Cytotherapy, 2015; ■■: ■■–■■

International Society for Cellular Therapy ISCT®

Evaluation of a cell-banking strategy for the production of clinical grade mesenchymal stromal cells from Wharton's jelly

IRENE OLIVER-VILA*, MARIA ISABEL COCA*, MARTA GRAU-VORSTER, NOÈLIA PUJALS-FONTS, MARTA CAMINAL, ALBA CASAMAYOR-GENESCÀ, ISABEL ORTEGA, LAURA REALES, ARNAU PLA, MARGARITA BLANCO, JOAN GARCÍA & JOAQUIM VIVES

Divisió de Teràpies Avançades/XCELIA, Banc de Sang i Teixits, Edifici Dr. Frederic Duran i Jordà, Passeig Taulat, 116, 08005 Barcelona, Spain

Abstract

Background aims. Umbilical cord (UC) has been proposed as a source of mesenchymal stromal cells (MSCs) for use in experimental cell-based therapies provided that its collection does not raise any risk to the donor, and, similar to bone marrow and lipoaspirates, UC-MSCs are multipotent cells with immuno-modulative properties. However, some of the challenges that make a broader use of UC-MSCs difficult include the limited availability of fresh starting tissue, time-consuming processing for successful derivation of cell lines, and the lack of information on identity, potency and genetic stability in extensively expanded UC-MSCs, which are necessary for banking relevant cell numbers for preclinical and clinical studies. *Methods.* Factors affecting the success of the derivation process (namely, time elapsed from birth to processing and weight of fragments), and methods for establishing a two-tiered system of Master Cell Bank and Working Cell Bank of UC-MSCs were analyzed. *Results.* Efficient derivation of UC-MSCs was achieved by using UC fragments larger than 7 g that were processed within 80 h from birth. Cells maintained their immunophenotype (being highly positive for CD105, CD90 and CD73 markers), multi-potentiality and immuno-modulative properties beyond 40 cumulative population doublings. No genetic abnormalities were found, as determined by G-banding karyotype, human telomerase reverse transcriptase activity was undetectable and no toxicity was observed *in vivo* after intravenous administration of UC-MSCs in athymic rats. *Discussion.* This works demonstrates the feasibility of the derivation and large-scale expansion of UC-MSCs from small and relatively old fragments of UC typically discarded from public cord blood banking programs.

Key Words: *cell culture, Good Manufacturing Practice, mesenchymal stromal cell, scale up, T-cell proliferation, umbilical cord, Wharton's jelly*

Introduction

Mesenchymal stromal cells (MSCs) are multipotent cells present in virtually all support and structural tissues of an organism and can be readily isolated and expanded *ex vivo*, with bone marrow (BM) and adipose tissue the most common sources of adult MSCs [1,2]. Cell-based therapy approaches taking advantages of MSCs derived from different tissues have been proposed for a variety of indications [3]. The identity of MSCs from any tissue source is commonly verified by compliance with the minimal criteria established by the International Society for Cellular Therapy, including (i) adherence to plastic in standard culture conditions; (ii) characteristic profile of surface antigen pattern expression, that is, highly positive (≥95%) for CD105, CD90, CD73 markers, and negative (≤2%) for CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR markers; and (iii) *in vitro* differentiation potential to become osteoblastic (bone cells), chondrocytic (cartilage cells) and adipocytic (fat cells) lineages [4]. Long considered a medical waste, the umbilical cord (UC) discarded after birth offers an accessible and easily collectable source of MSCs [5], thus avoiding painful and invasive interventions, in contrast to BM-MSC isolation and lipoaspirates [6]. Moreover, UC-MSCs are thought to be more primitive MSCs than those isolated from BM or lipoaspirates, and thus they are not expected to carry somatic mutations and show the immunological immaturity of

*These authors contributed equally to this work.

Correspondence: **Joaquim Vives**, PhD, Divisió de Teràpies Avançades/XCELIA, Banc de Sang i Teixits, Edifici Dr. Frederic Duran i Jordà, Passeig Taulat, 116, 08005 Barcelona, Spain. E-mail: jvives@bst.cat

(Received 3 August 2015; accepted 2 October 2015)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2015.10.001

newborn cells because of the total lack of expression of HLA-DR antigens [7]. This feature, in combination with the immuno-modulative properties of UC-MSCs, makes them candidates for allogeneic cell therapy [8]. However, little information is currently available in the literature regarding parameters of culture kinetics and the effect of cumulative population doublings on genetic stability of MSCs, maintenance of their identity, their multi-potentiality or their immuno-modulative properties. This knowledge is essential for large-scale expansion protocols to produce a homogeneous population of cells for clinical use with defined and reproducible characteristics [9,10]. Indeed, a relationship between high cumulative population doubling (CPD) values and chromosomic instability and loss of multi-potentiality has been reported in BM-MSCs [11–13].

In the present study, we addressed these issues by developing a simple, effective and reproducible method for UC-MSC derivation from Wharton's jelly, followed by the generation of a two-tiered system of Master Cell Bank (MCB) and Working Cell Bank (WCB), compatible with the Good Manufacturing Practice (GMP)-compliant production environment. This process has the potential to yield a large number of doses to use in preclinical and clinical studies in a space-, time- and cost-efficient manner that is suitable for implementation in small laboratories, particularly for academia, hospitals and blood banks. UC-MSCs derived using this method have been extensively characterized at different passages, as follows: morphology, cell growth and metabolic profile, immunophenotype, multipotentiality, immunomodulation capacity, genetic stability, *in vivo* subchronic toxicity assessment and biodistribution.

Methods

Source tissue and MSC derivation and expansion

UC tissue collected within the Concordia Program (http://www.bancsang.net/professionals/en_concordia) with appropriate donor informed consent was used for sourcing UC-MSCs. UC samples were washed twice with sterile phosphate-buffered saline (PBS; Gibco) to remove any traces of blood and then transferred to a 100-mm tissue culture plate (Corning). The tissue was cut longitudinally and split open to expose the inner surface so that the two arteries and the vein could be removed by pulling them gently. Wharton's jelly was scrapped with a surgical scalpel, spread uniformly over the culture plate, and incubated for approximately 30 min at 37°C. After the incubation, 10 mL of *derivation medium* consisting of Dulbecco's Modified Eagle's Medium (Gibco) containing 2 mmol/L glutamine were added and supplemented with 2×10^4 UI/mL penicillin (Invitrogen), 20 mg/mL streptomycin (Invitrogen), 120 µg/mL amphotericin B (Invitrogen) and 20% human serum B (hSerB, Banc de Sang i Teixits). After 2–5 days, a washing step with saline solution was performed, and 10 mL of fresh derivation medium was added. From this point, the culture medium was replaced every 3–4 days. Cells were further expanded *in vitro* by seeding cell culture flasks at 1000 cell/cm^2 in derivation medium. When the total number of cells reached at least 5×10^6, they were frozen in cryovials producing the "active ingredient" (AI). Further expansion was performed after thawing for the generation of the MCB and WCB [14] using expansion medium composed by Dulbecco's Modified Eagle's Medium containing 2 mmol/L glutamine and supplemented with 10% hSerB. All cultures were maintained at 37°C and 5% CO_2 in humidified incubators. The medium was changed every 3–4 days, and trypsinization was performed when reached 70–90% confluence. Cell number and viability were determined by the haemocytometer-based trypan blue dye exclusion assay. CPD was determined as CPD = [ln (initial cell density/final cell density)]/ln 2 [15]. Cell diameter, cell volume and total cell count was determined using the Scepter Handheld Automated Cell Counter (Millipore).

Flow cytometry

Fluorescence-activated cell sorter (FACS) analysis was performed to evaluate expression of surface markers CD31 (555445, BD), CD45 (HI30, BD Biosciences), CD90 (5E10, BD Biosciences), CD73 (AD2, BD Biosciences), CD105 (43A4E1 clone, Miltenyi) and HLA-DR (TU36, BD Biosciences) in a FACSCalibur flow cytometer (BD Biosciences). Phycoerythrin-conjugated immunoglobulin G1 (×40, BD Biosciences) and fluorescein isothiocyanate–conjugated immunoglobulin G2bk (27–35, BD Biosciences) antibodies were used as isotype controls.

Lymphocyte proliferation assays

The immunomodulation potential of UC-MSCs was analyzed with mononuclear cells (MNCs) obtained by ficoll gradient centrifugation (Lymphocyte Isolation Solution, Rafer) from the buffy-coat of healthy blood donors. Briefly, after washing cells with PBS, cells were resuspended at 2.5×10^6 cells/mL and labeled with 0.625 µmol/L carboxy-fluorescein diacetate succinimidyl ester (CFSE) for 10 min using the CellTrace CFSE Cell Proliferation Kit (Molecular Probes). MNCs were then washed with 5 volumes of expansion medium, resuspended at $1–2 \times 10^7$ cells/mL with expansion medium and incubated for 12 min at 37°C. A final wash with 5 volumes of expansion medium was performed, and CFSE-MNCs were resuspended in expansion medium