Journal of Biomechanics 44 (2011) 2150–2157

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Biomechanical effects of flow and coculture on human aortic and cord blood-derived endothelial cells

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ARTICLE INFO

Article history:
Accepted 23 May 2011

Keywords:
Endothelial progenitor cells
Smooth muscle cells
Mechanics
Atomic force microscopy
Cytoskeleton
Stress fiber

ABSTRACT

Human endothelial cells derived from umbilical cord blood (hCB-ECs) represent a promising cell source for endothelialization of tissue engineered blood vessels. hCB-ECs cultured directly above human aortic smooth muscle cells (SMCs), which model native and tissue engineered blood vessels, produce a confluent endothelium that responds to flow like normal human aortic endothelial cells (HAECs). The objective of this study was to quantify the elastic modulus of hCB-ECs cocultured with SMCs under static and flow conditions using atomic force microscopy (AFM). Cytoskeleton structures were assessed by AFM cell surface imaging and immunofluorescence of F-actin. The elastic moduli of hCB-ECs and HAECs were similar and significantly smaller than the value for SMCs in monoculture under static conditions ($p < 0.05$). In coculture, hCB-ECs and HAECs became significantly stiffer with moduli 160–180% larger than their corresponding values in monoculture. While the moduli of hCB-ECs and HAECs almost doubled in monoculture and flow condition, their corresponding values in coculture declined after exposure to flow. Both the number and diameter of cortical stress fiber per cell width increased in coculture and/or flow conditions, whereas the subcortical stress fiber density throughout the cell interior increased by a smaller amount. These findings indicate that changes to biomechanical properties in coculture and/or exposure to flow are correlated with changes in the cortical stress fiber density. For ECs, fluid shear stress appeared to have greater effect on the elastic modulus than the presence of SMCs and changes to the elastic modulus in coculture may be due to EC–SMC communication.

1. Introduction

Endothelial progenitor cells (EPCs), which can be easily isolated from peripheral or umbilical cord blood and expanded (Ingram et al., 2004), represent a promising source of endothelial cells (ECs) for vascular regeneration and tissue engineering (Kaushal et al., 2001; Schmidt et al., 2004; Melero-Martin et al., 2008). ECs derived from late outgrowth umbilical cord blood EPCs (hCB-ECs) have a higher proliferative potential than peripheral blood-derived EPCs and early outgrowth cord blood-derived EPCs (Ingram et al., 2004; Yoder et al., 2007). hCB-ECs express EC-specific markers such as platelet endothelial cell adhesion molecule (PECAM), von Willebrand Factor and VE-cadherin (Brown et al., 2009). Unlike early outgrowth EPCs, hCB-ECs do not express cell surface markers found on blood monocytes and macrophages (Yoder et al., 2007; Brown et al., 2009).

We found that the following exposure to 15 dyne/cm^2 for 48 h, hCB-ECs oriented and elongated in the direction of flow, and expressed similar numbers of $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins as well as antithrombotic and anti-inflammatory genes compared to human aortic ECs (HAECs; Brown et al., 2009). When injected intravenously in immune compromised mice, hCB-ECs accelerated vein graft reendothelialization and prevented vein graft thrombosis (Brown et al., 2010).

In native blood vessels, the biomechanical environment of ECs and smooth muscle cells (SMCs) influences their biological responses and likely regulates key functions of engineered blood vessels (Davies, 1995; Nerem, 2003; Li et al., 2005; Haga et al., 2007; Kliche et al., 2011). ECs convert the shear stress resulting from blood flow into intracellular signals that affect gene expression and cellular function such as proliferation, apoptosis, migration, permeability, cell alignment and mechanical properties. ECs' mechanical properties depend on the cytoskeletal structures, vary over different regions of cells and are increased after exposure to flow (Sato et al., 2000; Mathur et al., 2001). Using a direct coculture of ECs on SMCs in which ECs or EPCs form a confluent monolayer on extracellular matrix produced by quiescent SMCs (Lavender et al., 2005; Wallace et al., 2007a; Brown

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doi:10.1016/j.jbiomech.2011.05.024