Available online at www.sciencedirect.com
ScienceDirect
Biomaterials 28 (2007) 162–174
Biomaterials
www.elsevier.com/locate/biomaterials

Spectroscopic translation of cell–material interactions

Josephine Allen, Yang Liu, Young L. Kim, Vladimir M. Turzhitsky, Vadim Backman, Guillermo A. Ameer*

Northwestern University, Biomedical Engineering Department, 2145 Sheridan Rd., Room E310, Evanston, IL 60208, USA

Received 21 April 2006; accepted 5 July 2006
Available online 1 August 2006

Abstract

The characterization of cellular interactions with a biomaterial surface is important to the development of novel biomaterials. Traditional methods used to characterize processes such as cellular adhesion and differentiation on biomaterials can be time consuming, and destructive, and are not amenable to quantitative assessment in situ. As the development of novel biomaterials shifts towards small-scale, combinatorial, and high throughput approaches, new techniques will be required to rapidly screen and characterize cell/biomaterial interactions. Towards this goal, we assessed the feasibility of using 4-dimensional elastic light-scattering fingerprinting (4D-ELF) to describe the differentiation of human aortic smooth muscle cells (HASMCs), as well as the adhesion, and apoptotic processes of human aortic endothelial cells (HAECs), in a quantitative and non-perturbing manner. HASMC and HAEC were cultured under conditions to induce cell differentiation, attachment, and apoptosis which were evaluated via immunohistochemistry, microscopy, biochemistry, and 4D-ELF. The results show that 4D-ELF detected changes in the size distributions of subcellular organelles and structures that were associated with these specific cellular processes. 4D-ELF is a novel way to assess cell phenotype, strength of adhesion, and the onset of apoptosis on a biomaterial surface and could potentially be used as a rapid and quantitative screening tool to provide a more in-depth understanding of cell/biomaterial interactions.

Keywords: Light scattering; Optics; Tissue engineering; Cell characterization; Cell morphology; Cell–substrate interaction

1. Introduction

Current methods to monitor cell adhesion, apoptosis and phenotypic differentiation on materials for tissue engineering include techniques such as histology, confocal-microscopy, RT-PCR, biochemical assays, and flow cytometry. Although commonly used in biomaterials research, these techniques can be destructive, often include artifacts due to preparation protocols, are time consuming, and often times are costly, particularly if many materials must be tested for their cell compatibility properties. With the advent of combinatorial approaches to the development of biomaterials, there is a need for accessible technologies that can rapidly provide a real-time, non-perturbing, quantitative assessment of cell–material interactions. Although based on different sensing principles, some examples of technologies or approaches that have been useful in assessing tissue microarchitecture include electrical cell–substrate impedance sensing (ECIM) [1], quartz crystal microscopy (QCM) [1], optical waveguide lightmode spectroscopy (OWLS) [1], optical coherence tomography [2], fluorescence spectroscopy, Raman spectroscopy [3,4], and angle-resolved low-coherence interferometry [5]. Collectively these techniques are a step in the right direction; however, they may still require some degree of sample preparation or modification prior to analysis.

A recently developed enhancement of light-scattering spectroscopy (LSS), referred to as 4-dimensional elastic light-scattering fingerprinting (4D-ELF), has been shown to discriminate between cancerous and normal cells by detecting and analyzing light back-scattered from subcellular structures [6]. LSS-based imaging has been shown to provide a quantitative assessment of subcellular events, such as organelle enlargement or increased chromatin content, in a real-time and non-destructive manner [7–12].

*Corresponding author. Tel.: +1 847 467 6719; fax: +1 847 491 4928.
E-mail address: g-ameer@northwestern.edu (G.A. Ameer).

0142-9612/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2006.07.002

Light-scattering signals are rich in information yielding unprecedented insights into the micro-architectural organization of cells interacting with substrates. The spectral analysis of light scattered by living tissues provides information about the size scale of internal structures within the cell. Light-scattering signals depend on the size, shape, and organization of intracellular structures, and are sensitive not only to the "scatterer" but also the immediate surrounding milieu (i.e. the concentration of solid particles, such as proteins) [13].

We hypothesize that interfacial cell/biomaterial interactions will affect sub-cellular structures in defined ways, giving rise to specific light-scattering "fingerprints" that can be used to describe an ongoing cellular process. Specifically, interactions during the process of cell attachment onto a substrate can be assessed through the possible detection of changes in cell shape [14,15], sub-cellular organelle distribution [16], nuclear deformation [17], focal adhesion complex formation [18–20], and the assembly of micro-filamentous stress fibers [18–20]. Interactions involved in phenotypic differentiation could be assessed through the possible detection of changes in organelle size, abundance, and distribution as well as the abundance of actin and myosin filaments [21–26]. Interactions resulting in cell death via apoptosis can also be assessed, through the possible detection of nuclear chromatin condensation, cytoplasmic shrinking, and changes in size and shape of the cell nuclei and cell cytoplasm [12,27,28].

The work presented herein describes how 4D-ELF can potentially allow the identification of vascular SMC and EC differentiation processes, based on characteristic 4D-ELF spectral fingerprints. A comparison of the 4D-ELF profiles of cultured vascular cells can provide a quick and easy means to monitor cellular growth and differentiation on new biomaterials, once these processes have been calibrated. Ultimately, the microstructure of the cells can be quantified to determine if the scaffold environment supports cellular attachment, growth, retention, proliferation, and healthy tissue formation.

2. Experimental

2.1. Assessment of cell differentiation

Human aortic smooth muscle cells (SMCs) (Cambrex, East Rutherford, NJ) were induced to a uniform state of either contractile or proliferative phenotypes by culturing them on glass cover slips coated with either 25 μg/ml laminin or 25 μg/ml fibronectin, respectively. SMCs were cultured in SM-basal media (SmBM), supplemented with human epidermal growth factor (hEGF), human fibroblast growth factor (hFGF), gentamicin/amphotericin-B (GA-1000), insulin, and 5% fetal bovine serum (FBS). Cells were cultured at 37 °C, 95% relative humidity, and 5% CO_2 for 5–11 days. Once the phenotypes were achieved, immunohistochemistry was done using primary antibodies to smooth muscle cell specific marker, smooth muscle α-actin (SM-α-actin). (Sigma Aldrich, Milwaukee, WI) SMC proliferation was assessed by incubating the cells grown on both laminin and fibronectin with culture media containing fluorescently labeled 5-bromo-2′-deoxy-uridine (BrDU). BrDU incorporates into newly synthesized DNA, and thus is an indicator of cell proliferation. The cells that incorporated BrDU were visualized via fluorescence microscopy.

2.2. Assessment of cell adhesion

Human aortic endothelial cells (HAECs) (Cambrex, East Rutherford, NJ) were cultured in EC basal media-2 (EBM-2), supplemented with hEGF, hydrocortisone, GA-1000, vascular endothelial growth factor, hFGF-B, R3-insulin growth factor-1, ascorbic acid, heparin, and 10% FBS, at 37 °C, 95% relative humidity and 5% CO_2 for 15, 30, 60 and 120 min on either 25 μg/ml laminin or fibronectin-coated glass cover slips. Following the attachment time, the HAECs were probed with primary antibodies to focal adhesion complex specific protein vinculin, and actin specific phalloidin (Chemicon, Temecula, CA) via immunohistochemistry.

The relative strength of adhesion of HAEC to laminin and fibronectin was assessed using a previously described centrifugation assay [29]. The wells of a black 96-well tissue culture polystyrene (TCP) plate were coated with either 25 μg/ml laminin or 25 μg/ml fibronectin. Prior to the assay, the HAEC were fluorescently labeled with membrane permeable Calcein-AM (Invitrogen-Molecular Probes, Carlsbad, CA). Labeled cells were seeded into the wells and allowed to attach to the substrate for the appropriate duration. The plates were subsequently inverted and centrifuged at 750 rpm, applying 72 g detachment force, for 5 min. The fluorescence intensity of each well was read at 494 nm prior to and following the detachment spin.

2.3. Assessment of cell apoptosis

Tumor necrosis factor-α (TNF-α), a pro-apoptotic agent, was used to induce the molecular events that occur in the early stages of apoptosis [30]. Lactate dehydrogenase release (LDH), light microscopy, and measurements of caspase-3 activity were used to assess the physical and biological characteristics of apoptosis. To determine the optimal concentration of TNF-α to induce apoptosis within the experimental time of 3 h, HAECs were cultured in EBM-2 (fully supplemented as described) supplemented with TNF-α (Sigma Aldrich, Milwaukee, WI) at concentrations of 0.1, 0.5, 1.0, 10 and 50 ng/ml. LDH released into the cell culture media due to cell membrane damage was quantified using a cytotoxicity detection kit (Roche Applied Science, Indianapolis, IN) and compared to media samples from cells not exposed to TNF-α.

Subcellular molecular events associated with apoptosis were assessed by detecting caspase-3 activity (Assay design, Anne Arbor, MI) in cell lysates of TNF-α-challenged cells. Following a 3-h incubation with TNF-α, the media was aspirated, the cells were washed with PBS, then lysed in 150 μl of lysis buffer containing 10% protease inhibitor (Sigma Aldrich, Milwaukee, WI) and 1% Triton-X (Sigma Aldrich, Milwaukee, WI). The cell lysate was kept on ice, until assayed for caspase-3 activity. Lysates from cells incubated in media containing no TNF-α served as the baseline control. 4D-ELF analysis was conducted using HAECs that did not demonstrate changes in cytoplasm or cell membrane integrity following exposure to TNF-α. Cell membrane integrity was assessed via light microscopy or the LDH assay.

2.4. Transmission electron microscopy (TEM)

Primary fixation of the SMCs and HAECs was done in 2% paraformaldehyde/2% gluteraldehyde. The cells were post-fixed in 2% osmium tetraoxide, dehydrated in graded ethanol (50–100%), and embedded in Epon resin. Sections were placed on 200 mesh copper grids, stained with uranyl acetate, lead citrate, and viewed on a Jeol 1220 TEM at 80 kV (Research Resources Center, University of Illinois at Chicago, Chicago, IL).

TEM images were quantified for specific structural characteristics. For the assessment of phenotype, the images of SMCs cultured on laminin and fibronectin were examined for the density of key organelles, mitochondria and rough endoplasmic reticulum (RER). The number of each organelle