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Brief Communication

A simple and serum-free protocol for cryopreservation of human umbilical cord as source of Wharton's jelly mesenchymal stem cells ☆

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ABSTRACT

Mesenchymal stromal cells (MSCs) show promise in cell-based transplantations and regenerative medicine applications. MSCs from Wharton's jelly (WJ) of umbilical cord can be easily harvested and exhibit greater proliferative activity than bone marrow MSCs. It is important to develop a practical cryopreservation technique to effectively store umbilical cord for potential future applications. Successful cryopreservation would allow access to umbilical cord from the same donor for repeated WJ MSC-based transplantations. For therapeutic applications, one should be able to obtain clinically-relevant quality and quantity of MSCs from cryopreserved tissues. In this study, we optimised a serum-free formulation of 10% dimethyl sulfoxide (DMSO) and 0.2 M sucrose for cryopreservation of umbilical cord tissue. Slow freezing and rapid thawing were adopted. MSCs harvested from WJ of cryopreserved umbilical cord could undergo robust expansion, differentiate to mesodermal lineages and express MSC-characteristic surface antigens. The cumulative cell yield, however, was less compared to corresponding fresh cord tissue.

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Introduction

Mesenchymal stromal cells (MSCs) are emerging as cells with great therapeutic promise due to their immunosuppressive and regenerative capacities [3] and MSC-based clinical trials for regenerative treatment of various human disorders have been initiated in different parts of the world [11]. Umbilical cord, a discarded tissue, is abundantly available and is non-controversial source of MSCs. MSCs have been harvested non-invasively in large numbers from different regions of the umbilical cord [12]. Wharton's jelly (WJ) is the cushioning material found between the blood vessels of the umbilical cord [12] and MSCs derived from WJ are associated with desirable properties like high harvest and proliferation rates and hypoimmunogenicity [4].

As allogeneic human MSCs do not usually evoke a strong immune response, their clinical use for cell-based transplantation may provide an alternative medical paradigm and change the course of treatment of a variety of disorders. Therefore, ready availability of frozen stores of allogeneic MSCs or their tissue sources would be greatly beneficial. Being able to conserve the umbilical cord tissue from a particular donor and recover the WJ–MSCs from it at any chosen time point in future is an indispensable step both towards fulfilling clinical as well as research needs. Cryopreservation is defined as the preservation of structurally intact living cells and tissues using very low temperatures and a good cryoprotectant should be able to penetrate the cells and yet have low toxicity [9]. A major challenge lies in carefully selecting a suitable cryprotectant. Serum is widely used in cryopreservation solutions. However, use of animal-derived products, such as foetal bovine serum (FBS), in not encouraged in clinical applications for humans due to safety reasons. Strong immune reactions and anaphylaxis have been reported in patients when transplanted with cells exposed to animal-derived products [10,7]. Autologous human serum (HS) is another option, though its usage is restricted by limited availability. Moreover, high degree of variability between batches of FBS or autologous HS can result in an overall inconsistent cryopreservation solution, thereby introducing variability in the performance of cryopreserved samples.

There are several investigations on cryopreservation of MSCs and cryopreservation of human MSCs using different serum-free cryopreservation solutions have been reported in the past. BM-MSCs have been cryopreserved in the absence of proteins or dimethyl sulfoxide (DMSO) by using a novel polyampholyte cryoprotective agent comprising of carboxylated poly-L-lysine (COOH–PLL) [8]. Liu et al. established the cryopreservation of BM-MSCs in 7.5% DMSO (v/v), 2.5% PEG (w/v), and 2% bovine serum albumin (BSA) (w/v) [6]. In addition, DMSO-free cryoprotectant (CPA) solution which contained

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ethylene glycol (EG), 1,2-propylene glycol (PG) and sucrose as basic CPAs, supplemented with polyvinyl alcohol (PVA) as an additive, were developed for the cryopreservation of umbilical cord blood-derived MSCs [13]. Finally, MSCs from human umbilical cord stroma have been successfully cryopreserved using multistep slow freezing using sucrose and DMSO or DMSO alone [1]. However, there are not many reports on cryopreservation of human umbilical cord tissue. While our manuscript was under preparation, an article addressing cryopreservation of human umbilical cord tissue [2] using 20% autologous plasma along with DMSO and sucrose got published. However, MSCs were isolated from the whole cord tissue and not specifically from the WJ portion. Within the umbilical cord, there are actually 5 separate regions which contain mesenchymal stromal cells [12].

The aim of the present work was to develop a novel cryopreservation technique using an optimal serum-free condition that would allow long term storage of human umbilical cord tissue without affecting the basic characteristics of the WJ–MSCs derived from the frozen cord tissue. The efficiency of cryopreservation was assessed by growth kinetics, surface marker expression and *in vitro* differentiation potential of MSCs isolated from the WJ of cryopreserved cord tissue.

Materials and methods

Umbilical cord cryopreservation protocol

Fresh human umbilical cords ($n = 6$) from both sexes were collected after birth (caesarean section or normal vaginal delivery) with informed consent using the guidelines approved by the Institutional Ethics Committee at IISER-Kolkata.

The cord was cut into 2–3 cm segments and after rinsing the cord segments in normal saline (0.9% w/v sodium chloride) to remove the blood and blood clots, they were asceptically stored in normal saline containing 1× Antibiotic–Antimycotic (Invitrogen, CA, USA) at 4 °C for about 2 h.

Next, cryopreservation solutions of the following compositions were prepared using normal saline as a diluent,

Solution A: 10% (vol/vol) Dimethyl sulfoxide (DMSO; Sigma Aldrich, St Louis, USA).
Solution B: 10% DMSO + 0.2 M sucrose (SRL, Mumbai, India).
Solution C: 10% DMSO + 0.5 M sucrose.
Solution D: 10% DMSO + 0.2 M sucrose + 10% foetal bovine serum (FBS; Hyclone, Victoria, Australia).
Solution E: 10% DMSO + 50% FBS.
Solution F: 5% glycerol (Invitrogen, CA, USA) + 0.2 M sucrose.

The cord pieces were transferred to a laminar flow hood and after disinfecting with 70% ethanol, they were added to the different cryopreservation solutions described above.

The cord pieces were frozen overnight in Nalgene's Cryo 1 °C Mr. Frosty freezing container at a slow cooling rate of 1 °C/min from RT to −80 °C and then they were transferred to liquid nitrogen and stored at −196 °C for up to 5–29 days. While thawing, the frozen cryovials were immediately immersed in a 37 °C water bath until the cryopreserved cord tissue thawed completely.

Isolation of WJ–MSCs

After rinsing thoroughly in PBS (Sigma), cord vessels were manually removed and the exposed mesenchymal tissue was cut into small explants and placed in a tissue culture dish with KnockOut Dulbecco's Modified Eagle's Medium (KnockOut DMEM-; Life Technologies, Grand Island, NY, USA), 2 mM L-glutamine (Life Technologies), 1× Antibiotic–Antimycotic (Invitrogen) and 10% FBS (Hyclone). All cultures were incubated at 37 °C with 5% humidified CO_2. After 7–20 days, once cells started to emerge, the cord explants were discarded and cells were passaged when they reached 70–80% confluence. In the subsequent passages, WJ–MSCs both from fresh and cryopreserved cord tissues were plated at seeding density of 5×10^4 cells/cm^2.

Growth kinetics

Growth kinetics was determined by calculating total cell yield, number of population doublings (PDs) and population doubling time (TD). The total number of cells at each passage was calculated

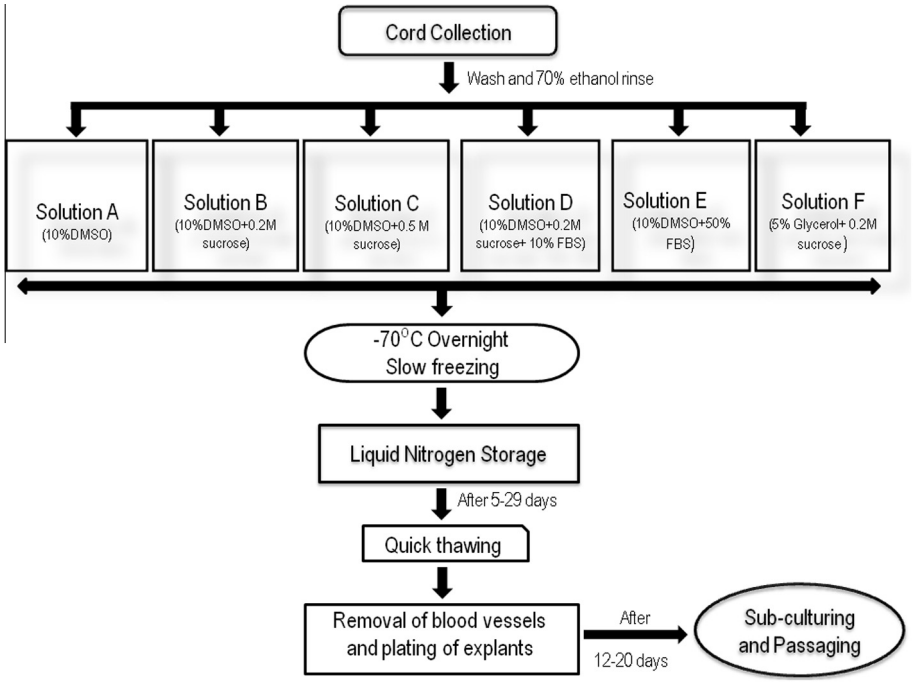

Fig. 1. Experimental design. Schematic representation of cryopreservation protocol for human umbilical cord samples.

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