Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/acme

Original Research Article

Chemical control of polyelectrolyte film properties for an effective cardiovascular implants endothelialization

A. Mzyk[a], R. Major[a,*], M. Kot[b], J. Gostek[c], P. Wilczek[d], B. Major[a]

[a]Institute of Metallurgy and Materials Science, Polish Academy of Sciences, 25 Reymonta Street, 30-059 Krakow, Poland

[b]AGH University of Science and Technology, Faculty of Mechanical Engineering and Robotics, Al. Mickiewicza 30, 30-059 Krakow, Poland

[c]The Henryk Niewodniczański Institute of Nuclear Physics, Polish Academy of Sciences, 152 Radzikowskiego Street, 31-342 Krakow, Poland

[d]Foundation for Cardiac Surgery Development, 345a Wolnosci Street, 41-800 Zabrze, Poland

ARTICLE INFO

Article history:
Received 4 July 2013
Accepted 20 September 2013
Available online 26 September 2013

Keywords:
Polyelectrolyte
Multilayer
Endothelium
Chemical cross-linking

ABSTRACT

The aim of this study was to improve properties of blood contacting materials such as polyurethane, in a form of intelligent, self-organizing and self-controlling coatings, which allow the selective mobilization and colonization of the endothelial cells on their surface. The prepared multilayer polyelectrolyte scaffolds were cross-linked chemically by EDC/NHS reagents in order to control their physicochemical properties and thus improving potential to endothelialization. Four types of coatings, i.e. non-cross-linked, cross-linked by 260 mM, 400 mM and 800 mM EDC reagent, were investigated. Their comparison was performed based on the results of the surface topography measurements using Atomic Force Microscopy (AFM), cellular morphology and proliferation analysis using Confocal Laser Scanning Microscopy (CLSM) and the mechanical properties examinations.

The optimal multilayer rigidity and surface roughness parameters were found for an effective control of the endothelial cells growth. Surface topography analysis indicated an increase in the coating's roughness due to application of higher EDC cross-linker concentrations. Mechanical studies revealed that cross-linking caused a significant increase in the hardness and elastic modulus. The results from the cellular experiments allowed the conformation that 400 mM cross-linked PLL/HA films possess desired properties.

1. Introduction

Current development in modern implantable cardiovascular devices is highly related to the biocompatibility improvement through manufacturing surfaces mimicking extracellular matrix (ECM) components. Besides providing structural support to the cells, the ECM also plays an important role in modulating numerous cellular functions including: cell adhesion, migration,

*Corresponding author at: Institute of Metallurgy and Materials Science, Polish Academy of Sciences, 25 Reymonta Street, 30–059 Krakow, Poland. Tel.: +48 12 2952800; fax: +48 12 2952804.
E-mail address: r.major@imim.pl (R. Major).

1644-9665/$ - see front matter © 2013 Politechnika Wrocławska. Published by Elsevier Urban & Partner Sp. z o.o. All rights reserved.
http://dx.doi.org/10.1016/j.acme.2013.09.006

proliferation and differentiation as was described by Richert et al. [1], Boudou et al. [2], Angelova and Hunkeler [3] and Major [4]. This dynamic microenvironment facilitates covering an internal surface of the implant with a cell monolayer which masks the material from an inflammatory response and results in tissue-like structure formation.

The regenerative medicine based on tissue engineering is expected to provide new treatment possibilities which will be appropriate for heart valves and vessels replacement or as bioactive extracorporeal cardiovascular devices, in the opinion of Minuth et al. [5] or Brendan and Michael [6]. The most common approach proposed by several research teams such as Valfre' et al. [7], Borger et al. [8], Riess et al. [9], Tominaga et al. [10] and Tsyganov et al. [11] include the native or modified porcine tissue or synthetic materials application. The limited life is an underlying problem with bioprosthesis due to structural changes such as tissue wear and calcification. On the other hand it has been widely described by Chandran [12] and Picart [13] that synthetic substitutes are very durable, but they are susceptible to thrombosis, structural failure, red blood cell and platelet destruction, tissue overgrowth, damage of endothelial lining and moreover their application requires a long-term anticoagulation therapy.

The cell settlement plays a key role in implant stability. Accordingly to a cardiovascular application, creation of a uniform endothelium layer on the inner surface would be favorable in order to mask the underlying material from an inflammatory response as was presented by Lodish et al. [14]. Moreover, Williams [15], Kaihara et al. [16] and Richardson et al. [17] have revealed that the cellular monolayer acts as a dynamic interface which actively regulates the hemostasis and thrombosis leading to the improvement of the artificial device integration. Therefore, the surface functionalization that tends to cover the surface of an implant by a confluent endothelial layer should become the crucial task on which the experimental efforts are concentrated. The most promising approach proposed by Crouzier et al. [18] and Yilgor et al. [19] refers to application of porous polyelectrolyte films created by a "layer by layer" technique using electrostatic interactions. This technique allows the creation of multi-layer tissue-like structures and is based on the sequential adsorption of successive layers of oppositely charged polyelectrolytes. Boudou et al. [2] and Richert et al. [20] have shown that parameters such as surface topography and charge, rigidity, and chemical composition could affect biological activity of polyelectrolyte films and in consequence could influence cell adhesion processes. The chemical cross-linking is one of the methods which allows to control the stiffness parameters. Richert et al. [21] have shown that Young's modulus of cross-linked films can be more than 10-fold larger than that of the native ones. Studies performed by Richert et al. [20,21], Engler et al. [22] and Semenov et al. [23] on poly-L-lysine (PLL)/hyaluronic acid (HA) coatings with primary chondrocytes, mesenchymal stem cells and smooth muscle cells showed a greater cell adhesion and spread on the cross-linked multilayers. Similar observations were made by Wissink et al. [24] for the endothelial cells proliferating on the cross-linked collagen coatings. Therefore, the cross-linking has been intensively studied and it was found by Boudou et al. [2] and Valfre' et al. [7] to enhance multilayer stability with respect to the enzymatic degradation, dehydratation and rigidity. Unfortunately, there is no information concerning the optimal surface topography and stiffness parameters for endothelial cells adhesion and proliferation on polyelectrolyte films consisting of PLL and HA layers.

In the present study, PLL and HA were used as promising materials for the cardiovascular implants surface modification. The polyelectrolyte scaffolds were cross-linked and the relationship between the applied concentration of chemical reagent and the changes in films properties such as surface topography, stiffness and potential to settlement by endothelial cells was determined.

2. Materials and methods

2.1. Materials

The following materials were used to produce polyelectrolyte coatings: the cationic poly-L-lysine purchased from Sigma-Aldrich and the anionic hyaluronic acid bought from LifeCore'sOnCore™, hereafter referred to as PLL and HA, respectively. The average molecular weight of the PLL was 15–30 kDa, HA was 176–350 kDa. Chemicals necessary for cross-linking i.e. 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide (EDC) and N-hydrosulfosuccinimide (NHS) were supplied from Sigma-Aldrich. Human Umbilical Vein Endothelial Cell line (HUVEC), culture medium and all supplements (EGM-2 BulletKit) were from Lonza. Cells morphology was assessed by an application of 4′,6-diamidino-2-phenylindole (DAPI) and phalloidin antibody from Invitrogen.

2.2. Porous scaffold manufacturing

Polyelectrolyte multilayered films were deposited by a "layer by layer" technique onto the 1.5 × 1.0 cm substrate material. Substrates were activated by 10 M NaOH and washed with pure Milli-Q water. PLL and HA were dissolved in 400 mM HEPES/0.15 M NaCl solution with concentrations of 0.5 and 1 mg/ml, respectively and pH of the solutions was set at pH 7.4 by adding 0.5 M NaOH. Films were manufactured by an automatic dipping machine using an alternately immersing substrate in solutions of PLL and HA for 8 min each. After each deposition step, the substrate was rinsed in 0.15 M NaCl solution buffered at pH 7.4 to remove excess polyelectrolyte. The process was repeated until a desired number of 12 bilayers were obtained. Multilayers were further processed for cross-linking or were left in a non-cross-linked state. Then additional layer of PLL was adsorbed. Afterwards, the samples were subjected to final rinsing steps and stored at 4 °C in 400 mM HEPES/0.15 M NaCl solution buffered at pH 7.4 until measurements were performed.

2.3. Chemical cross-linking

The chemical stability of multilayer polyelectrolyte films was caused by the chemical cross-linking process. In all the experiments, properties of both types of samples (cross-linked and native) were determined. The cross-linking of the layers was performed with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysulphosuccinimide (NHS) according to the process described elsewhere [18,16]. Reagents were prepared in