Cryobiology 72 (2016) 93–99

Contents lists available at ScienceDirect

Cryobiology

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

A study of the osmotic characteristics, water permeability, and cryoprotectant permeability of human vaginal immune cells

Zhiquan Shu [a, b], Sean M. Hughes [c], Cifeng Fang [a], Jinghua Huang [a, e], Baiwen Fu [a, e], Gang Zhao [f], Michael Fialkow [c], Gretchen Lentz [c], Florian Hladik [c, d, **], Dayong Gao [a, *]

[a] Department of Mechanical Engineering, University of Washington, Seattle, WA 98195, USA
[b] School of Mechanical and Materials Engineering, Washington State University, Everett, WA 98201, USA
[c] Department of Obstetrics and Gynecology, University of Washington, Seattle, WA 98195, USA
[d] Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA
[e] College of Information Technology, Beijing Union University, Beijing 100101, China
[f] Department of Electronic Science & Technology, University of Science and Technology of China, Hefei 230027, China

ARTICLE INFO

Article history:
Received 2 September 2015
Received in revised form
9 March 2016
Accepted 10 March 2016
Available online 11 March 2016

Keywords:
Cryopreservation
Human vaginal mucosa
T cell
Macrophage
Cryobiological characteristics
Microfluidic perfusion channel

ABSTRACT

Cryopreservation of specimens taken from the genital tract of women is important for studying mucosal immunity during HIV prevention trials. However, it is unclear whether the current, empirically developed cryopreservation procedures for peripheral blood cells are also ideal for genital specimens. The optimal cryopreservation protocol depends on the cryobiological features of the cells. Thus, we obtained tissue specimens from vaginal repair surgeries, isolated and flow cytometry-purified immune cells, and determined fundamental cryobiological characteristics of vaginal $CD3^+$ T cells and $CD14^+$ macrophages using a microfluidic device. The osmotically inactive volumes of the two cell types (V_b) were determined relative to the initial cell volume (V_0) by exposing the cells to hypotonic and hypertonic saline solutions, evaluating the equilibrium volume, and applying the Boyle van't Hoff relationship. The cell membrane permeability to water (L_p) and to four different cryoprotective agent (CPA) solutions (P_s) at room temperature were also measured. Results indicated V_b values of 0.516 V_0 and 0.457 V_0 for mucosal T cells and macrophages, respectively. L_p values at room temperature were 0.196 and 0.295 μm/min/atm for T cells and macrophages, respectively. Both cell types had high P_s values for the three CPAs, dimethyl sulfoxide (DMSO), propylene glycol (PG) and ethylene glycol (EG) (minimum of 0.418×10^{-3} cm/min), but transport of the fourth CPA, glycerol, occurred 50–150 times more slowly. Thus, DMSO, PG, and EG are better options than glycerol in avoiding severe cell volume excursion and osmotic injury during CPA addition and removal for cryopreservation of human vaginal immune cells.

1. Introduction

In HIV vaccine and microbicide trials, immune responses are typically evaluated in the peripheral blood, despite the most important immune responses being at the sites of viral entry, namely the genital and rectal mucosae. Sophisticated analyses of fresh mucosal cell and tissue samples are currently being done, but cryopreservation is little used [19,22,27]. Cryopreservation of mucosal specimens is critically important for immunological studies because it allows samples obtained at different times and trial sites to be preserved, shipped and stored for later analysis at a central laboratory. However, it is not clear whether the currently-used cryopreservation strategies, which were originally developed for peripheral blood mononuclear cells (PBMC), are ideal for mucosal specimens. Publications reporting functional cell-based assays performed with cryopreserved mucosal specimens are limited and inconsistent [3,8,14,20].

To optimize the cryopreservation of mucosal cells, it is necessary to have a quantitative understanding of their biophysical response to the freezing process [15,16,18]. According to Mazur's "Two-Factor Hypothesis", the cellular response to freezing is governed by

* Corresponding author. Department of Mechanical Engineering, University of Washington, Seattle, WA 98195, USA
** Corresponding author. Department of Obstetrics and Gynecology, University of Washington, Seattle, WA 98195, USA.
E-mail addresses: fhladik@fredhutch.org (F. Hladik), dayong@u.washington.edu (D. Gao).

http://dx.doi.org/10.1016/j.cryobiol.2016.03.003

intrinsic properties of the cells, including the portion of the cell volume that does not respond to osmotic pressure (V_b), the permeability of the cell membrane to water (L_p) and the permeability of the membrane to cryoprotective agents (CPAs; P_s) [17]. These properties are unknown for mucosal immune cells.

Our hypothesis is that understanding the fundamental cryobiological characteristics of mucosal immune cells will allow the development of an improved cryopreservation procedure. In this work, the cryobiological properties of mucosal immune cells were determined using a microfluidic device developed in our group [1,2]. Since the female genital tract is one of the most common sites of sexual HIV transmission, the cells assessed were isolated from the human vagina. Specifically, two cell populations that are central to adaptive cellular immunity and HIV susceptibility, T lymphocytes and macrophages, were isolated and their osmotically inactive cell volume (V_b), cell membrane permeability to water (L_p), and cell membrane permeability to CPAs (P_s) were determined. Four widely used CPAs – dimethyl sulfoxide (DMSO), glycerol, propylene glycol (PG) and ethylene glycol (EG) – were tested.

2. Materials and methods

2.1. Human vaginal mucosal specimens

Human vaginal tissues were obtained from healthy women undergoing vaginal repair surgeries in the Department of Obstetrics and Gynecology at the University of Washington. These tissues, which would otherwise have been discarded, were collected without any identifying patient information under a waiver of consent approved by the Institutional Review Boards of the University of Washington and the Fred Hutchinson Cancer Research Center.

2.2. Isolation and sorting of vaginal T cells and macrophages

Vaginal tissues were maintained in saline and on ice during transport and dissection. The stroma was removed from the epithelium, leaving epithelial pieces about 2 mm thick. These were subsequently cut into pieces of about 1 × 1 mm and stored overnight in cell culture medium at 4 °C. The next morning, cells were isolated using an enzymatic digestion protocol [22]. Briefly, tissues were incubated in collagenase type II digestion medium (700 collagen units per mL; Sigma, St. Louis, MO) with 500–1000 units per mL DNase I (Sigma) at 37 °C with shaking for 30 min; tissues were disrupted by passage through a blunt needle and syringe, and the resulting cell suspensions were separated from undigested tissue pieces by filtration through a 70 μm strainer. Remaining tissue pieces were re-digested up to three additional times. Vaginal T cells and macrophages were purified from the bulk cell population by flow cytometric sorting, after staining with CD45 APC, CD3 FITC, and CD14 PE-Cy7 (all mouse anti-human from BD Biosciences, San Jose, CA, USA) and 0.1 μg/mL 4′,6-diamidino-2-phenylindole (DAPI) for viability. All antibodies were titrated before use and used at the minimum saturating dose. Live $CD45^+CD3^+CD14^-$ and live $CD45^+CD3^-CD14^+$ events were sorted on a four laser BD FACSAria II (408, 488, 535, and 633 nm). The sorted cells were suspended in 1× PBS at 10,000 cells/mL, stored at 4 °C, and used for the following experiments within 8 h.

2.3. The microfluidic perfusion system

Cell membrane permeabilities were measured with a microfluidic perfusion chamber we developed previously [1]. The microfluidic device was fabricated using soft lithography. The height of the microfluidic perfusion chamber was 15 μm to accommodate a monolayer of the expected cell sizes (8–12 μm). At the edge of the chamber, the channel height was shortened to 3 μm to trap the cells but still allow fluid to flow.

During experiments, the microfluidic device was immobilized on the stage of the microscopy (DM IRB, Leica, Buffalo Grove, IL). A droplet of cell suspension (~10 μL) was added gently to the inlet reservoir. The fluid was withdrawn continuously by a digitally controlled syringe pump (PHD 2000 Infusion, Harvard Apparatus, Holliston, MA) with a flow rate of 40 μL/h in order to stably trap cells in the chamber. After 10–15 cells were trapped and aligned in front of the block, 0.5 mL perfusion solution was added into the inlet reservoir, avoiding any violent perturbation to the fluid flow. The fluid was drawn into the chamber continuously by the syringe pump. The cell volume excursion history was recorded by a CCD camera (Phantom v310, Vision Research, Wayne, NJ) at 24 frames/second until osmotic equilibrium was obtained, generally within 2 min. All the experiments were done at room temperature (~22 °C).

In order to measure the osmotically inactive cell volume (V_b) and the cell membrane permeability to water (L_p), trapped cells were perfused with hypotonic and hypertonic saline solutions (0.7× PBS, 2× PBS and 3× PBS). To determine the cell membrane permeability to DMSO, glycerol, PG, and EG, dilutions of these chemicals were prepared in 0.9% NaCl saline solution. The osmolalities of the solutions were measured by an osmometer (Wescor Inc., Logan, UT) based on vapor pressure assessment (Table 1).

2.4. Image analysis

After video capture, the videos were converted to image frames using Cine Viewer software (Vision Research, Wayne, NJ). Cells were cropped from each frame of the image. The cropped images were enhanced and processed to find the cell boundary (see Fig. 1). In order to detect the cell boundary precisely, the "Active Contour (dual-snake)" algorithm was applied [7]. Thereafter, the two-dimensional cell area was evaluated by pixel counting and then converted to three-dimensional cell surface area and volume based on the assumption of spherical cell shape. All the image processing was performed with MATLAB software (MathWorks, Natick, MA).

In order to assess the hypothesis of spherical cell shape, the sphericity of T cells and macrophages (cell images at the beginning of each experiment) was evaluated, which was defined as $2\pi \cdot r_{equ}/p_{act}$. Here, r_{equ} is the equivalent cell radius calculated with the two-dimensional cell area based on image analysis, and p_{act} is the actual cell perimeter.

2.5. Determination of osmotically inactive cell volume (V_b)

V_b, the osmotically inactive volume of the cell (μm^3), can be determined by the Boyle van't Hoff relationship. Assuming the cell acts as an ideal osmometer, the osmotic response of the cell volume during hypertonic shrinkage can be described as

Table 1
Perfusion solutions and osmolalities.

Perfusion solutions	Osmolality (mOsm/kg-H_2O)
0.7× PBS	201
1× PBS	297
2× PBS	605
3× PBS	881
10% (v/v) DMSO in 0.9% NaCl	1823
1.5 M glycerol in 0.9% NaCl	1956
1.5 M ethylene glycol in 0.9% NaCl	1761
1.5 M propylene glycol in 0.9% NaCl	1575