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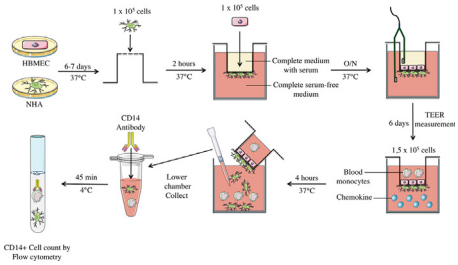
Optimization of an in vitro human blood–brain barrier model: Application to blood monocyte transmigration assays

Alexandre Paradis [1], David Leblanc [1], Nancy Dumais *

Département de Biologie, Faculté des Sciences, Université de Sherbrooke, Sherbrooke, QC, Canada J1 K 2R1

GRAPHICAL ABSTRACT



ABSTRACT

The blood–brain barrier (BBB) is a selectively permeable barrier that separates the circulating blood from the extracellular fluid of the brain and is an essential component in brain homeostasis. In vitro BBB models are valuable supporting tools that can precede and complement animal and human studies of the development and progression of the central nervous system diseases. At present, mono-, co-, and tri-culture models that use porcine, murine, or human cells have been developed. We have optimized a two-dimensional model of the human BBB using primary human brain microvascular endothelial cells and normal human astrocytes. We have validated the effectiveness of our model with transmigration assays of human blood monocytes toward CCL19, a natural ligand of the chemokine receptor CCR7. This model offers the following advantages:

- It is simple, convenient, and requires small quantities of material, reagents, and primary cells.
- It can be used to monitor cell migration through the BBB.

* Corresponding author at: Département de Biologie, Faculté des Sciences, Université de Sherbrooke, 2500 boul de l'Université, Sherbrooke, QC, Canada J1 K 2R1. Tel.: +1 819 821 8000x63711.

E-mail address: Nancy.Dumais@USherbrooke.ca (N. Dumais).

[1] These authors contributed equally to this work.

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- It can be used to assess brain capillary permeability in the presence of xenobiotic, pro-inflammatory, or other substances.

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Method details

Introduction

Mimicking the physiology and functional responses of the blood–brain barrier (BBB) in vitro is a challenging task. Many techniques have been described including an in silico model, immobilized artificial membrane chromatography, and a parallel artificial membrane used for predicting drug permeability across specific physiological membranes in vivo [1,2]. Over the years, new cell culture techniques and improved technologies have provided the necessary tools to create more realistic in vitro cell-based BBB models to advance our understanding of BBB physiology and function [1–4]. Highly purified populations of cultured human brain cells (human brain microvascular endothelial cells [HBMEC]) and normal human astrocytes (NHA) exhibit excellent characteristics for studying the developmental and pathophysiological processes of the BBB. These cultures are more tedious to grow than other cell lines and require many technical skills to establish an appropriate BBB model. However, primary human cells used in co-culture provide a more realistic BBB model than mono-cultures and cell line co-cultures [1–4].

Here, we describe a method based on the BBB model developed initially by Persidsky et al. and Ifergan et al. [3–5]. Primary human endothelial cells and astrocytes are co-cultured using Thincerts™ tissue culture inserts to obtain a selective and tight in vitro model of the human BBB. This method includes a detailed protocol to facilitate the generation of a functional in vitro BBB model optimized to perform transmigration assays of human blood monocytes in response to chemokines. The method is outlined below, in two steps.

Step 1. Preparation of confluent human brain microvascular endothelial cells (HBMEC) and normal human astrocytes (NHA)

Materials and reagents

- Human Brain Microvascular Endothelial Cells (Cell Systems, Cat# ACBRI-376, Kirkland, WA, USA).
- Normal human astrocytes (Cell Systems, Cat# ACBRI-371).
- Complete Classic Medium Kit With Serum and CultureBoost (Cell Systems, Cat# C4Z0-500).
- CSC complete serum-free medium with RocketFuel (Cell Systems, Cat# SF-4Z0-500).
- "BAC-OFF" antibiotic (Cell Systems, Cat# 4Z0-643).
- Attachment Factors (Cell Systems, Cat# 4Z0-210).
- Passage Reagent Group™ (Cell Systems, Cat# 4Z0-800).
- Tissue culture dish, 150 mm (Life Technologies, Cat# 130183, Burlington, ON, Canada).
- PRIMARIA™ tissue culture dishes, 100 mm (Life Technologies, Cat# 353803).
- Cell scrapers (Sarstedt, Cat# 83.1830, Nümbrecht, NW, Germany).

Procedure

1. Preheat Attachment Factors at 37 °C.
2. Completely coat 100-mm adherent cell plates with 1.5 or 2.0 mL preheated Attachment Factors for HBMEC and NHA, respectively.