Physical interactions of cells with lyotropic liquid crystals

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Abstract

A soft substrate method using lyotropic liquid crystals has been developed to sense the cells traction forces. Lyotropic liquid crystals was determined as a biocompatible material and provide high flexibility and elasticity to transduce forces from the cytoskeleton of the cells to the liquid crystals membrane.

Introduction

Many soft substrate method such patterned PDMS, polyacrylamide and collagen sheet have been used to measure cells exertion force (K.A. Beningo, at al., 2002). However, these methods used are either lack of resolution or required pre-treatment of Extracellular Matrix Proteins in which the distribution of the proteins density could mediate the strength of cells adhesion (A. Engler, at al., 2004). A new soft substrate technique based on liquid crystals without functionalisation with ECM proteins is proposed to transduce cells adhesion and contraction forces. Lyotropic liquid crystal will function more effectively for the following two reasons: (a) Cell membranes are constructed from amphiphile molecules of lyotropic liquid crystals. Hence, liquid crystal might form a good adhesion layer. (b) Shear sensitive liquid crystals might be elastic, flexible and sensitive to external perturbations. One of the objectives in the sensor development is to investigate the physical interaction of cells with the liquid crystal membrane and in turn, showed the flexibility of the material. In order to study the cells-liquid crystals membrane interaction, an enzyme, polymerization and depolymerisation drugs would be applied to the cells and causing a response of the liquid crystals in sensing the cells physiological changes during attachment to the substrate.

Materials and methods

Biocompatible lyotropic liquid crystals (C. F. Soon, at al., 2009a) were prepared by spreading 1µl of cholesteryl ester liquid crystal (C. F. Soon, at al., 2009b) on a petri dish and the liquid crystals were immersed in 6ml of RPMI-1640 media which was supplemented with L-glutamine, Fungizone, Penicillin and Streptomycin for cell culture. Cytochalasin B (Sigma Aldrich) were diluted in 0.083% (v/v) ethanol (35mg/ml). Before the application of cytochalasin B, Human keratinocytes cell line (HaCaTs) were cultured on cholesteryl ester liquid crystal in RPMI-1640 culture media at a density of 2 x 10^4cells/ml. The RPMI-1640 media was supplemented with Fetal Calf Serum, L-glutamine, Fungizone, Penicillin and Streptomycin for cell culture. Two flask of similar cultures were prepared for the control and Cytochalasin-B treatments. Subsequently, they were incubated at 37^{0}C for 24 hours. After incubation, the cultured cells were treated with 5µl of 30µM Cytochalasin B for one hour at 37^{0}C. 0.083 (v/v)% of Ethanol was applied to the cells as control under the same culture conditions. These experiments were repeated three times. Under similar cell culture conditions, independent experiments were conducted with the application of 0.25% Trypsin-EDTA solution to a substrate cultured with HaCaTs. 1% formaldehyde that could fix the cells was applied to the cells cultured on liquid crystals at 37^{0}C to investigate the material would creep within 96 hours of treatment. Time lapsed images were taken with phase contrast microscopy for the three experiments.

Result and Discussions

HaCaT cells was found attached to the liquid crystals membrane. The adhesion layer or the liquid crystals membrane exhibited the Lyotropic smectic phase (focal conic texture) under the illumination of crossed polarising microscope (Figure 1). On addition of Cytochalasin B to one of the cultures caused a remarkable change in cell morphology and distribution of liquid crystals deformation lines in comparison with the control (Figure 2). Cytochalasin B caused the cells to shorten the deformation lines on LC (Figure 2b). Cytochalasin-B reduced the forces exerted by the cells on the liquid crystals indicating that the liquid crystal surface could be used to sense forces generated internally by actin filaments. These findings was supported by an additional experiment in which the forces induced by the cells was inhibited by the trypsin (Figure 2c) indicating the forces induced on the liquid crystals by the actin filaments were transmitted to the surface via proteins couplings, i.e., focal contacts. Cells treated with Cytochalasin B remained spread, however, cells treated with trypsin develop rounded morphology off the surface. Cells morphologies were also distinctly different in both treatments. This correlated well with the known findings that adhesion proteins on the cells membrane must be first established during a cell attachment and further strengthened by the cytoskeletons (A. Bershadsky, et al., 2003). After 24

hours of treatment with 1% formaldehyde, a drop of deformation length was observed on most of the deformation lines induced. There were evidences (L. Paljarvi at el., 1979 and C.H. Fox at el., 1985) in literature that the cells continue to shrink up to 16 hours after fixation in formaldehyde. Therefore, shrinkage of cells has caused shortening of deformation lines within 24 hours of formaldehyde treatment. The measurements were as presented in Figure 3. The cells maintained the deformations through out the 24-72 hours treatment but deformation lines decreased slightly after 72 hours (Figure 3). A decrease in deformation lines after 72 hours might be due to the deterioration of the biological material even though the cells stress was fixed or it could be due to the cells were fixed in a lower concentration of formaldehyde.

CONCLUSIONS

This work has provided a clear indication that HaCaTs adhesion and spreading on the lyotropic liquid crystals were facilitated by adhesion proteins and forces generated by cells to the liquid crystals were associated with the forces generated by cytoskeleton. Creep effects on the liquid crystals membrane were not found. Future work is to determine the group of integrins and focal adhesions involved.

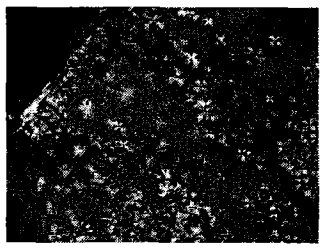

Figure 1 Lyotpropic liquid crystals ripping of the bulk lyotropic liquid crystals imaged with crossed polarising microscopy. (Scale bar: 25μm)

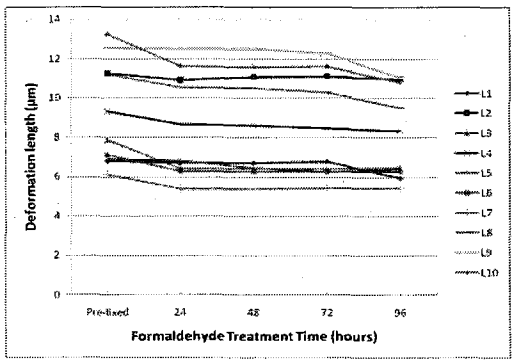

Figure 3 Deformation length due to the contraction of cells after treatment with 1% formaldehyde for 4 days.

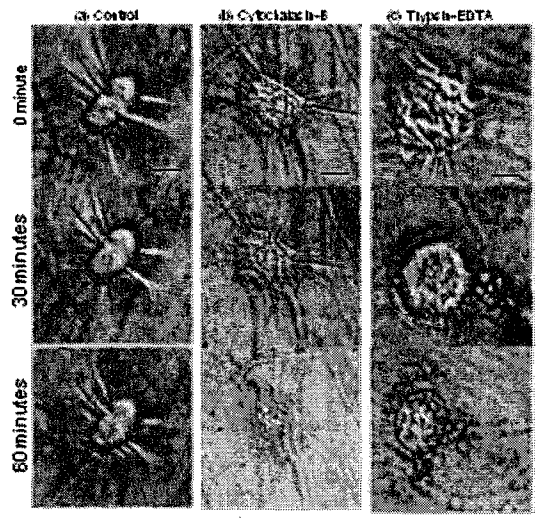

Figure 2 Application of single dose (a) 0.083% Ethanol (Control), (b) 30μM Cytochalasin B in 0.083% Ethanol, (c) 0.25% Trypsin-EDTA solution on HaCaTs adhered on lyotropic liquid crystals in 60minutes treatment. (Scale bar: 25μm)

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