Available online at www.sciencedirect.com
ScienceDirect
Acta Biomaterialia 4 (2008) 182–191
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Regulation of endothelial cell phenotype by biomimetic matrix coated on biomaterials for cardiovascular tissue engineering

Chennazhy Krishna Prasad, Lissy K. Krishnan *

Thrombosis Research Unit, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum 695 012, India

Received 8 December 2006; received in revised form 24 May 2007; accepted 30 May 2007
Available online 3 July 2007

Abstract

One major weakness that all cardiovascular replacements have in common is the lack of endothelial cell (EC) growth and post-implant remodeling of the device. The emerging field of tissue engineering focuses on the in vitro generation of functional organ replacements using living endothelial cells and other vascular cells for which nondegradable or biodegradable scaffold base materials are used. In this paper, it is demonstrated that some of the cardiovascular device materials in clinical use lack the ability to promote endothelial cell growth in vitro. We previously established a biomimetic matrix composition which supports the growth of human umbilical vein endothelial cells (HUVECs) while maintaining normal physiology in vitro. Here the effectiveness of the same coating to preserve the normal antithrombotic phenotype of endothelial cells grown on biomaterials was evaluated. The up/down-regulation of two prothrombotic and two antithrombotic molecules by HUVECs grown on bare material surfaces were compared with that on composite-coated materials. The suitability of this approach for blood-contacting applications was investigated by in vitro blood compatibility studies as recommended in ISO10993 part 4, by putting an EC-seeded surface in contact with human whole blood. It is demonstrated that EC-seeded bare material surfaces are prothrombotic, whereas surfaces pre-coated with biomimetic molecules facilitated maintenance of the normal EC phenotype and reduced the risk of platelet adhesion and activation of blood coagulation. The results presented here suggest that matrix composed of biomimetic adhesive proteins and growth factors is suitable for cardiovascular tissue engineering to improve biological function, irrespective of the material chosen to meet the mechanical properties of the device.

Keywords: Biomimetic matrix; Tissue engineering; Endothelial cells; Blood compatibility; Biomaterials

1. Introduction

Implanted cardiovascular devices such as artificial hearts, ventricular assist devices, heart valves, small diameter vascular grafts and stents encountered biocompatibility problems. The problems are exacerbated by long implantation periods, which are associated with substantial risk of thromboemboli and thrombotic occlusion caused by the lack of an endothelial lining and the flow abnormalities that result from a rigid outflow structure [1–7]. To minimize these risks, chronic anticoagulation therapy is prescribed which renders patients vulnerable to potentially serious hemorrhagic complications [8,9].

Recent experimental studies indicate the feasibility of developing an "organoid" consisting of a biomaterial lattice coated with a monolayer of cells. These materials can be constructed "two-dimensionally", i.e. as surfaces colonized by cells, such as heart valves or vascular prostheses lined by a mature, naturally thromboresistant, non-immunogenic and semi-permeable endothelial layer [10–13]. This approach has the potential for improving biocompatibility of various types of vascular implants and allowing the use

* Corresponding author. Tel.: +91 471 2520219; fax: +91 471 2341814.
E-mail address: lissykk@sctimst.ac.in (L.K. Krishnan).

1742-7061/$ - see front matter
doi:10.1016/j.actbio.2007.05.012

of biodegradable materials to construct completely biological implants. However, efficient attachment, survival and release of biologically active agents by endothelial cells (ECs) are dependent on improved methods of surface modification.

Several in vitro studies have indicated that most of the metallic and polymeric materials do not support endothelial cell growth unless a suitable protein composition mimicking the extracellular matrix (ECM) is deposited to enhance EC adhesion [14,15]. Another problem that limits the success of in vitro EC seeding is that in culture, endothelial cells transform and lose essential cellular functions [16].

An effective method of promoting the integration and adhesion of the cells onto the device is to immobilize agents such as ECM proteins and peptides directly onto the device surface [17,18]. In a previous study, we standardized a composite matrix using human umbilical vein endothelial cell (HUVEC) culture, which supports good actin organization and transduces signals for proliferation, viability and maintenance of the normal antithrombotic EC phenotype [19]. In this study, the effectiveness of the same matrix composition for endothelialization of biomaterials used for cardiovascular implants is evaluated. The specific objective of this study was to demonstrate that, unlike ECs grown on bare materials, ECs on material coated with a biomimetic matrix composed of fibrin, fibronectin and growth factors are able to maintain their normal antithrombotic phenotype by quantitative expression of mRNA. The potential of this approach for blood-contacting applications was investigated by in vitro blood compatibility studies as recommended in ISO10993 part 4, which includes coagulation analysis and platelet adhesion studies.

2. Materials and methods

MCDB 131 medium, trypsin-EDTA and antibiotics were from GIBCO BRL (USA). Growth factor (GF) used for EC culture in this study was prepared from bovine hypothalamus according to the method of Maciag et al. [20], obtained as a mixture of vascular endothelial cell growth factor (VEGF) and fibroblast growth factor (FGF). Fetal bovine serum (FBS) was from GIBCO BRL. The complete medium contained 20% FBS, 200 μg ml^{-1} growth factor and 10 μl ml^{-1} antibiotics. Radioisotope ^{125}I was obtained from American Radio Chemicals (USA). Absolute alcohol, lactoperoxidase, Sepharose CL-2B, H_2O_2 and glutaraldehyde were from Sigma Chemicals (USA).

2.1. Endothelial cell culture

HUVECs were isolated using the method of Jaffe et al. [21]. Harvested primary cells were initially grown on 2% gelatin-coated tissue culture plates in a humidified incubator under 5% CO_2 at 37 °C using complete MCDB 131 medium containing 20% FBS, antibiotics and 200 μg ml^{-1} growth factor [19]. All the cells used for the study were from passages 3–5.

2.2. Study materials

Polished (0.1 μ Ra) titanium (Ti) discs of 18 mm diameter, ultrahigh molecular weight polyethylene (UHMWPE) discs of Himount 1900 grade and of 15 mm diameter, and Ti discs of 15 mm diameter coated with diamond-like carbon (DLC) by plasma-enhanced vapor deposition method were used.

2.3. Preparation of composite-coated surfaces

To coat the surface with composite matrix, materials were kept immersed in bovine thrombin (~2.5 IU cm^{-2}) and incubated for 30 min. After the incubation period, excess solution was removed and the surface was added with a thin layer of plasma cryoprecipitate (20 μl cm^{-2} area) containing 5 mg fibrinogen and 200 μg fibronectin in each ml, premixed with gelatin (0.02% w/v) and growth factor (200 μg ml^{-1}). Fibrin was allowed to polymerize at 37 °C for 30 min before the materials were frozen at −70 °C for at least 2 h and lyophilized. Titanium, UHMWPE and DLC–Ti pieces were coated using the same method and compositions. The coated and freeze-dried materials were stored at 4 °C until the cell seeding was done.

2.4. Endothelial cell culture on bare and composite-coated materials

Before seeding endothelial cells, all the materials were washed with serum-free medium, ECs harvested from 25 cm^2 culture flasks were counted using a hemocytometer and ~4×10^4 cells cm^{-2} were seeded onto each material. After 6 h, the unattached cells were removed and fresh complete medium was added. Culture conditions were similar as described earlier. Medium was changed every alternate day, and cells were allowed to grow for 5 days before the studies described below were performed. At this seeding density, cells from passages 3 to 5 grown on gelatin coated culture flasks usually attain confluence by 3–4 days [19].

2.5. Cell adhesion and spreading

HUVEC was stained for F-actin with Texas red phalloidin (Molecular Probes) and the actin filament assembly analyzed microscopically. Third passage cells (2×10^4 cells cm^{-2} area), were used for seeding on each material. For analysis of cell adhesion behavior, 24 h after seeding, each material was washed with phosphate-buffered saline (PBS) and fixed using 3.7% paraformaldehyde. Manufacturer's instructions were followed for staining and surfaces were observed under a confocal microscope (LSM 510 Meta, Carl Zeiss, Germany).

The fluorescence intensity at specific area was estimated by taking a series of "Z" sections and computing the fluorescence intensity profile of a selected area containing 10 cells each at each section using LSM 510 Meta software (Ver. 3.5.0.223).

ID	Title	Pages
1984	Regulation of endothelial cell phenotype by biomimetic matrix coated on biomaterials for cardiovascular tissue engineering	10