Cryobiology xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Cryobiology

journal homepage: www.elsevier.com/locate/ycryo

Serum-free cryopreservation of human amniotic epithelial cells before and after isolation from their natural scaffold ☆

Hassan Niknejad *, Tina Deihim, Habibollah Peirovi, Hassan Abolghasemi

Nanomedicine and Tissue Engineering Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 1 November 2012
Accepted 7 May 2013
Available online xxxx

Keywords:
Amniotic membrane
Epithelial cells
Cryopreservation
Serum
Amniotic fluid

ABSTRACT

Amniotic epithelial cells are a promising source for stem cell-based therapy through their potential capacity to differentiate into the cell lineages of all three germ layers. Long-term preservation is necessary to have a ready-to-use source of stem cells, when required. Reduced differentiation capability, decrease of viability and use of fetal bovine serum (FBS) are three drawbacks of clinical application of cryopreserved stem cells. In this study, we used human amniotic fluid instead of animal serum, and evaluated viability and multipotency of amniotic epithelial cells after cryopreservation in suspension and compared with those cryopreserved on their natural scaffold (in situ cryopreservation). There was no significant difference in viability of the cells cryopreserved in amniotic fluid and FBS. Also, the same results were achieved for expression of pluripotency marker OCT-4 when FBS was replaced by amniotic fluid in the samples with the same cryoprotectant. The cells cryopreserved in presence of scaffold had a higher level of viability compared to the cells cryopreserved in suspension. Although, the number of the cells expressed OCT-4 significantly decreased within cryopreservation in suspension, no decrease in expression of OCT-4 was observed when the cells cryopreserved with their natural scaffold. Upon culturing of post-thawed cells in specific lineage differentiating mediums, the markers of neuronal, hepatic, cardiomyocytic and pancreatic were found in differentiated cells. These results show that replacement of FBS by amniotic fluid and in situ cryopreservation of amniotic epithelial cells is an effective approach to overcome limitations related to long-term preservation including differentiation during cryopreservation and decrease of viability.

© 2013 Elsevier Inc. All rights reserved.

Introduction

Amniotic membrane (AM) is the innermost membrane lining the placenta facing the fetus. This membrane consists of an epithelium containing a single layer of cuboidal to columnar cells, a thick basement membrane, and an avascular stroma. The basement membrane and stromal matrix of the AM contain different types of extracellular matrix (ECM) proteins such as collagen I, collagen III, collagen IV, laminin, fibronectin and perlecan [26]. Due to promoting of wound healing, bacteriostatic activity, inducing of epithelialization, scar inhibition and low immunogenicity, the AM has been successfully employed in a variety of clinical applications such as wound and burn therapy, reconstruction of epidermis and mucosal surfaces, abdominal surgery and neurosurgery and ophthalmologic fields [2,18,27]. One of the reasons which makes the AM as biomaterial with such vast applications is its epithelium.

Human amniotic epithelial cells (hAECs) express SSEA-3, SSEA-4, TRA1–60, TRA1–80 as stem cell surface markers and OCT-4 and Nanog as pluripotent stem cell markers [20,26]. Although AECs have the capability of colony formation, they are not tumorigenic. In vitro differentiation of the hAECs into the three germ layers have frequently been reported [20,21,24]. These unique properties make hAECs as a potential cell source for therapeutic applications. However, long-term preservation of these cells for immediate use and controlling of possible infections which are within window period is an important issue which remains to be evaluated.

Decrease of viability, differentiation during cryopreservation and use of xenogenic materials in cryopreservation media are the three major problems in cell banking preparation. In the recent years, new techniques for cryopreservation have been developed in order to solve these challenges.

Applying ECM proteins and other soluble and insoluble factors within cryopreservation is a new method to obtain high yield of viable cells [12]. ECM ingredients have important roles in cell differentiation, migration, and survival and employing these

☆ This study was supported by Nanomedicine and Tissue Engineering Research Center of Shahid Beheshti University of Medical Sciences.

* Corresponding author. Address: Nanomedicine and Tissue Engineering Research Center, Taleghani Hospital, Velenjak, P.O. Box 1985711151, Tehran, Iran. Fax: +98 21 22439848.

E-mail address: niknejad@sbmu.ac.ir (H. Niknejad).

0011-2240/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cryobiol.2013.05.001

Please cite this article in press as: H. Niknejad et al., Serum-free cryopreservation of human amniotic epithelial cells before and after isolation from their natural scaffold, Cryobiology (2013), http://dx.doi.org/10.1016/j.cryobiol.2013.05.001

ARTICLE IN PRESS

macromolecules in cell preservation medium improves viability and suppresses differentiation during cryopreservation. A variety of scaffolds with ECM proteins have been used in cryopreservation of fibroblasts [13,22], hepatocytes [1] and embryonic stem cells [8,12]. The results from these investigations have been demonstrated that cryopreservation with scaffold optimizes long-term preservation of the cells. Therefore, the first aim of this study was to cryopreserve the whole amniotic membrane and evaluate the viability of hAECs while they stayed on their natural scaffold (with enriched ECM proteins) within cryopreservation.

Animal serums are employed in lots of the current cryopreservation protocols with concentrations ranging from 5% to 90% (v/v) [4]. Although, fetal bovine serum (FBS) is a natural mix of growth factors, hormones and nutrients supporting cell survival, it contains many uncharacterized components which might be hazardous to recipient of cryopreserved cells. Inducing recipient immune system due to using of the cells cryopreserved with animal serum is a massive limitation in cell replacement therapy. It has been shown that antibodies against FBS components could be produced in recipient serum [15,33]. It has also been demonstrated that nonhuman sialic acid Neu5Gc as an antigenic material is expressed on the surface of cells preserved in FBS-containing mediums [17]. Furthermore, risk of transmitting animal pathogens such as bovine spongiform encephalopathy prion is another drawback of FBS [33]. These limitations have made it desirable to seek alternatives to animal serum. Amniotic fluid (AF) is a complex biological fluid containing growth factors, proteins, peptides, carbohydrates, lipids, hormones, and electrolytes that protects the fetus physically and biochemically and assists in fetal development [19]. In addition, AF prepared of human sources is a non-xenogenic material and could be a suitable substitute for animal serum in xeno-free cell banking. Therefore, another aim of this study was to employ amniotic fluid as a substitute for FBS in cryopreservation of hAECs.

Materials and methods

Preparation of the AM and AF

Human placentas ($n = 20$) with gestational age 36–38 weeks were obtained directly after elective Caesarean sections. All experiments were in accordance with the Shahid Beheshti University of Medical Sciences guidelines. Serological tests in all mothers were negative for human immunodeficiency virus types I and II, human hepatitis B and C, and syphilis. To avoid cross-contamination, each placenta was processed individually. All procedures were performed under sterile conditions. The AM was peeled from chorion and rinsed several times with cold sterile phosphate buffered saline (PBS) containing 50 μg/ml penicillin, 50 μg/ml streptomycin, 100 μg/ml neomycin and 2.5 μg/ml amphotericin B to remove blood remnants. The AM was placed epithelial side up on nitrocellulose membranes before being divided into pieces of appropriate size.

AF samples were obtained by surgeon in aseptic condition at the time of Cesarean section by direct syringe aspiration from the intact amnion and transferred to lab at 4 °C. AF was centrifuged at 2000*g* for 10 min and supernatants were filtered-sterilized by 0.22 μm filters. AF was deactivated similar to animal serum deactivation protocol at 56 °C for 20 min. The AMs and AF were collected after obtaining informed consent from the donors.

Isolation of hAECs

For isolation of hAECs, the AM was cut into several pieces and incubated at 37 °C with 0.15% trypsin–EDTA (TrypNik, Yavandan Andisheh). Cells from the first 10 min of digestion were discarded to exclude debris. The solution from the second and third 40-min digests was pooled and centrifuged at 1500*g* for 12 min. Cells were washed and re-suspended in PBS. The purity of the isolated cells was determined by immunostaining with antibody against the epithelial marker pan-cytokeratin conjugated with FITC (1:100; Sigma–Aldrich).

Preparation of cryoprotectant solutions

We employed Me_2SO (Merck) and Glycerol (Merck) as conventional cryoprotectant agents (CPAs) in three major groups: 10% (v/v) Me_2SO, 50% (v/v) Glycerol and 10% (v/v) Me_2SO+50% (v/v) Glycerol. As shown in Table 1, in each CPA group, six different combinations of FBS 10% (Gibco), AF 10% and Dulbecco Modified Eagle's Medium (DMEM; Sigma–Aldrich) 10% were involved. PBS was used to reach the final concentration of 100%. All freezing vials contained 1 mL of final freezing medium after addition of the cells.

Cryopreservation of hAECs

To investigate the effects of cryopreservation on hAECs in suspension and adhered to basement membrane (their natural scaffold), hAECs were cryopreserved in two positions: with-scaffold and without-scaffold. To prepare with-scaffold samples, the AM was cut into pieces of 0.5 × 0.5 cm, which were placed epithelial side up on nitrocellulose membranes. The numbers of hAECs in each sample were 2×10^7 cell/0.5 cm^2 as counted by a calibrated microscope with digital camera (Zeiss, Jena, Germany). The same number of the cells was cryopreserved in suspension, which we nominated without-scaffold group. After 4 min of incubation at room temperature, all samples were cooled to −80 °C in a controlled-rate freezer (Planer, Sunbury on Thames, United Kingdom) at the rate of −1 °C/min and kept overnight. The samples were then transferred to a liquid nitrogen tank for 12 months before thawing in a water bath at 37 °C and further analysis. Immediately after being thawed, each sample was diluted with an equal volume of a solution containing 2.5% (wt/vol) human albumin (Cutter) and 5% (wt/vol) Dextran 40 (Baxter Health Care) in PBS and then centrifuged at 400*g* for 8 min. The supernatant was removed, and the sedimented cells were resuspended slowly in fresh albumin/dextran solution for further analyses. The AM samples were also washed several times with fresh albumin/dextran solution. Isolation of the cells from with-scaffold samples after cryopreservation was done using the same protocol, as described for the fresh amniotic membrane.

MTT assay

To evaluate whether the different cryopreservation conditions affect the viability of hAECs, cell viability was measured by MTT assay before and after cryopreservation. The cells were dispersed in

Table 1
Ingredients of cryopreservation vials in each cryoprotectant group. There are three main groups including: Glycerol (50%), DMSO (10%) and Glycerol (50%) + DMSO (10%). In each of them, FBS and/or amniotic fluid (AF) and/or DMEM were added. PBS was used to reach the final concentration of 100%.

Group	FBS (10%)	AF (10%)	DMEM (10%)
1	✓	–	–
2	–	✓	–
3	✓	✓	–
4	✓	–	✓
5	–	✓	✓
6	✓	✓	✓

Please cite this article in press as: H. Niknejad et al., Serum-free cryopreservation of human amniotic epithelial cells before and after isolation from their natural scaffold, Cryobiology (2013), http://dx.doi.org/10.1016/j.cryobiol.2013.05.001

Download English Version:

https://daneshyari.com/en/article/10928566

Download Persian Version:

https://daneshyari.com/article/10928566

Daneshyari.com