestine submucosa*

Introduction

Stem cell–based regenerative therapies likely represent the future of healthcare as we move further into an era of personalized medicine. Mesenchymal stromal cells (MSCs) have garnered interest for clinical use due to their differentiation capabilities, wound healing and immunomodulatory properties [1–7]. Our previous work demonstrated that placenta-derived mesenchymal stromal cells (PMSCs) represent a readily available, allogeneic cell source that is compatible with existing delivery vehicles [8,9]. Additionally, compared with bone marrow MSCs, isolation of PMSCs is relatively noninvasive and yields larger cell numbers [10]. Studies have shown that early gestation PMSCs display superior immunomodulatory, neuroprotective and wound-healing capabilities compared with later gestation PMSCs and MSCs from other sources [5,8,9,11–18].

The therapeutic effect of MSCs is thought to result from paracrine secretion of unique cytokines and growth factors promoting endogenous healing and growth [19,20] and thus localized rather than systemic delivery of these cells can potentiate their effect. Use of a matrix delivery vehicle promotes precise cell delivery and improves cell retention and survival [21].

Porcine small intestine submucosa-derived extracellular matrix (SIS-ECM; Cook Biotech) is a commercially available, US Food and Drug Administration (FDA)-approved vehicle compatible for use with MSCs [22–26]. SIS-ECM is a natural candidate for cell delivery because it is a decellularized biomaterial that retains its inherently porous quality, allowing new cells to repopulate and thrive [25]. Studies

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(Received 18 November 2016; accepted 3 March 2017)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2017.03.003

have shown that MSCs seeded onto SIS-ECM have increased secretion of vascular endothelial growth factor (VEGF) when compared with other culture conditions [25,27].

PMSCs seeded onto SIS-ECM represents an optimal combination of an ideal cell source with a functional delivery vehicle for allogeneic cell therapy; however, there lacks a common manufacturing protocol and stringent quality control measures [28]. In this study we describe the development of a Current Good Manufacturing Process (CGMP)-compliant method to isolate, expand and bank early gestation PMSCs. In addition, we characterize the viability, proliferation and neuroprotective growth factor secretory profile of PMSCs seeded on SIS-ECM.

Materials and methods

Isolation, expansion and banking of PMSCs from human early gestation placenta

Discarded early gestation placental tissue (15–19 weeks) was collected at the University of California, Davis Medical Center. Cell isolation occurred within 24 h of tissue collection from three donor tissues using an explant culture method. Chorionic villus tissue was carefully dissected from placental tissue and washed in sterile 1X phosphate-buffered saline (PBS) containing 100 U/mL penicillin and 100 µg/mL streptomycin (P-S; Thermo Fisher Scientific). Then, 0.5 g of tissue was transferred to a clean 100 mm petri dish and minced into <1 mm^3 fragments before being collected in a suspension of complete culture media and transferred into a tissue culture-treated T150 flask. Flasks were rocked gently to ensure even distribution and incubated in a humidified 37°C, 5% CO_2 incubator. Four flasks were cultured to initiate each PMSC lot, and media changed every 3–4 days for 2–4 weeks until flasks were confluent. Complete culture media for PMSCs consisted of Dulbecco's Modified Eagle's Medium (DMEM) high glucose with 5% fetal bovine serum (FBS; Hyclone), 20 ng/mL recombinant human basic fibroblast growth factor (R&D Systems), 20 ng/mL recombinant human epidermal growth factor (R&D Systems), and P-S.

PMSCs were cryopreserved throughout the manufacturing process at passages one (P1), two (P2) and four (P4). When explant cultures became confluent, cells were harvested and filtered through 70 µm nylon filters to remove tissue debris. Pellets were pooled before counting with a hemocytometer using a 1:1 dilution in trypan blue 0.4% solution. Six T150 flasks were re-seeded with cells at P1 at a density of 4×10^3 cells/cm^2, and remaining cells were cryopreserved generating a small P1 cell bank. PMSCs were then expanded and cryopreserved at P2 in the Master Cell Bank (MCB). After qualification at P2–P4, PMSCs from the MCB were expanded and cryopreserved in a Working Cell Bank (WCB) at P4. PMSC cryopreservation was accomplished via resuspension in a solution of 10% dimethyl sulfoxide (DMSO) and 90% FBS and controlled freezing at −80°C using Mr. Frosty freeze containers (Thermo Fisher Scientific). Viability of cells from MCB and WCB was assessed at 6 months from their generation using trypan blue exclusion viability counts.

Sterility, endotoxin and mycoplasma screening

Sterility and endotoxin levels of PMSC culture supernatants collected at P2–P3 were assessed by the UC Davis Institute for Regenerative Cures (IRC) Quality Control Laboratory. 14-day sterility testing was completed according to United States Pharmacopeia Chapter 71 standards. Endotoxin testing was performed on an Endosafe-PTS system (Charles River Laboratories) and concentrations reported as Endotoxin Units (EU)/mL, where <0.5 EU/mL was considered acceptable. *Mycoplasma* screening was performed at the UC Davis Comparative Pathology Lab. Screened specimens included 3×10^6 cells and 500 µL of supernatant that were delivered on ice to the Comparative Pathology Lab within 2–3 h of collection before being screened using quantitative PCR (qPCR) for the presence of *Mycoplasma* species.

Karyotyping

Cells were karyotyped at the UC Davis IRC Karyotyping Core. PMSCs were seeded in T75 flasks at 1×10^4 cells/cm^2 24–48 h prior to being treated with Colcemid (Thermo Fisher Scientific) to arrest mitotic cells in metaphase. Cells were detached from flasks using TrypLE Select (Thermo Fisher Scientific) and treated with a 0.067 mol/L KCl hypotonic solution before fixation with 3:1 methanol:acetic acid solution. Fixed cells were then dropped on slides and slides were aged before trypsin-Giemsa stain and microscope analysis. Microscopy included analysis of 20 metaphase spreads and karyotype of two metaphases.

Growth kinetics

PMSC growth kinetics were calculated using cell counts obtained from P1–P4 during expansion for cell banking. The following formulas were used to calculate kinetics data, where T is equal to the time in hours, n is equal to the final cell number divided by the initial seeded number and "initial" refers to the starting cell number at each passage:

1. $Doubling\ Time\ (DT) = T * log(2)/log(n)$
2. $Population\ Doublings\ (PD) = log(n)/log(2)$
3. $Log\ Cell\ Number = Initial * 2^{PD}$