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Capability of human umbilical cord blood progenitor-derived endothelial cells to form an efficient lining on a polyester vascular graft in vitro

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Abstract

One of the goals of vascular tissue engineering is to create functional conduits for small-diameter bypass grafting. The present biocompatibility study was undertaken to check the ability of cord blood progenitor-derived endothelial cells (PDECs) to take the place of endothelial cells in vascular tissue engineering. After isolation, culture and characterization of endothelial progenitor cells, the following parameters were explored, with a commercial knitted polyester prosthesis (Polymaille® C, Laboratoires Pérouse, France) impregnated with collagen: cell adhesion and proliferation, colonization, cell retention on exposure to flow, and the ability of PDECs to be regulated by arterial shear stress via mRNA levels. PDECs were able to adhere to commercial collagen-coated vascular grafts in serum-free conditions, and were maintained but did not proliferate when seeded at 2.0×10^5 cm^{-2}. Cellularized conduits were analyzed by histology and histochemical staining, demonstrating collagen impregnation and the endothelial characteristics of the colonizing cells. Thirty-six hours after cell seeding the grafts were maintained for 6 h of either static conditions (controls) or application of pulsatile laminar shear stress, which restored the integrity of the monolayer. Finally, quantitative real-time RT-PCR analysis performed at 4 and 8 h from cells lining grafts showed that MMP1 mRNA only was increased at 4 h whereas vWF, VE-cadherin and KDR were not significantly modified at 4 and 8 h. Our results show that human cord blood PDECs are capable of forming an efficient lining and to withstand shear stress.

Keywords: Progenitor-derived endothelial cells; Biocompatibility evaluation; Vascular grafts; Shear stress; Gene regulation

1. Introduction

The isolation of endothelial progenitor cells (EPCs) from human peripheral and umbilical cord blood [1] has generated great hope in the fields of cellular therapies and regenerative medicine, as these precursors have been demonstrated to be of particular benefit in animal models, as well as in some clinical studies, after injection into the circulatory system or at the site of injury in case of ischemic vascular diseases.

The outlook for EPC-based therapy for cardiovascular disease tissue engineering as well as for cancer has been the subject of recent reviews [2–7]. If EPCs directly injected are able to differentiate in situ, they could also differentiate in vitro, and be proposed for applications in the biomedical field. Indeed, despite numerous attempts all over the world through different approaches over a number of decades [8,9], the engineering of a small-caliber blood vessel substitute for use in peripheral and coronary bypass surgery is still a challenge. As for a normal blood vessel, non-thrombogenicity is provided by the vascular endothelium; thus, to

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construct blood vessels by tissue engineering, one technique used is the seeding of endothelial cells (ECs). At present, EC seeding of synthetic prostheses can be performed, in humans, with autologous EC, requiring the harvesting of cells, followed by culturing and amplification for several weeks and then seeding onto coated grafts [10–12]. As large amounts of seeded ECs are needed, a reliable source of these cells is a major concern in vessel tissue engineering.

Isolating EPCs are relatively easy, and their capacity to expand in culture while retaining their ability to switch into ECs has led to their application in developing constructs. In line with this, they have been used for the endothelialization of vascular artificial stents [13], human pulmonary valves [14] and prostheses [15–17] instead of mature endothelial cells. Furthermore, the first clinical trials have been reported using EPCs antibody-coated coronary stents [18,19]. In the present work our overall hypothesis was that umbilical cord blood progenitor-derived ECs (PDECs) could represent a reliable cell source for seeding, with the following benefits and for the following reasons: the umbilical cord contains a richer source of stem cells that can easily be extracted and cryopreserved [20] than peripheral blood or bone marrow [21]. It provides immediately available cells, with no risk to the donor and a low risk of transmitting infectious diseases [22]. These cells have a higher proliferation rate and superior colony-forming ability than peripheral blood-derived EPCs [23], and possess a superior in vivo potential to form normal functional long-lasting vessels in vivo, as recently demonstrated in SCID mice [24].

In vivo, ECs are continuously exposed to mechanical (tangential fluid shear stress, cyclic circumferential strain and blood pressure) and biochemical (VEGF, IL-1, TNFα, etc.) stimuli, which are important modulators of vascular cell functions, growth and structure at both the protein and mRNA levels. The mechanisms by which ECs sense mechanical stimuli and convert them into biochemical signals have been partly elucidated [25,26]. In particular, vascular endothelial growth factor receptor 2 (VEGFR2, Flk1/KDR) and vascular endothelial cadherin (VE-cadherin) of the adherens junction were shown to act as shear stress cotransducers and belong to a mechanosensory complex [27].

Thus, the capability of umbilical cord blood-derived EPCs to form an endothelial cell lining on a commercial vascular prosthesis in vitro, and their ability to withstand the shear stress of the blood flow and to be responsive to shear stress at mRNA levels were the subjects of investigation in the present study.

2. Materials and methods

2.1. Isolation, culture and characterization of PDECs

Human umbilical cord blood samples were collected from donors in accordance with the French legislation. The samples were immediately processed in the laboratory for isolation of EPCs according to the procedure described by Bompais et al. [28]. PDECs were characterized as endothelial by immunofluorescent stainings for von Willebrand Factor (vWF), KDR, CD31 and incorporation of Dil-Ac-LDL, and by FACS analysis for VE-cadherin, as in our previous papers [29,30].

2.2. Vascular graft under test

The vascular graft POLYMAILLE® C is a knitted polyester prosthesis, kindly provided by Laboratoires Pérouse (France), impregnated with collagens I + III of bovine origin that was sterile at the time of use.

2.3. Cytotoxicity, cell attachment and proliferation assessments

Cytotoxicity assessment was performed according to standard ISO/EN 10993 part 5 guidelines using PDECs plated in 96-well plates (seeding density: 2.0×10^5 cm^{-2}) and grown until confluency, obtained 4 days after seeding. Briefly, cell monolayers were incubated for 24 h at 37 °C (six wells per series and concentration) with the original extract (i.e. pure undiluted) or to a dilution series of the original extract. At the end of the incubation period, the material or control extracts were removed and two quantitative colorimetric tests were performed: the cell viability (Neutral Red assay) and the cell metabolic (tetrazolium-based) activity (MTT assay).

For cell attachment measurement, circular pieces were stamped from prostheses to fit the bottom of 48-well plates. In order to avoid cell adhesion to the plastic of wells, which could occur during seeding, an agarose layer was prepared and poured into the wells as previously described [31]. Cells were seeded on the inner surface of patches and controls (culture plates coated with gelatin (0.2% (w/v)) at two different densities (0.2 or 2.0×10^5 cm^{-2}, corresponding to cell seeding density (CSD) 1 and CSD2, respectively) in serum-free medium (M199, GIBCO®) for 1, 3 and 24 h ($n = 8$). At the end of the incubation period, quantitative attachment tests were performed as previously described [32] and compared with the controls.

Cell proliferation was assessed on circular pieces of prostheses fitted the bottom of 24-well plates previously filled with an agarose layer as above and compared with gelatin-coated control wells. Cells were seeded at two different densities (0.2 or 2.0×10^5 cm^{-2}) in ECGM-MV2 medium PromoCell® and cultured for 1, 2, 4, 7 and 9 days ($n = 6$) with medium changes every 3 days. At the end of the incubation period, cell proliferation was evaluated with the MTT test because of a satisfactory correlation between cell numbers and absorbance [33].

2.4. Grafts endothelialization and flow experiments

The POLYMAILLE® C graft (6 mm internal diameter and 20–25 cm length) was filled with the PDEC suspension: 2×10^6 cells ml^{-1} of ECGM-MV2 medium representing a

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