Colloids and Surfaces B: Biointerfaces 144 (2016) 46–56

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Interfacial energetics approach for analysis of endothelial cell and segmental polyurethane interactions

Michael J. Hill[a], Calvin Cheah[a], Debanjan Sarkar[a,b,*]

[a] Department of Biomedical Engineering, University at Buffalo, The State University of New York, Buffalo, NY 14260, USA
[b] Department of Chemical and Biological Engineering, University at Buffalo, The State University of New York, Buffalo, NY 14260, USA

ARTICLE INFO

Article history:
Received 14 January 2016
Received in revised form 24 March 2016
Accepted 30 March 2016
Available online 31 March 2016

Keywords:
Surface
Energetics
Endothelial
Network
Polyurethane
Adhesion

ABSTRACT

Understanding the physicochemical interactions between endothelial cells and biomaterials is vital for regenerative medicine applications. Particularly, physical interactions between the substratum interface and spontaneously deposited biomacromolecules as well as between the induced biomolecular interface and the cell in terms of surface energetics are important factors to regulate cellular functions. In this study, we examined the physical interactions between endothelial cells and segmental polyurethanes (PUs) using L-tyrosine based PUs to examine the structure-property relations in terms of PU surface energies and endothelial cell organization. Since, contact angle analysis used to probe surface energetics provides incomplete interpretation and understanding of the physical interactions, we sought a combinatorial surface energetics approach utilizing water contact angle, Zisman's critical surface tension (CST), Kaelble's numerical method, and van Oss-Good-Chaudhury theory (vOGCT), and applied to both substrata and serum adsorbed matrix to correlate human umbilical vein endothelial cell (HUVEC) behavior with surface energetics of L-tyrosine based PU surfaces. We determined that, while water contact angle of substratum or adsorbed matrix did not correlate well with HUVEC behavior, overall higher polarity according to the numerical method as well as Lewis base character of the substratum explained increased HUVEC interaction and monolayer formation as opposed to organization into networks. Cell interaction was also interpreted in terms of the combined effects of substratum and adsorbed matrix polarity and Lewis acid-base character to determine the effect of PU segments.

1. Introduction

Interactions between endothelial cells (ECs) and the biomaterial substratum are important for various biomedical applications, including endothelialization of artificial blood vessels, vascularization of tissue engineering scaffolds, and understanding of angiopathies [1–3]. In-vitro, EC can either proliferate into a monolayer, differentiate into capillary-like networks which eventually lift from the substratum surface to form 3-dimensional tubes, or undergo anoikis [4,5].

Effect of substratum stiffness on endothelial cell response has been extensively studied to show that substratum modulus must be sufficiently high to induce survival and capillary formation but sufficiently low to prevent proliferation into a monolayer [6–8]. However, EC and other cell types also spread and display distinct phenotypical features at liquid–liquid interfaces in the presence or absence of surface active molecules depending on the chemistry of the organic phase, highlighting the significance of the physicochemical relationship between cell and the underlying surface [9–11]. These interactions between the biomaterial interface and the fate of ECs, in terms of surface energetics are not well understood. This may be due to the complexity of the relationship between substratum molecular structure and the character of the resultant force field which emerges on the scale of biomacromolecular interactions [12].

Water adhesion to the biomaterial surface has often been implicated in cell-material interactions with a range of values for optimal water contact angle (θ_w) reported. For example, on non-stoichiometric silicon oxide ECs adhere and proliferate more on hydrophilic surfaces with an optimal θ_w of ~26° while they migrate further on hydrophobic surfaces ($\theta_w > 65°$) [13]. However, alkylated cellulose showed that greater adhesion and proliferation occurred on surfaces with θ_w of 48°, while greater migration, cell alignment, and eventually capillary formation occurred on surfaces with θ_w of ~32° [14]. Other studies have implicated hydrophilic surfaces

* Corresponding author at: Department of Biomedical Engineering, University at Buffalo, The State University of New York, Buffalo, NY 14260, USA.
E-mail addresses: debanjan@buffalo.edu, debanjans@gmail.com (D. Sarkar).

http://dx.doi.org/10.1016/j.colsurfb.2016.03.082
0927-7765/

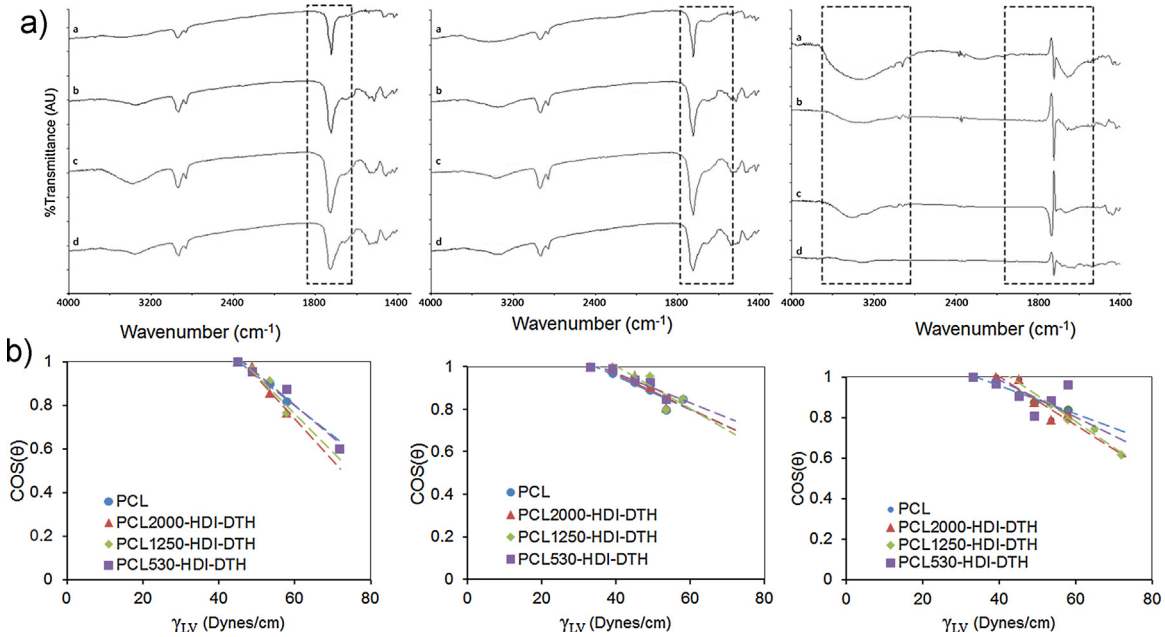

Fig. 1. IR Spectra and Zisman Plots Top IR spectra of a.) PCL, b.) PCL2000-HDI-DTH, c.) PCL1250-HDI-DTH, d.)PCL530-HDI-DTH. left to right: IR spectra of bare PU film, spectra of adsorbed protein, and the difference spectra between the two. Bottom, left to right: Zisman plot of bare PU, plot of serum interfaces after 4 h, plot of serum interfaces after 48 h.

in capillary formation or a θ_w between 55–65° resulting in optimal adhesion/proliferation [15–17]. This anomalous observation indicates water contact angle is not a predictive indicator of EC response on the biomaterial surface. One explanation is that water adhesion may not reflect adhesive outcomes between mutually dehydrated substratum surfaces and biomacromolecules or cells. [18] Alternatively, contact angle analyses using multiple liquids e.g. critical surface tension of Zisman (CST), Fowkes' method of additive polar γ^p and dispersion γ^p component analysis (Owens-Wendt [19], Wu's method [20], Kaelble numerical method [12,21]) [22], and more recently the Lifshitz-Van der Waals γ^{LW} and Lewis acid-base component γ^{ab} of van Oss-Good-Chaudhury theory (vOGCT) have been used to correlate substratum surface free energy to cell adhesion and in particular to EC adhesion in the past, with contradictory results. [23–29] Additionally, most practical biomaterial surfaces are non-uniform and the role of surface heterogeneity in interfacial relations is not well agreed upon [30,31].

In this study, we used a combinatorial strategy by using these approaches to analyze the EC response on segmental polyurethanes (PUs). PUs are extensively used as biodegradable materials, particularly for applications requiring EC e.g. vascular grafts, tissue scaffolds [32–34]. Structurally, PUs consist of the "hard" and "soft" segments which can be modulated to tune the physicochemical and mechanical properties [35]. By altering the chemical structure and composition of PU segments, inter and intra-segmental interactions can be regulated. While surface energy of different PUs has been studied for various applications [34,36,37], correlations between structure and resultant interfacial properties are not yet established with respect to endothelial cell organization. Therefore, further study of how PU structure and interfacial energetics influence EC phenotypical traits is warranted, particularly with EC types which are studied for regenerative medicine such as human umbilical vein endothelial cells (HUVEC) [38]. To establish these correlations, we will use L-tyrosine based polyurethanes which are developed as biodegradable PUs, with polycaprolactone (PCL) as soft segment and hexamethylene diisocyanate (HDI) and desaminotyrosine tyrosyl hexyl ester (DTH) as hard segment [35,39,40]. Utilizing the slowly advancing contact angle method we examined the correlations between EC organization and macroscopic surface energy of both substratum and serum adsorbed matrix on segmented PU surfaces.

2. Materials and methods

2.1. Materials

PCL with 1250 MW. was purchased from Polysciences while 530 and 2000 MW. were purchased from Sigma Aldrich. Hexamethylene diisocyanate (HDI), dimethyl formamide, and 1-*E*thyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) were purchased from TCI and dimethyl sulfoxide (DMSO), L-tyrosine, and 3-(4-hydroxyphenyl)propionic acid (DAT) were purchased from Alfa Aesar. The 15 mm coverslips were purchased from Warner Instruments, while all other chemicals and solvents were purchased at Sigma Aldrich and used as received unless otherwise noted. Cryopreserved human umbilical vein endothelial cells (HUVEC) and Endothelial Cell Growth Medium containing 2% serum with growth factor supplement (containing epidermal and basic fibroblast growth factor and no VEGF) (EC medium) were purchased from Promo Cell. Cells were typically used between passage 4–7. Matrigel was purchased from Corning at a concentration of 10 mg/ml and 4′,6-diamidino-2-phenylindole (DAPI) was purchased from Invitrogen CA.

2.2. PU synthesis and substrate preparation

DTH was synthesized according to established protocols [40,41]. PUs were synthesized according to a previously published protocol via a two-step polycondensation reaction between different PCL (MW: 530, 1250, 2000) as polyol and HDI as aliphatic diisocyanate, followed by DTH as chain extender [35]. Since the polyurethanes were synthesized with 1:1 molar ratio of soft and hard segment, increasing PCL molecular weight resulted in decreased hard segment fraction in the polymer. Pure PCL (MW:1250) and polymer synthesized from condensation of HDI and DTH (i.e. HDI-DTH) were used as control substrates for PUs. MW of the PU were