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Quantitative analysis of composite umbilical cord tissue health using a standardized explant approach and an assay of metabolic activity

MATTHEW L. SKILES[1], KATHERINE S. BROWN[1], WILLIAM TATZ[2], KRISTEN SWINGLE[3] & HEATHER L. BROWN[1]

[1]*Scientific and Medical Affairs, Cbr Systems, Inc., South San Francisco, California, USA,* [2]*Laboratory Operations, Cbr Systems, Inc., Tucson, Arizona, USA, and* [3]*Consumer Sales and Operations, Cbr Systems, Inc., Tucson, Arizona, USA*

Abstract

Background. Umbilical cord (UC) tissue can be collected in a noninvasive procedure and is enriched in progenitor cells with potential therapeutic value. Mesenchymal stromal cells (MSCs) can be reliably harvested from fresh or cryopreserved UC tissue by explant outgrowth with no apparent impact on functionality. A number of stem cell banks offer cryopreservation of UC tissue, alongside cord blood, for future cell-based applications. In this setting, measuring and monitoring UC quality is critical. *Materials and Methods.* UC explants were evaluated using a plating and scoring system accounting for cell attachment and proliferation. Explant scores for fresh and cryopreserved-then-thawed tissue from the same UC were compared. Metabolic activity of composite UC tissue was also assayed after exposure of the tissue to conditions anticipated to affect UC quality and compared with explant scores within the same UC. *Results.* All fresh and cryopreserved tissues yielded MSC-like cells, and cryopreservation of the tissue did not prevent the ability to isolate MSCs by the explant method. Thawed UC tissue scores were 91% (±0.6%; P = 0.0009) that of the fresh, biologically identical tissue. Within the same UC, explant scores correlated well to both cell yield (R^2 = 0.85) and tissue metabolic activity (R^2 = 0.69). *Discussion.* A uniform explant scoring assay can provide information about the quality of composite UC tissue. Such quantitative measurement is useful for analysis of tissue variability and process monitoring. Additionally, a metabolic assay of UC tissue health provides results that correlate well to explant scoring results.

Key Words: *cryopreservation, mesenchymal stromal cells, stem cell banking, umbilical cord tissue*

Introduction

Considerable pre-clinical evidence suggests the safety and efficacy of mesenchymal stromal cells (MSCs) across a wide variety of conditions, due primarily to trophic and immune modulatory effects. It is estimated that more than 450 clinical trials are now evaluating MSCs as part of a therapeutic intervention [1] . MSCs can be found in many different tissues, including bone marrow, adipose tissue, dental pulp and newborn tissues such as umbilical cord (UC) blood, UC tissue and placental tissue [2]. Bone marrow and adipose tissue remain the most often used MSC sources for clinical applications. Yet several groups have observed that MSC prevalence and function are negatively affected by increased age and certain chronic disease conditions [3–5]. In contrast, MSCs isolated from newborn tissue sources, including placental and UC tissue, have demonstrated an enhanced proliferative capacity and in general have had less risk of exposure to virus and toxins compared with their counterparts isolated from aged adult tissues [6]. Thus, preservation of newborn tissues as a source of MSCs may be advantageous for future clinical applications.

Large numbers of MSCs can be consistently isolated from UC tissue, which also serves as a source of other progenitor cell populations [7–12]. Approaches for isolating MSCs from UC tissue include enzymatic digestion and tissue explanting, with recent reports also demonstrating tool-aided mechanical tissue dissociation techniques [2,13]. Compared with tissue digestion or dissociation, isolation of MSCs from UC by an explant approach may minimize cellular stresses and damage that can impact cellular function [2,14–16]. Furthermore, explant isolation results in MSC populations that are more homogeneous than extracts collected by digestion or mechanical dissociation [17,18]. That explanted UC tissues consistently

Correspondence: **Matthew L. Skiles**, PhD, Scientific and Medical Affairs, Cbr Systems, Inc., 6550 S. Bay Colony Drive, Suite 160, Tucson, AZ 85756 USA.
E-mail: mskiles@cordblood.com

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produce clonogenic cells and that these cells possess MSC qualities, including morphology, immunophenotype, proliferation kinetics, differentiation potential and immunosuppressive activity, has been well established [6,14,16–21]. It has also been shown that MSCs can be harvested from cryopreserved-then-thawed UC tissues by the explant approach without any obvious functional impact [12,16,22,23].

In recognition of the unique benefits that UC tissue may offer as rich source of MSCs, numerous UC blood banks, including some public banks, have established UC tissue storage programs [13,24]. A popular banking strategy has been to cryopreserve and store the UC tissue as a whole, composite material. This approach minimizes front-end tissue manipulation, reduces processing time and expense and allows for isolation of the progenitor population when the therapeutic need is known. Moreover, it has been proposed that composite UC tissue acts as an ideal and natural storage compartment with maintenance of the stem cell niche [25]. Within the banking setting, it is essential that UC tissue health is maintained throughout the collection, processing and storage steps so that therapeutic cells can be isolated from the tissue when needed. However, unlike the cord blood storage industry, which has been established for over two decades and for which approved transplant indications are defined, the cord tissue storage industry has yet to solidify consensus processing and handling protocols, quality metrics or release criteria, in part due to the complicated nature of assessing composite tissue health.

In pursuing the potential of UC MSCs in regenerative therapies, the research literature has largely overlooked the distinction between UC MSC quality (characteristics such as population doubling time, immunophenotype, differentiation capacity and immunomodulation of cells derived from UC tissue) and UC tissue quality (overall tissue viability and the propensity with which desirable cells are harvestable from the UC tissue). While the former may ultimately dictate use criteria for particular clinical indications, the latter will be essential for the success of a sound banking platform that can support clinical applications by reliably storing and supplying clinical material. Developing baseline expectations for MSC harvests from UC and how various factors, such as donor variation, collection and transport procedures, and processing and freezing protocols impact the ability to harvest MSCs from UC will allow for the optimization and standardization of UC tissue banking. Therefore, it will be important to use assays that can consistently and reliably measure UC tissue health. The current work presents, to our knowledge, the first report of approaches for quantifying the quality of composite UC tissue. We first demonstrate that within a cord blood banking setting, co-collection of UC tissue along with UC blood does not detrimentally impact the quality of cord blood collection. Then, using a standardized UC explant protocol, which measures a tissue's propensity to yield MSC-like cells, and a quantifiable metabolic assay, which indicates a tissue's overall health status, we show that the assays are able to measure and compare the relative quality of UC tissues and identify impacts to UC tissue quality caused by various treatment scenarios. We believe that these approaches represent a starting point for the development of such quality assays as will be required for clinical application of MSCs derived from UC tissue.

Materials and methods

Cord blood collection unit characteristics

A random sampling of 1000 cord blood collections processed and stored between July 1 and December 31, 2014 was selected for analysis from a larger inventory. All collections were from consenting mothers into gravity bags prefilled with 500 units of lyophilized heparin and transported to a processing facility in Tucson, Arizona. Cord blood was processed on the AutoXpress® Platform (Cesca Therapeutics, Inc.), and post-processing aliquots were assessed for total nucleated cell (TNC) counts on a Sysmex hematology analyzer (model XE-2100L, Sysmex America) and for viability using 7-aminoactinomycin D (7-AAD) staining. CD34+ cell content was enumerated on an FC 500 flow cytometer (Beckman Coulter, Inc.) with the Beckman Coulter Stem-Kit. Birth weights of donors in either collection scenario were not significantly different (data not shown), and cord blood collection volume was normalized by birth weight. Units more than 48 hours old at time of processing or processed as a protocol exception were excluded from the analysis. All units were collected from births >37 weeks gestation, based on self-reported data.

UC tissue collection and cryopreservation

Donated UC tissue units were collected from consenting mothers following either a surgical or vaginal delivery (>37 weeks gestation) and transported to a processing facility at ambient temperatures in a buffered saline solution containing an aminoglycoside antibiotic at typical concentration. Upon receipt, the cords were decontaminated by a stepwise series of immersion rinses, first in Dulbecco's phosphate-buffered saline (DPBS), then in 70% ethanol and finally in DPBS again. After a final DPBS rinse, the cords were segmented into small sections (0.3 cm^3). Cord tissue sections were submerged in a dimethyl sulfoxide (DMSO)-based, clinical-grade cryopreservation solution (CryoStor CS10, BioLife Solutions) prior to