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Interfacial study of cell adhesion to liquid crystals using widefield surface plasmon resonance microscopy

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ABSTRACT

Widefield surface plasmon resonance (WSPR) microscopy provides high resolution imaging of interfacial interactions. We report the application of the WSPR imaging system in the study of the interaction between keratinocytes and liquid crystals (LC). Imaging of fixed keratinocytes cultured on gold coated surface plasmon substrates functionalized with a thin film of liquid crystals was performed in air using a 1.45 NA objective based system. Focal adhesion of the cells adhered to glass and LC were further studied using immunofluorescence staining of the vinculin. The imaging system was also simulated with 2×2 scattering matrix to investigate the optical reflection of the resonant plasmonic wave via the glass/gold/cell and glass/gold/LC/cell layers. WSPR imaging indicated that keratinocytes are less spread and formed distinct topography of cell–liquid crystal couplings when cultured on liquid crystal coated substrates. The simulation indicates that glass/LC shifted the surface plasmon excitation angle to 75.39° as compared to glass/air interface at 44°. The WSPR microcopy reveals that the cells remodelled their topography of adhesion at different interfaces.

1. Introduction

A challenge to cell based biosensor development is being able to achieve effective sensing of cell responses within support media which are chemically and rheologically compatible. To this end, our previous work has examined the emerging application of liquid crystal thin films in single cell force sensing using conventional optical microcopy [5]. These liquid crystal based biosensors enable cell adhesion and contractile activity to change the organization of the liquid crystals leading to the detection of cell responses [1–5]. Our study [5] indicated that cholesteryl ester liquid crystals can support cell adhesion and allow the detection of cellular contractions without pre-coating the liquid crystals with adhesion ligands. Although cell-surface interactions have recently been examined with liquid crystals functioning as the force transducer, it is not understood how soft substrates such as liquid crystals affect the organization of the focal adhesions that enable cell attachment to a culture substrate.

Surface plasmons (SP) are highly sensitive to dielectric permittivity changes on a metal-dielectric surface [6]. Thus, surface plasmon technology has been used in the development of light microscope system in which differences in the optical densities of the imaging target alter the way p-polarized light couples into surface plasmons. This results in varying amount of light reflected from a metallic coated surface such as a gold coated glass substrate [7]. This means that surface plasmon microscopy allows the acquisition of a sample image in which contrast is dependent on the relative intensity of the reflected light, and this in turn is dependent on the macromolecular interfacial optical density. Surface Plasmon microscopes allow high contrast imaging of antigen-antibody bindings in cells [8], monitoring local impedance changes in relation to the dynamics of cellular process [9] and determining the mass of the fibronectin absorbed on glass substrate [10]. Attempts to interrogate the surface interactions of cells using surface plasmon microscope have been made, in which, the system incorporated an aluminium coated prism [11]. Although this system enabled reasonably high resolution imaging of the cell-surface interface, the microscope was limited by poor

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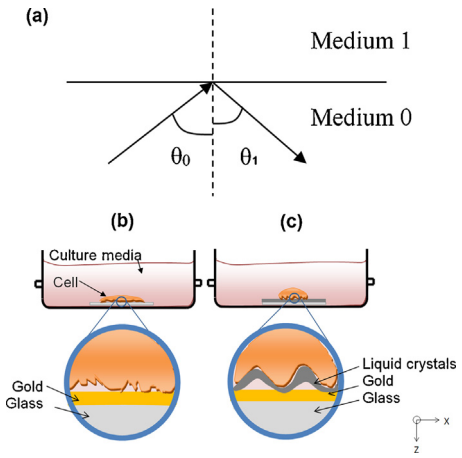

Fig. 1. (a) An incident light strikes interface of medium 0/1 at an angle θ_o and reflected in medium 0 at angle θ_1. The graphical representations of cells culture in the (b) absence and (c) presence of liquid crystals on gold substrates.

contrast resolution of cellular structure due to the wide angles over which p-polarized light excited surface plasmons. To circumvent this optical limitation, the Widefield Surface Plasmon Microscopy (WSPR) was developed [12]. This microscope uses a high numerical aperture lens (either NA 1.45 or 1.65) and a system in which p-polarized light at a wavelength of 633 nm is applied via a rotatable diffuser before striking to gold coated substrate. The WSPR has been used to imaging of lamellipodium of human keratinocytes with good contrast at resolutions down to 500 nm [12]. To date, surface plasmon microscopy has not been used to examine cells on liquid crystals.

The novel aim in this study is the use of widefield surface plasmon microscopy to capture interfacial images of cells grown on liquid crystals in order to examine the restructuring of the adhesion plaques of cells cultured on soft substrates. In order to support the observations obtained by WSPR microscopy, immunocytochemical staining against vinculin will be used to confirm the outcomes of the WSPR study in relation to any observed changes on focal adhesion distribution. In order to understand the effects of the optical reflectivity when adding a liquid crystal layer to a glass/gold substrates interfacial substrate, Fresnel's equation will be used to determine the reflectance of light incident on a multilayer system consists of a glass/gold and glass/gold/liquid crystal system. In consideration of the multilayer reflection, a 2×2 scattering matrix technique will be used to solve the reflective coefficients for the multilayer system.

2. Materials and methods

2.1. Simulation of widefield surface plasmon resonance profiles

Simulation of the WSPR optical function was performed using MATLAB Integrated Development Environment software. Fresnel's equations were used to compute the reflectance of light incident on a multilayer system consisting of either a layer of liquid crystal extending beyond the evanescent field on a gold coated glass cover slip or a simple gold coated glass cover slip. Surface plasmons can be excited at the metal-dielectric interface when an incident light strikes on the interface at a specific angle. At the resonance angle, the energy carried by the photons of light is transferred to the interfacial electrons generating a surface plasmons wave. The efficiency of this coupling can be measured in a surface plasmon microscope by measuring the light reflected by a gold coated surface.

Consider a ray of incident light striking at the interface between medium 0 and 1 (Fig. 1a). Each has a refractive index of N_0 and N_1 respectively. The incident wave vector with amplitude of E_0 arrived to the interface at an angle of θ_o with respect to the normal plane.

The Fresnel's coefficients of the reflectance of the s-polarized and p-polarized light are given by

$$r_{01S} = \left|\frac{E_0^-}{E_0^+}\right| = \frac{N_0 \cos\theta_0 - N_1 \cos\theta_1}{N_0 \cos\theta_0 + N_1 \cos\theta_1} \quad (1)$$

$$r_{01P} = \left|\frac{E_0^-}{E_0^+}\right| = \frac{N_1 \cos\theta_0 - N_0 \cos\theta_1}{N_1 \cos\theta_0 + N_0 \cos\theta_1} \quad (2)$$

where r_{01s} and r_{01p} are the reflection coefficients for s-polarized and p-polarized light, respectively. θ_o and θ_1 are the angles of incident and refraction, respectively as obtained from Snell's law [13].

The method for computing the reflectance of the glass/gold/air and glass/gold/LC systems (Fig. 1a) is based on the application of 2×2 scattering matrix [14]. The equation can be expressed as the product of the interface matrices, I and the layer matrices, L. It describes the effects of light interaction with individual interfaces and layers of the entire stratified structures as

$$\begin{vmatrix} E_0^+ \\ E_0^- \end{vmatrix} = \begin{bmatrix} S_{11} & S_{12} \\ S_{21} & S_{22} \end{bmatrix} \begin{bmatrix} E_n^+ \\ 0 \end{bmatrix} \quad (3)$$

From Eq. (3), we obtained

$$E_0^+ = S_{11} E_n^+ \quad (4)$$

and

$$E_0^- = S_{21} E_n^+ \quad (5)$$

Therefore,

$$r = \frac{E_0^-}{E_0^+} = \frac{S_{21}}{S_{11}} \quad (6)$$

$$t = \frac{1}{S_{11}} \quad (7)$$

The matrix S for a three layers system (1, 2 and 3) is

$$S = I_0 L_1 I_{12} L_2 I_{23} L_3 \quad (8)$$

The matrix I of an interface between two medium a and b is given by

$$I_{ab} = \frac{1}{t_{ab}} \begin{bmatrix} 1 & r_{ab} \\ r_{ab} & 1 \end{bmatrix} \quad (9)$$

where the layer matrix for layer L_b is given by

$$L_b = \begin{bmatrix} e^{i\beta} & 0 \\ 0 & e^{i\beta} \end{bmatrix} \quad (10)$$

where β is the phase shift that is dependent on the layer thickness (d_b), index of refraction (N_b) and angle of refraction (θ_b), therefore,

$$\beta = K N_b d_b \cos\theta_b \quad (11)$$

and

$$K = \frac{2\pi}{\lambda} \quad (12)$$

where K is the wavevector and λ is the wavelength of incident light.