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Research paper

Mechano-regulatory cellular behaviors of NIH/3T3 in response to the storage modulus of liquid crystalline substrates

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ABSTRACT

The extent of substrate stiffness has been shown to be predominant in regulating cellular behaviors. Previous studies have used matrices such as elastomers or hydrogels to understand cell behavior. Herein, liquid crystalline matrices that resemble movable morphology of biomembrane and viscoelasticity were fabricated with tunable storage modulus for the evaluation of the modulus-driven cell behaviors. Our results demonstrated that NIH/3T3 cells showed a hypersensitive response to the storage modulus of liquid crystalline substrates by the alteration in attachment, spreading, proliferation and viability, polarization, cell cycle and apoptosis, and activity of mechano-transduction-related signal molecules including FAK, paxillin and ERK. The octyl hydroxypropyl cellulose substrates (OPC-1-5) with intermediate storage modulus of 12,312 Pa and 7228 Pa (OPC-2 and OPC-3 respectively) could provide more beneficial adhesion conditions leading to a larger spreading area, more elongated morphology and higher proliferation rates possibly through paxillin-ERK pathway, whereas the substrates with the highest or lowest storage modulus (16,723 Pa, OPC-1; and 41 Pa, OPC-5, respectively) appeared unfavorable for cell growth. Our study provides insights into the mechanism of modulus-driven cellular behaviors for better design of bioengineered cell substrates.

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1. Introduction

Natural extracellular matrix (ECM) provides a microenvironment where residing cells can sense external mechanical stimuli and respond to changes in the balance of cellular forces to influence many fundamental cellular processes, including cellular adhesion, morphology, proliferation and gene expression (Ghosh et al., 2007). The interactions between cells and their surrounding substrate trigger numerous responses to regulate cell behaviors and fates (Feugier et al., 2005). These ECM–cellular interactions are greatly influenced by not only the chemical, but also the physical properties of the ECM, such as viscoelasticity, wettability and surface energy (Pegueroles et al., 2010; Lim et al., 2005; Prager-Khoutorsky et al., 2011). It was shown recently that the cell–ECM biointerface dominates cell–substrate interaction (Li et al., 2014) and is responsible for transmitting environment signals to the cell via transmembrane ECM receptors acting as mechanoreceptors through a process termed mechano-transduction, in which the interfacial stiffness or elasticity is an important property that can greatly affect cell fate and activity (Banerjee et al., 2009; Gu et al., 2012; Pegueroles et al., 2010; Li et al., 2014). *In vitro* studies have shown that cells can respond to substrate rigidity *via* mechano-regulatory process to affect changes in cell-shape (Allioux-Guérin et al., 2009; Ghosh et al., 2007; Prager-Khoutorsky et al., 2011) cell migration, differentiation and even apoptosis (Keogh et al., 2010;Vogel and Sheetz, 2006), indicating that substrate stiffness plays an important role in cell fate and properties and should be considered a key design parameter in the development of bioengineered substrates (Ghosh et al., 2007; Peng et al., 2012).

However, most conventional synthetic polymers based on elastomers or hydrogels are unable to achieve the satisfactory rigidity and flexibility similar to that of natural ECM. It is well known that cells are neither purely elastic nor viscous but are viscoelastic, and ECM as biopolymers with higher storage modulus exhibits power-law mechanical responses similar to cells (Lange and Fabry, 2013). From this point of view, there is an urgent need to take the rigidity and flexibility into account in the design of novel matrices that mimic biological systems to provide a potentially more beneficial environment for regulation of cell growth and cell function.

Various studies have indicated that many cellular components, such as cell membranes, DNA and phospholipids, exist in liquid-crystal (LC) phases, as this types of anisotropic viscoelastic material are likely to be soft elastic solid, which make liquid crystals potentially useful for engineering interfaces for living cells (Lockwood et al., 2006). Our previous work demonstrated that cholesteryl liquid crystal-based ester presented a strong capability of providing affinity for cell attachment and enhancing physical properties of cells during attachment and proliferation (Han et al., 2012). Moreover, it has also been found experimentally that liquid crystals are highly sensitive to the interactions between the mesogens forming the liquid crystal and a confining interface, and these liquid crystalline materials can reorganize under the influence of stress comparable in magnitude to those transmitted from cells to their extracellular environments (Lockwood et al., 2006). Nevertheless, the elasticity effects of these types of liquid crystalline substrates on cell behaviors are rarely studied.

Herein, to study the effect of liquid-crystalline substrates on cellular behavior, a series of liquid crystalline matrices that resemble the movable morphology of natural biomembrane surface were fabricated with tunable storage modulus for the evaluation of mechanical cell sensing and modulus-driven cell behaviors. Compared with the pure elastic hydrogels such as polyacrylamide (PAM) or polydimethylsiloxane (PDMS) previously utilized, (Allioux-Guérin et al., 2009; Prager-Khoutorsky et al., 2011; Keogh et al., 2010; Vogel and Sheetz, 2006) liquid crystalline matrices more closely mimick in vivo tissue mechanics by resembling the movable morphology of biomembrane and consisting of both elastic component (storage modulus, G') and dissipative component (viscous modulus, G''). In addition to test the cell spreading, polarization, proliferation, cycle and apoptosis on OPC substrates, we also examined the phosphorylation levels of mechanotransduction signal molecules including focal adhesion kinase (FAK), paxillin and ERK, which may provide insight into the complex system of mechanical cell sensing (Provenzano et al., 2009).

2. Materials and methods

2.1. Preparation of OPC substrates

A series of octyl hydroxypropyl cellulose esters (OPCs) with five different degree of substitution (DS), accordingly coded as OPC-1, OPC-2, OPC-3, OPC-4 and OPC-5, were prepared *via* esterification between hydroxypropyl cellulose (HPC) (Sigma-Aldrich, M_w=100,000 g/mol, St. Louis, MO) and octanoyl chloride (OC) (Sigma-Aldrich, St. Louis, MO) as previously described (Huang et al., 2007). The concentration of OC was varied to adjust the DS of OPCs for regulating the stiffness of the substrates.

Briefly, HPC of 5.0 g (corresponding to 41.71 mmol of hydroxyl groups) was dissolved in 30 mL acetone with mild stir, and then OC was quickly added to the solution of HPC by using a syringe. After 2 h of reflux at 55 °C, the reaction mixture was poured into 200 mL distilled water. After removing the liquid phase, the cream color sticky mass was dialyzed in tetrahydrofuran (THF) to remove the residual OC and then dried at 60 °C (30 mbar, 48 h).

OPC substrates were prepared *via* a solution-casting method. Each sample and THF was stirred together for 1 h at 20 °C to obtain clear and homogeneous mixture with a concentration of 5%. The solutions were cast onto clean glass culture dishes and the solvents evaporated for 24 h at 50 °C. The resulting membranes were further dried in vacuum at 60 °C for 24 h to eliminate residual solvent. The average thickness of the membranes was approximately 100-μm measured by thickness gauge.

2.2. Measurement of DS

Elemental analysis (EA) of OPC-1-5 was conducted using a vario EL elemental analyzer to analyze the C, H and O elements for the calculation of DS of OPC-1-5.