Cryobiology 69 (2014) 442–450

Contents lists available at ScienceDirect

Cryobiology

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

Measurement of the biophysical properties of porcine adipose-derived stem cells by a microperfusion system ☆

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ARTICLE INFO

Article history:
Received 10 June 2014
Accepted 6 October 2014
Available online 23 October 2014

Keywords:
Biophysical properties
Adipose-derived stem cell
Cryopreservation
Microperfusion system

ABSTRACT

Adipose-derived stem cells (ADSCs), which are an accessible source of adult stem cells with capacities for self-renewal and differentiation into various cell types, have a promising potential in tissue engineering and regenerative medicine strategies. To meet the clinical demand for ADSCs, cryopreservation has been applied for long-term ADSC preservation. To optimize the addition, removal, freezing, and thawing of cryoprotective agents (CPAs) applied to ADSCs, we measured the transport properties of porcine ADSCs (pADSCs). The cell responses of pADSCs to hypertonic phosphate-buffered saline and common CPAs, dimethyl sulfoxide, ethylene glycol, and glycerol were measured by a microperfusion system at temperatures of 28, 18, 8, and −2 °C. We determined the osmotically inactive cell volume (V_b), hydraulic conductivity (L_p), and CPA permeability (P_s) at various temperatures in a two-parameter model. Then, we quantitatively analyzed the effect of temperature on the transport properties of the pADSC membrane. Biophysical parameters were used to optimize CPA addition, removal, and freezing processes to minimize excessive shrinkage of pADSCs during cryopreservation. The biophysical properties of pADSCs have a great potential for effective optimization of cryopreservation procedures.

Introduction

Stem cells are characterized by the ability to self-renew and migrate to locations where tissues have suffered damage from injury or sickness and differentiate into various somatic cells to repair damaged tissue, thus helping to maintain homeostasis [12,13]. In mammalians, embryonic and adult stem cells have the ability to maintain homeostasis [48]. Adipose-derived stem cells (ADSCs) are an accessible source of adult stem cells that can easily regenerate adipose tissue on a scaffold [49]. Studies have shown that ADSCs can differentiate into osteoblasts and chondrocytes [36,61], islet cells [24,32,54], and partial hepatic lineage cells *in vitro* or *in vivo* [28,47]. Notably, subsequent studies have indicated that ADSCs can be induced to differentiate into neuronal cells, neuronal-like cells, and cardiomyocytes [29,45,51,59,62].

Soft-tissue reconstruction is commonly applied in plastic surgery to recreate tissue damaged by deep burns, tumor resection, or trauma [1,22,23,44]. A conventional strategy is long-term cryopreservation of adipose tissue to provide an available resource to graft fat [3,6,8,53]. However, alloplastic implants cause foreign body reactions and autologous implants undergo an unpredicted degree of resorption [21,26]. Several studies have focused on the use of ADSCs as an autologous cell source in tissue engineering and regenerative medicine strategies. Furthermore, ADSCs can self-renew and differentiate into various cell lineages, and may be used as a potential tool for drug screening to investigate the pharmacodynamic effects on differentiation, development, regeneration, and toxicity [31,43].

Although ADSCs can be isolated from adipose tissue by liposuction or a needle biopsy [2], fleshly isolated ADSCs cannot meet the needs of tissue engineering and regenerative medicine. Thus, to vastly improve the applications of ADSCs, new techniques need to meet the increasing demands for sufficient numbers of stem cells as well as long-term preservation. Cryopreservation is a highly effective technology for preservation of tissues and cells

☆ *Statement of funding:* This work was supported by the National Natural Science Foundation of China (Nos. 51276179, 51476160), and the Fundamental Research Funds for the Central Universities (WK 2100230009).

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http://dx.doi.org/10.1016/j.cryobiol.2014.10.002
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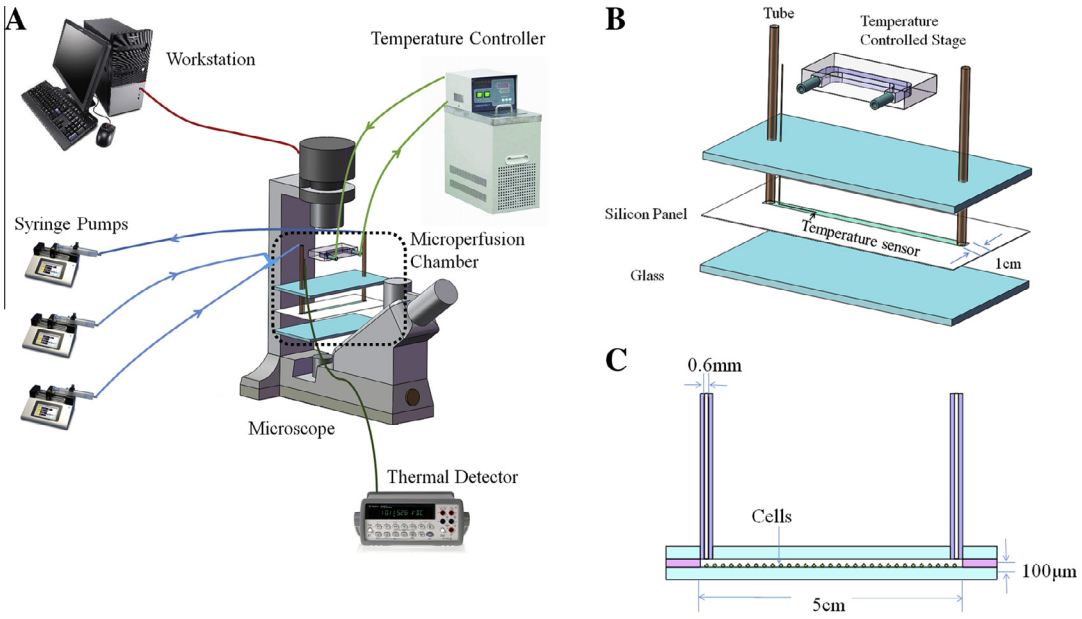

Fig. 1. Schematic of the microperfusion system and chamber. (A) The microperfusion system included a microperfusion chamber, inverted microscope, video camera, and temperature controller. (B, C) The subunit and size of the microperfusion chamber.

including adult stem cells. During cryopreservation, cells undergo shrinkage over their limit as well as intracellular ice formation (IIF) [37,38,55]. Cryoprotective agents (CPAs) minimize the freezing damage but change the transport properties of the cell membrane. Therefore, it is essential to measure the transport properties of the cell membrane to optimize CPA addition and removal as well as freezing and thawing processes. Because of their physical and metabolic similarities to humans, pigs are an ideal animal model for disease and regenerative medicine research [19]. In this study, a microperfusion system was used to measure the transport properties of the porcine ADSC (pADSC) membrane. The membrane permeability coefficient to water (L_p), membrane permeability coefficient (P_s) in the presence of dimethyl sulfoxide (Me_2SO), ethylene glycol (EG), and glycerol, and the activation energies and reference membrane coefficient were investigated at 28, 18, 8, and −2 °C. The effects of temperature on the transport properties of the pADSC membrane were analyzed and the cryobiological parameters were used to simulate the cell response during CPA addition and removal. Furthermore, we were able to predict these phenomena during freezing.

Materials and methods

Theory of the cell membrane transport model

Kedem and Katchalsky improved the conventional permeability equation to study the transport of both water and CPAs through the cell membrane [27]. In the *K–K* model, hydraulic conductivity, solute permeability, and reflection coefficient were introduced to describe the transport properties of cell membranes. Water and solute fluxes were considered to interact with

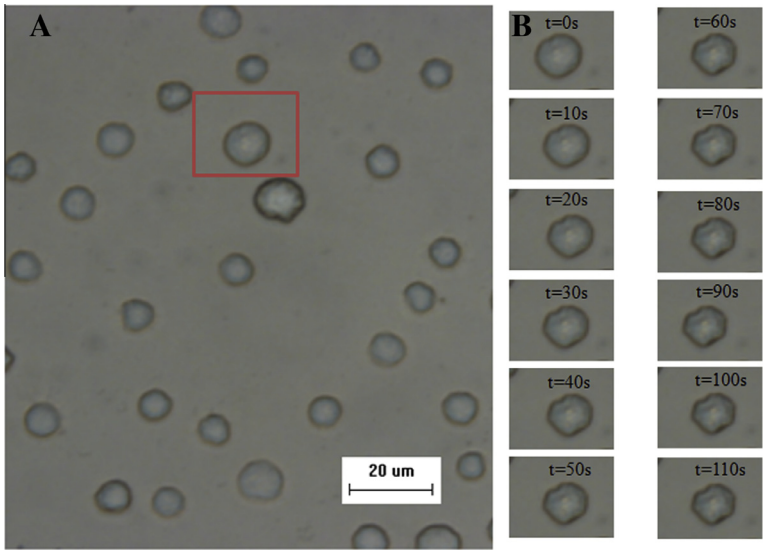

Fig. 2. Transient osmotic response of pADSCs. (A) Morphology of pADSCs immobilized on the silicified glass. (B) Volume changes of pADSC during the osmotic shift from PBS to 3× PBS at 8 °C.