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Umbilical cord tissue–derived mesenchymal stromal cells maintain immunomodulatory and angiogenic potencies after cryopreservation and subsequent thawing

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Abstract

Background aims. The effect of cryopreservation on mesenchymal stromal cell (MSC) therapeutic properties has become highly controversial. However, data thus far have indiscriminately involved the assessment of different types of MSCs with distinct production processes. This study assumed that MSC-based products are affected differently depending on the tissue source and manufacturing process and analyzed the effect of cryopreservation on a specific population of umbilical cord tissue–derived MSCs (UC-MSCs), UCX®. *Methods.* Cell phenotype was assessed by flow cytometry through the evaluation of the expression of relevant surface markers such as CD14, CD19, CD31, CD34, CD44, CD45, CD90, CD105, CD146, CD200, CD273, CD274 and HLA-DR. Immunomodulatory activity was analyzed *in vitro* through the ability to inhibit activated T cells and *in vivo* by the ability to reverse the signs of inflammation in an adjuvant-induced arthritis (AIA) model. Angiogenic potential was evaluated *in vitro* using a human umbilical vein endothelial cell–based angiogenesis assay, and *in vivo* using a mouse model for hindlimb ischemia. *Results.* Phenotype and immunomodulatory and angiogenic potencies of this specific UC-MSC population were not impaired by cryopreservation and subsequent thawing, both *in vitro* and *in vivo*. *Discussion.* This study suggests that potency impairment related to cryopreservation in a given tissue source can be avoided by the production process. The results have positive implications for the development of advanced-therapy medicinal products.

Key Words: *advanced-therapy medicinal product, cryopreservation, mesenchymal stromal cells, potency, thawing*

Introduction

Mesenchymal stromal cells (MSCs) are promising active substances for advanced therapy medicinal products (ATMPs), mainly because of their immunomodulatory, anti-inflammatory, pro-angiogenic and regenerative properties. Currently, there are approximately 700 clinical trial entries using "mesenchymal cells" in the search tool at the ClinicalTrials.gov website, consisting of trials promoted by both academic institutions and industry alike for a wide variety of indications. Umbilical cord tissue–derived expanded MSCs, UCX®, consists of a specific population of MSCs from the umbilical cord tissue (UC-MSCs), isolated according to a patented technology that is the basis for several allogeneic ATMPs under development [1,2]. Preclinical studies using UCX® have demonstrated the safety and quality of the cells [2], as well as their efficacy in animal models for several immune-related and cardiovascular diseases. Specifically, UCX® have demonstrated an anti-inflammatory effect in both acute and chronic arthritis models [3], leading to a drastic reduction of local and

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systemic arthritic manifestations. They have promoted recovery of cardiac function upon intramyocardial administration in an acute myocardial infarction murine model, mostly due to a paracrine activity that activated pro-angiogenesis and anti-apoptotic mechanisms [4]. In addition, an UCX® pro-angiogenic effect has recently been demonstrated in a murine model of hindlimb ischemia [5], whereas the paracrine effects of UCX® cells have also been noted in a wounded skin *in vivo* model, where they have promoted healing via a mechanism that could implicate the recruitment of other systemic/endogenous MSCs [6].

Given their recognized potential for cellular therapy and regenerative medicine, it is necessary to cryopreserve and bank MSCs to ensure their immediate availability. A remarkable amount of research and resources have been expended toward optimizing the cryopreservation/thawing processes, as well as developing Good Manufacturing Practices (GMP) to ensure that MSCs retain their safety and therapeutic characteristics after cryopreservation. Nevertheless, the results are controversial. Reports have indicated that cryopreservation of bone marrow–derived MSCs (BM-MSCs) impaired their immunosuppressive properties due to a heat-shock response [7] and resulted in their faster complement-mediated elimination after blood exposure [8]. Similar claims have been made for adipose tissue-derived MSCs (AT-MSCs), where administration of freshly thawed cryopreserved AT-MSCs was associated with significant adverse effects in contrast to administration of cultured AT-MSCs, which resulted in improvement in renal function without adverse effects [9]. However, the belief that cryopreservation negatively affects the performance of MSCs has recently been challenged. One study has shown that xenogen-free, GMP-grade BM-MSCs can undergo cryopreservation without altering their characteristics and immune-modulating potential [10]. More recently, an *in vivo* analysis comparing freshly thawed and cultured BM-MSCs in a mouse model of allergic airway inflammation also demonstrated no difference between freshly thawed and cultured cells [11]. Most importantly, in a phase II clinical study using BM-MSCs in acute graft-versus-host disease in which either cultured or freshly thawed cells were infused, there was no report of clinical differences observed between the two groups [12].

UCX® cells are specifically meant to serve as a basis for off-the-shelf cryopreserved products, and the challenge is to ensure that cryopreserved cells retain the therapeutic potential of their freshly isolated counterparts. In previous preclinical studies, UCX® were not freshly thawed immediately before *in vivo* use, meaning they were typically administered immediately after a culture step [2–6]. In the present study, we set out to test whether the phenotype and therapeutic potency of our specific UC-MSC population is affected by our cryopreservation and/or thawing procedures, comparing cultured cells with freshly thawed cells in terms of their immunophenotype as well as their immunomodulatory and angiogenic potencies, both *in vitro* and *in vivo*.

Methods

Umbilical cord samples

This study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee at the Cascais Hospital Dr. José de Almeida. Umbilical cord donations were obtained with written informed consent according to Directive 2004/23/EC of the European Parliament (transposed to the Portuguese Law 12/2009 of March 26).

Isolation of UCX® cells

Human UCX cells were isolated according to patented proprietary method developed by ECBio as described in Soares *et al* [1]. Briefly, human umbilical cords, obtained after full-term natural or cesarian delivery births, were depleted of blood, cut in transversal sections and digested with animal component-free collagenase NB4 (Serva) and TrypLE Select (Gibco, Life Technologies). After overnight incubation at 37°C under 7% CO_2 in a humidified incubator, non-adherent cells were removed, and fresh medium was added, α-Minimum Essential basal medium with 1 g/L glucose (Biochrom), 2 mmol/L glutamine (Sigma-Aldrich) complemented with 20% fetal bovine serum (FBS) (Gibco). Change of culture medium was performed twice weekly. Cells were passaged when 80–90% confluence was observed.

Cryopreservation and thawing of UCX® cells

UCX® cells used in this study were cultured up to passage 7 and tested between passage 5 and 7. In near confluent cultures, cells were detached using TrypLE Select (Gibco). Approximately 3×10^6 cells were centrifuged at 200*g*, 4°C for 10 min and cryopreserved in 10% dimethylsulfoxide (WAK-Chemie) and 90% FBS (Gibco). Cryopreservation was performed in a Controlled Rate Freezer (CRF) (IceCube14S, Sylab) using a freezing profile described by Freimark *et al* [13].

During thawing, cryovials were removed from the liquid N_2 container and placed immediately in the water bath at 37°C until cell suspension was almost entirely thawed and diluted with MSC medium, counted and plated in a culture flask (Nunc, Thermo Fisher Scientific). Cell suspension was then either (i) washed and suspended in the vehicle for further assays