Cytotherapy, 2014; 0: 1–7

International Society for Cellular Therapy ISCT®

Human platelet lysate is a feasible candidate to replace fetal calf serum as medium supplement for blood vascular and lymphatic endothelial cells

PABLO HOFBAUER[1], SABRINA RIEDL[1], KARIN WITZENEDER[2], FLORIAN HILDNER[1,2], SUSANNE WOLBANK[1], MARION GROEGER[3], CHRISTIAN GABRIEL[2], HEINZ REDL[1] & WOLFGANG HOLNTHONER[1,4]

[1]*Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Austrian Cluster for Tissue Regeneration, Vienna, Austria,* [2]*Red Cross Blood Transfusion Service of Upper Austria, Austrian Cluster for Tissue Regeneration, Linz, Austria,* [3]*Division of General Dermatology, Department of Dermatology, Medical University of Vienna, Vienna, Austria, and* [4]*Department of Biochemical Engineering, University of Applied Sciences Technikum Wien, Vienna, Austria*

Abstract

Background aims. As angiogenic and lymphangiogenic key players, endothelial cells (ECs) are promising candidates for vascular regenerative therapies. To culture ECs *in vitro*, fetal calf serum (FCS) is most often used. However, some critical aspects of FCS usage, such as possible internalization of xenogeneic proteins and prions, must be considered. Therefore, the aim of this project was to determine if human platelet lysate (hPL) is a suitable alternative to FCS as medium supplement for the culture of blood vascular and lymphatic endothelial cells. *Methods.* The usability of hPL was tested by analysis of endothelial surface marker expression, metabolic activity and vasculogenic potential of outgrowth ECs (OECs), human umbilical vein ECs (HUVECs), and lymphatic ECs (LECs). *Results.* Expression of EC markers CD31, VEGFR2, VE-cadherin and CD146 did not differ significantly between the EC types cultured in FCS or hPL. In addition, OECs, HUVECs and LECs formed tube-like structures on Matrigel when cultured in hPL and FCS. With the use of 3-(4,5-**d**imethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid assays, we found that the metabolic activity of OECs and LECs was slightly decreased when hPL was used. However, HUVECs and LECs did not show a significant decrease in metabolic activity, and HUVECs showed a slightly higher activity at low seeding densities. *Conclusions.* The use of hPL on different EC types did not reveal any substantial negative effects on EC behavior. Thus, hPL appears to be a favorable candidate to replace FCS as a medium supplement in the culture of ECs.

Key Words: *angiogenesis, endothelial cells, fetal calf serum, human platelet lysate, regenerative medicine*

Introduction

To facilitate cell proliferation *in vitro*, an adequate cell culture medium must be provided. Fetal calf serum (FCS) is currently most often used to supplement cell culture media with factors such as hormones and growth factors, which are necessary for optimal growth (1,2). However, this carries certain risks, such as internalization of xenogeneic proteins, possible virus and prion transmission or other immunological risks, which negatively influence the applicability of FCS for cellular therapy (1–3). Additionally, several ethical concerns have been raised concerning the harvesting of FCS, which has been shown to significantly harm the fetus (2).

These issues have led to a search for alternatives to animal serum and have been promoted by regulatory authorities and the research community (2–5). One possible alternative to animal serum is the use of human platelet lysate (hPL). Activated platelets are known to release a wide range of growth factors, hormones and other active molecules to sites of injury by exocytosis. These factors include platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), insulin-like growth factor-1 (IGF-1), epithelial growth factor (EGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and transforming growth factor-β1 (TGF-β1) (2,3). The release of these growth factors, together with the fact that most of the potent mitogenic factors

Correspondence: **Wolfgang Holnthoner**, PhD, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Austrian Cluster for Tissue Regeneration, Donaueschingenstrasse 13, 1200 Vienna, Austria. E-mail: wolfgang.holnthoner@trauma.lbg.ac.at

(Received 24 September 2013; accepted 29 April 2014)

http://dx.doi.org/10.1016/j.jcyt.2014.04.009

found in serum are released by activated platelets, makes hPL a reasonable candidate to substitute animal serum. Other advantages include the human origin of hPL and thus no risk of xenogeneic protein, virus or prion transmission (2,3). Furthermore, all blood donors are thoroughly tested for, among others, human viruses such as hepatitis A, B and C or human immunodeficiency virus as other components of whole blood are used for transfusion. Finally, human thrombocyte concentrates can be stored in blood banks for up to 7 days for clinical use and must be routinely discarded afterward. These expired thrombocyte concentrates can then be used to produce hPL from waste material (3).

The suitability of hPL in cell culture has already been shown for different cell types, including stem and somatic cells, and is in the process of becoming routine practice. Cells successfully propagated in hPL-containing media include adipose-derived stem cells (ASCs), chondrocytes, fibroblasts, kidney cells, monocytes, keratinocytes and certain endothelial cells derived from human umbilical cord as well as peripheral blood (3,5–8). However, a comparison of blood vascular and lymphatic endothelial cells (ECs) has not been conducted until now.

ECs in general form the endothelium, which builds the inner cellular lining of blood and lymphatic vessels. There are two main types of ECs, namely blood ECs and lymphatic ECs (LECs), and some of their important functions include angiogenesis and lymphangiogenesis. For therapeutic intervention, endothelial progenitor cells such as outgrowth ECs (OEC) should help to overcome the challenges of cellular therapies aiming at improving angiogenesis and vasculogenesis in tissue-engineered constructs and ischemic tissues (9–13).

Therefore, we hypothesized that hPL is a feasible candidate to replace FCS as a medium supplement in the culture of human primary ECs derived from blood, macrovascular and lymphatic origin.

Methods

Production of hPL

Human PL was prepared from 36 buffy coats of whole blood donations by centrifugation at 3933*g* for 11 min after informed consent was obtained. Six buffy coats with identical blood group and Rhesus factor were pooled and centrifuged at 442*g* for 7 min to obtain platelet-rich plasma, which was then stored at −80°C until the donors were tested negative for serology. Finally, the pools were thawed at 37°C, centrifuged at 5348*g* for 7 min and pooled to make up a pool of 36. Aliquots were prepared and stored at −80°C. Before use, aliquots were thawed at 37°C, centrifuged at 2000*g* for 10 min and filtered with a 0.22-μm sterile filter (Millipore, Vienna, Austria). The final hPL could be characterized with a pH level between 6.9–8.0 and a total protein concentration between 3–8 g/dL. Growth factor analysis for platelet-derived growth factor–BB (PDGF-BB), TGF-β1, bFGF, IGF-1 and EGF revealed concentrations of 18.11 ± 5.33 ng/mL, 32.74 ± 7.07 ng/mL, 363.47 ± 23.55 pg/mL, 33.14 ± 2.26 ng/mL and 13.96 ± 0.05 pg/mL, respectively.

Isolation and culture of ECs

A local ethics committee approved the cell isolation, and all further experiments and written informed consents were collected from all blood donors. OECs were isolated as described elsewhere (14,15). Briefly, 30 mL of human peripheral blood were taken from healthy donors and separated with cold Lymphocyte Separation Medium (LSM 1077, PAA Laboratories, Pasching, Austria). After washing with 1× phosphate-buffered saline (PBS, PAA Laboratories) the cells were resuspended in 1 mL of Endothelial Growth Medium (EGM)-2 (Lonza, Walkersville, MD, USA) supplemented with 20% FCS (PAA Laboratories) and transferred to a well plate precoated with 2 μg/mL human fibronectin (Sigma-Aldrich, St Louis, MO, USA) at a density of approximately 5×10^5 cells/cm^2. OECs were expanded up to passages 6–8 with the use of different media as described below. LECs were isolated from human foreskin by means of podoplanin selection as described elsewhere (11,16); they were immortalized by means of stable integration of human telomerase (16) and used up to passages 39–42 for all experiments. Additionally, primary LECs were also used for all experiments up to passage 10 to corroborate the results. Human umbilical vein ECs (HUVECs) were purchased from Lonza (C2519A, pooled donor) and used between passages 7–13. Moreover, we isolated HUVECs from an independent donor according to Petzelbauer *et al.* (17), and these cells were also used for all experiments. All cells were cultured in EGM-2 medium containing hydrocortisone, VEGF, hFGF-B, R3–IGF-1, ascorbic acid, hEGF, gentamicin and amphotericin (GA-1000) supplemented with either 5% FCS, 5% FCS + 4 U/mL heparin (Heparin Immuno, EBEWE Pharma GmbH, Unterach, Austria) or 5% hPL + 4 U/mL heparin. These three conditions are referred to as "FCS," "FCS+heparin" and "hPL+heparin" throughout this report for simplifying purposes. Both media supplemented with FCS or FCS+heparin were used as controls because it is necessary to heparinize the hPL medium to avoid gelation during culture. Additionally, FCS is routinely used for the expansion of ECs and therefore represents the