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Biophysical characteristics of cells cultured on cholesteryl ester liquid crystals

Chin Fhong Soon[a,d,*], Wan Ibtisam Wan Omar[a], Rebecca F. Berends[d], Nafarizal Nayan[a], Hatijah Basri[a], Kian Sek Tee[a], Mansour Youseffi[b], Nick Blagden[c], Morgan Clive Thomas Denyer[d]

[a] Biosensor and Bioengineering Laboratory, MiNT-SRC Research Center, Universiti Tun Hussein Onn Malaysia, 86400 Parit Raja, Batu Pahat, Johor, Malaysia
[b] School of Engineering, Design and Technology-Medical Engineering, University of Bradford, Bradford BD7 1DP, United Kingdom
[c] Lincoln School of Pharmacy, University of Lincoln, Brayford Pool, Lincoln, Lincolnshire LN6 7TS, United Kingdom
[d] School of Medical Sciences, University of Bradford, Bradford BD7 1DP, United Kingdom

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ABSTRACT

This study aimed at examining the biophysical characteristics of human derived keratinocytes (HaCaT) cultured on cholesteryl ester liquid crystals (CELC). CELC was previously shown to improve sensitivity in sensing cell contractions. Characteristics of the cell integrin expressions and presence of extracellular matrix (ECM) proteins on the liquid crystals were interrogated using various immunocytochemical techniques. The investigation was followed by characterization of the chemical properties of the liquid crystals (LC) after immersion in cell culture media using Fourier transform infrared spectroscopy (FTIR). The surface morphology of cells adhered to the LC was studied using atomic force microscopy (AFM). Consistent with the expressions of the integrins α2, α3 and β1, extracellular matrix proteins (laminin, collagen type IV and fibronectin) were found secreted by the HaCaT onto CELC and these proteins were also secreted by cells cultured on the glass substrates. FTIR analysis of the LC revealed the existence of spectrum assigned to cholesterol and ester moieties that are essential compounds for the metabolizing activities of keratinocytes. The immunostainings indicated that cell adhesion on the LC is mediated by self-secreted ECM proteins. As revealed by the AFM imaging, the constraint in cell membrane spread on the LC leads to the increase in cell surface roughness and thickness of cell membrane. The biophysical expressions of cells on biocompatible CELC suggested that CELC could be a new class of biological relevant material.

1. Introduction

Exploring new potentials of liquid crystals in bioengineering and biosensing is attracting increasing research interest. A number of these studies have examined interactions of biological entities with liquid crystals (Brake et al., 2003; Lockwood et al., 2006; Woolverton et al., 2005). The work presented in Lockwood et al. (2006) demonstrated the application of nematic liquid crystals in imaging reorganization of cells. Based on our recent work reported in Soon et al. (2013a), we discovered that cholesteryl ester liquid crystals (CELC) allow the adhesion of cells and they are sensitive to the physical restructuring of single cells. The application of the liquid crystals could be extended as a new class of bio-physical relevant adhesion substrate which do not require pre-conditioning with ligands. In the context of biocompatibility, CELC has been shown to be thermally stable between 20 °C and 50 °C, non-toxic and capable of providing affinity for cell attachment (Soon et al., 2009, 2011). In many respects, liquid crystals are biomimetic material sharing characteristics with the phospholipid bilayer (Guille et al., 2005; Small, 1977). In addition, cholesterol moieties were positively described with several attractive advantages in enhancing the physical properties of cells during attachment and proliferations (Hwang et al., 2002). However, the chemical property of the CELC used in cell culture remains unknown. In this work, the elements in the CELC were examined using Fourier transform infrared spectroscopy (FTIR) and cross-polarized microscopy. Although new applications of liquid crystals have been unveiled, little is known about the expressions of surface proteins and effects onto morphological changes due to the interactions with the liquid crystals. Driven by the positive reports of CELC in supporting cell adhesion, it is necessary to further investigate the biophysical characteristics of cells after interaction with CELC. In this paper, the integrins and extracellular matrix (ECM) protein expressions for the adherent cells on the liquid crystals

* Corresponding author at: Biosensor and Bioengineering Laboratory, MiNT-SRC Research Center, Universiti Tun Hussein Onn Malaysia, 86400 Parit Raja, Batu Pahat, Johor, Malaysia. Tel.: +60 4538614; fax: +60 4536060.
E-mail addresses: fionasoon@gmail.com, soon@uthm.edu.my (C.F. Soon).

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using various immunostaining techniques were studied. In order to study cell responses on the liquid crystals, the morphological effects and adhesion characteristics of cells on the soft substrate were investigated using atomic force microscopy (AFM).

2. Materials and methods

2.1. Cell culture

Human keratinocyte cell lines were purchased from cell line services (CLS, Germany). The cells were maintained in 25 cm^2 tissue-treated culture flask containing Roswell Park Memorial Institute-1640 (RPMI-1640, Sigma–Aldrich, UK) culture media supplemented with foetal calf serum (PromoCell, UK), L-glutamine (2 mM, Sigma–Aldrich, UK), fungizone (2.5 mg/l, Sigma–Aldrich, UK), penicillin (100 units/ml, Sigma–Aldrich, UK) and streptomycin (100 mg/ml, Sigma–Aldrich, UK). Upon reaching confluency, the media was removed from the cell culture flask and the flask was washed three times with Hanks Balance Salt Solution (HBSS). After removing the HBSS solution, 1 ml of trypsin (0.5 mg/ml) was deposited into the flask and the flask was incubated in a humidified 5% CO_2 atmosphere for about 6 minutes. Subsequently, the flask was examined under a phase contrast microscope (Nikon T100) to ensure that all the cells were detached from the surface of the culture flask. 5 ml of RPMI 1640 Medium were then deposited to halt the trypsinization process. Subsequently, the cell suspensions were transferred to a centrifuge tube and centrifuged for 5 min at 1600 rpm. When this process was completed, supernatant were discarded and the cells were re-suspended in 6 ml of culture medium. Eventually, the cell suspension was ready for the subsequent experiments.

2.2. Preparation of liquid crystal substrates

Cholesteryl ester liquid crystals were synthesized from a mixture of cholesteryl chloride (25 wt% of $C_{27}H_{45}Cl$), Cholesteryl Pelargonate (38 wt% of $C_{36}H_{62}O_2$) and Cholesteryl Oleyl Carbonate (38 wt% of $C_{46}H_{80}O_3$). All cholesteryl liquid crystals were purchased from Sigma–Aldrich. The melting points for these liquid crystals are 94–96 °C, 74–77 °C and 118 °C, respectively. The crystalline or solid mixtures of CELC in vials were heated up to the melting temperatures on a hot stage. The solid mixtures turned into a turbid fluidic media at approximately 70 °C and then became isotropic (clear yellowish liquid) at the highest melting temperature, 118 °C. Whilst CELC was in the isotropic phase, 5 μl of the fluid mixtures was spread at a thickness of approximately 100 μm on glass cover slips using a cell scraper (Corning Incorporation). The thickness of the liquid crystal coating was controlled by using thin gold grids with a thickness of 100 μm (Agar Scientific, UK) and confirmed by using a microscopy focusing technique. Liquid crystal substrates at a thickness (t) of ~100 μm were prepared and each placed in a petri dish ready for the following experiments.

2.3. Immunofluorescence staining of integrins

HaCaT cells were prepared as described previously and plated in two petri dishes, each containing a plain glass cover slip and a glass cover slip coated with liquid crystals at a density of 1.2×10^3 cells/cm^2. A negative control was prepared by seeding similar cell density on a glass cover slip. After approximately 3 days when the cells had reached sub-confluency, the glass cover slips were removed from the petri dishes and washed twice with Hanks Balanced Salt Solution (HBSS, Sigma–Aldrich). Sub-confluent cells were fixed in 1% formaldehyde for 6 min and permeabilized in 0.1% Triton X-100 for 3 min. In the staining procedure, cells were rinsed and treated with a blocking solution. For integrins receptor staining, the cells were bathed with 2% of bovine serum albumin (BSA, Sigma–Aldrich, UK) and 2% goat serum (Sigma–Aldrich, UK), respectively. After blocking, cells were washed three times in HBSS and incubated in 50 μl of primary antibody for 24 h in a humidity chamber at 5 °C. The procedures were repeated except for the primary antibody for the negative control. After blocking, cells were washed three times in HBSS and incubated in 50 μl of primary antibody for 24 h in a humidity chamber at 5 °C. The procedures were repeated except that the primary antibody was omitted but substituted with HBSS for the negative controls. The primary antibodies used to stain the integrins (α2, α3, α5 and β1) were primary mouse monoclonal antibodies raised against CD49b (1:250 in 1% BSA for α2), CD49c (1:250 in 1% BSA for α3), CD49e (1:5000 in 1% BSA for α5) and CD29 (1:2500 in 1% BSA for β1). All primary antibodies were obtained from Biosciences Incorporated. These experiments were repeated three times. After incubation for 24 h, the substrates were washed three times with HBSS, blotted and incubated with goat anti-mouse Immunoglobulin (IgG) secondary antibody labelled with Alexor Fluor 488 (5 μg/ml diluted in 1% BSA, Sigma–Aldrich, UK) for one hour. After staining, the substrates were subjected to three five minutes washes in HBSS before mounting onto the glass slides with DAPI dihydrochloride (0.1 μg/ml diluted in HBSS). These experiments were repeated three times.

All the immunofluorescence staining were observed using a Nikon Eclipse 80i fluorescence microscopy under dark field (DF) at 40× magnification and images were captured with ACT-2u software. The exposure time of the DF imaging for the blue (nuclei) and green (integrins) staining was fixed at 8 s and 15 s, respectively. Blue and green staining images were digitally merged using ImageJ software.

2.4. Laminin staining

A sterilized cover slip with LC and without LC coating each was placed in a petri dish and seeded with cells at a density of 1.3×10^3 cells/cm^2. A negative control was prepared by culturing similar cell density on a glass cover slip. After reaching sub-confluency, the cells were washed, fixed and blotted as described earlier. All the reagents used were from a laminin staining kit (Sigma–Aldrich). 3% hydrogen peroxide was applied to the cells plated on a glass substrate for 5 min, followed by two washes and blotting. Then, the cells were incubated with 2% BSA for 10 min and the excess reagent was discarded. Subsequently, the substrate was incubated with rabbit anti laminin primary antibody for 60 min. In a negative control, this staining for rabbit anti laminin primary antibody was not undertaken. After incubation, the cover slip was washed, blotted and treated with a biotin conjugated goat anti rabbit IgG secondary antibody for 20 min. Again, the cells were washed, blotted and treated with peroxidase reagent. After a short incubation of 20 min, a mixture of substrate reagents was added to the cover slip for 20 min. The mixture contained 4 ml of distilled water, 100 μl of acetate buffer, 5 μl of AEC chromogen and 3% hydrogen peroxidase. After the final incubation, the cover slip was rinsed in HBSS for 5 min and photographed using a Nikon-100 inverted phase contrast microscope and the associated digital camera. Same procedures were repeated for staining cells seeded on the liquid crystal coated substrate and both experiments were repeated three times.

2.5. Immunoperoxidase staining of collagen type IV

Cells and liquid crystal substrates were prepared as described in the procedures used in staining the immunoperoxidase staining of laminin. A sterilized glass cover slip with LC and two glass cover slips without LCs (control and negative control) were placed in three separate petri dishes and each seeded with cells at a density of 1.5×10^3 cells/cm^2. After incubation at 37 °C for 24 h,

the cells were washed, fixed and blotted. After blotting, the cells were blocked with 2% BSA for 10 min and the cover slips were blotted. Then, the cells on all the cover slips except the negative control were incubated with monoclonal anti-collagen type IV antibody raised in mouse (1:400, Sigma–Aldrich, UK) for 60 min. The cells were then washed, blotted and followed by treatment in anti-mouse IgG peroxidase conjugate antibody developed in goat (1:2000, Sigma–Aldrich, UK) for 20 min. Then, the cells were washed, blotted and incubated with 2 drops of peroxidase reagent (IMMH7, Sigma–Aldrich, UK) for 20 min. Subsequently, a mixture of substrate reagents was added to the cover slips for 20 min and washed with HBSS for 5 min. The cover slips were photographed using Nikon-100 inverted phase contrast microscope at 10× magnification. Similar procedures were repeated three times for cells cultured on LC coated substrates, controls and negative controls. These experiments were repeated three times.

2.6. Immunofluorescence staining of fibronectin

For the immuno-staining of fibronectin, HaCaT cells were cultured on a plain glass cover slip coated with liquid crystals and two plain glass cover slips (control and negative control) using similar procedures described for the immuno-staining of integrins. After incubation at 37 °C for 24 h, sub-confluent cells were washed, fixed and permeabilized as described previously. Subsequently, cells were rinsed and blocked with a 2% goat serum solution. After blocking, cells were washed three times in HBSS and incubated in 50 μl of mouse anti-fibronectin primary antibody (Biosciences Incorporated) for 24 h in a humidity chamber at 5 °C. The substrates were washed three times with HBSS, blotted and incubated with goat anti-mouse IgG secondary antibody labelled with Alexor Fluor 488 (5 μg/ml diluted in 1% BSA) for one hour. After staining, the substrate was subjected to three five minutes washes in HBSS before mounting onto the glass slides with DAPI dihydrochloride (0.1 μg/ml diluted in HBSS). These experiments were repeated three times.

2.7. Preparation of liquid crystal substrate for polarizing microscopy

CELC coated substrates were prepared according to the procedures described previously. The glass cover slip coated with CELC were immersed in a petri dish containing 6 ml of RPMI-1640 culture media (Sigma–Aldrich, UK) and this resulted in the formation of lyotropic liquid crystals (Soon et al., 2011). Presumably, cholesteryl ester liquid crystal has a chemical structure of a cholesterol that consists of a hydrophilic head except that the hydrophobic tail is an ester bond instead of a hydroxyl group ($^-$OH), while lyotropic liquid crystals are a class of liquid crystals that form in the presence of a solvent (Small, 1977). The surface of the CELC after interaction with the cell culture media was studied using a Zeiss Axioplan2 cross-polarizing microscope. A digital camera attached to the microscope and controlled by the AxioVision version 4.6 software was used to record the photomicrographs of the liquid crystalline phases.

2.8. Liquid crystal sample preparation and FTIR

A vial containing 1 ml of CELC with an addition of 5 ml RPMI-1640 media and a control sample (without culture media) were incubated in a humidified 5% CO_2 incubator at 37 °C for 24 h. After incubation, a pipette was used to remove samples from the surface of the liquid crystals interacted with the cell culture media. Having the LC sample sandwiched in between a pair of circular cell windows (CAF_2 disks), FTIR spectrum of the LC was obtained in a Perkin Elmer Spectrum 2000 spectrophotometer over a range of wavenumbers from 4000 to 600 cm^{-1}. The acquisition of the data was performed in 16 scans and the resolution in wave number was set at 4 cm^{-1} in the Spectrum software. Similar experiments were repeated three times for the samples incubated in the cell culture media.

2.9. Characterizing cell surface with atomic force microscopy

HaCaT cells were prepared as described earlier in this paper and these cells were cultured in a Petri dish each containing a glass and liquid crystal coated substrates at a cell density of 1.3×10^3 cells/cm^2. At sub-confluency, both substrates were removed and rinsed with HBSS. The cells cultured on both substrates were fixed with 1% formaldehyde for 6 min followed by serial alcohol dehydration at 25%, 50%, 75% and 90%. After the final alcohol dehydration, the cells were left air dry in a ESCO streamline biological safety cabinet. An atomic force microscope (AFM, Park System XE-100) was used to study the surface morphology of cells cultured on plain glass and liquid crystal coated glass substrates. Under tapping mode, the cells were scanned with a non-contact silicon cantilevers with a radius of 10 nm. At a scan rate of 0.5 Hz, the boundary region of the adherent cells were scanned at a maximum area of 40 μm × 40 μm in which, the scanning head of the AFM can handle. AFM imaging was repeated for each randomly selected cell from three independent experiments with and without the presence of liquid crystals (refers to Supplementary material). The line profiles of the cell membrane in the AFM images were obtained via the data acquisition programme (XE software) which was linked to the AFM system. Cell thickness was measured from ten randomly selected locations of each cell membrane via the XE software and expressed as mean ± standard deviation (SD). To compare the means of cell membrane thickness, *t*-test analysis was performed in the Statistical Package for Social Sciences (SPSS statistic version 20) software. Differences in the means were significant at $p < 0.05$.

3. Results and discussion

The biophysical characteristics of human keratinocytes on liquid crystals from the perspective of the integrins associated with cell attachment have been determined. The objective of integrin staining experiments is to investigate if the same suites of integrins and ECM proteins are expressed by the cells cultured on the liquid crystal substrates when compared with those cells cultured on plain glass substrates. For cells cultured on the liquid crystals, the immuno-stained micrographs of α2, α3, α5 and β1 integrins (Fig. 1c, f, i, l) seem to be similar to the adherent cells cultured on controls (plain glass substrates) (Fig. 1b, e, h, k) in comparison with the negative controls (plain glass substrates treated without primary antibody) (Fig. 1a, d, g, j). Similar patchy and diffused appearances of integrins were observed for the cells cultured on control and LC coated substrates (Fig. 1). In this work, synthetic ECM proteins were not applied to the cultures of cells on the plain glass and LC coated substrates. However, Fig. 2 shows that collagen type IV, laminin and fibronectin proteins are found present on both glass and liquid crystal substrates. This indicates that cells cultured on both substrates secreted collagen type IV, laminin and fibronectin.

Heterodimers α2β1 and α3β1 are integrins used by HaCaT cells to bind to collagen type IV and laminin (O'Toole, 2001; Kainulainen et al., 1998; Kirfel and Herzog, 2004) while α5β1 recognizes fibronectin (Kainulainen et al., 1998; Larjava and Oikarinen, 1998). These three groups of integrins (α2β1, α3β1 and α5β1) are found in cells cultured on control (Fig. 1b, e, h, k) and LC substrates (Fig. 1c, f, i, l). The results show that β1 integrin expressions are commonly present in the sub-confluent cells on both glass and liquid crystal substrates. In fact, integrin β1 is usually co-localized

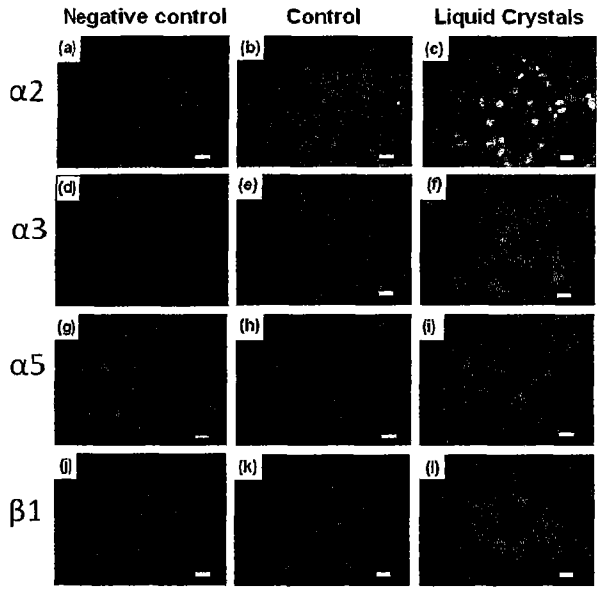

Fig. 1. Micrographs of immunocytochemical staining against α2, α3, α5, β1, fibronectin and laminin for (a, d, g, j) negative controls, HaCaT cells cultured on (b, e, h, k) glass cover slips and (c, f, i, l) liquid crystal substrates, respectively. (scale bar = 25 μm) (enlarged exert, scale bar = 25 μm).

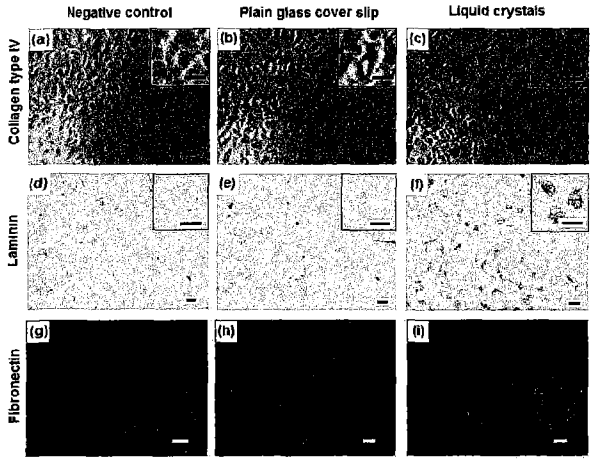

Fig. 2. Micrographs of immuno-staining against collagen type IV, laminin and fibronectin for (a, d, g) negative controls, HaCaT cells cultured on (b, e, f) glass cover slips and (c, f, i) liquid crystal substrates, respectively (scale bar = 25 μm) (enlarged exert, scale bar = 25 μm).

with α integrins sub-unit corresponding to the type of ECM proteins present (O'Toole, 2001; Kirfel and Herzog, 2004; Burridge et al., 1997). Our result shows that culturing HaCaT cells on liquid crystals stimulated α3 integrins expressions and laminin depositions. α3β1 is one of the specific receptors that bind to the laminin (DeHart et al., 2003). α3 integrin expression on the LC substrate in response to the laminin deposition has promoted affinity for cell attachment, which in turn, enabled the formation of stable adhesion together with the support from the cytoskeleton (Nguyen et al., 2000; O'Toole, 2001). Although there might be some slight differences in the staining intensity of integrins and ECM proteins as indicated in the immuno-stained micrographs, however, further quantifications using enzyme-linked immunosorbent assay (ELISA) and western blot analysis require extensive work which is beyond the scope of this work. In this work, synthetic ECM proteins were not applied to the cultures of cells on the plain glass and LC coated substrates. However, the results show that collagen type IV, laminin and fibronectin proteins are found in the cells cultured on both glass and liquid crystal substrates. This indicates that cells cultured on both substrates secreted the three main ECM proteins hence, forming a provisional basement membrane in the in vitro culture. Deposition of laminin over exposed collagen is required in the formation of basement membrane (Frank and Carter, 2004). This enabled HaCaT cells to attach to the LC surface via the naturally produced ECM proteins. In this study, cells on soft liquid crystal layer may have secreted sufficient ECM proteins to optimize their attachment to the liquid crystal substrate. Via the naturally produced ECM proteins in vitro, this might have made the liquid crystals biologically viable. Results in this study showed consistently with other studies (Li et al., 2004; Stanley et al., 1982a,b) that keratinocytes are capable to generate self-derived ECM proteins in vitro.

After the CELC were immersed in the cell culture media, a translucent film was seen overlying the surface of the CELC and this film is the adhesion layer where the keratinocytes attached to (Fig. 3a). In the cross-polarized microscopy, we found a similar film formed on top of the cholesteric liquid crystals substrate characterized by oily streaks and this film exhibited smectic phase (Fig. 3b). The liquid crystal film appears dark with a few white bands of streaks and some dark focal conic texture in cross-polarized microscopy (Fig. 3b) which showed that this lipid characterized a mixture of smectic and cholesteric phase. This is due to the multiple layers of water molecules interlacing the smectic phase of the lyotropic liquid crystals and they appeared as streaks when viewed down the cross-polarizer (Asher and Pershan, 1979). This film known as the lamellar mesophase consists of bilayers of lipid molecules in high water content and they are uniform under defect free condition (Hyde, 2001). The deposition of endogenous ECM molecules on the LC would require the cells to have an affinity to the substrate. This study showed that CELC after immersion in media formed lyotropic layers associated with the amphiphilic molecules re-orientation, such that the hydrophobic head (cholesterol entity) orientated towards the water and hydrophobic hydrocarbon chain (ester bond) orientated to the bulk layer. This self-assembly mechanism transforms the polarized LC into the uni-lamellar or multi-lamellar layer interlaced by water molecules in a lyotropic system (Asher and Pershan, 1979). The binary system (water-lipid interface) has been described as a translucent layer, basically showing fluid like or isotropic properties (Munoz and Alfaro, 2000) and this is in good agreement with our observation (Fig. 3) in which the lamellar molecules have self-organized into an ordered system where the hydrophilic layer forms an adhesion substrate to the keratinocytes. The fact that the cell membrane consists of lyotropic liquid crystals distributed with cholesterol moieties may explain the affinity of cells for CELC.

In addition to the polarizing microscopy, Fourier transform infrared spectroscopy of CELC was performed to determine which chemical properties of the CELC enabled cell attachment. This experiment is important to determine whether incubation in culture media modified those chemical properties of liquid crystals and hence, enhanced cell attachment. Fig. 4 shows the FTIR spectrum for the control and three samples of CELC incubated in cell culture media at 37 °C. For this experiment, the results showed that CELC samples have no significant difference with control. A peak broadly spread from 3600 down to 3100 cm^{-1} was found for all the spectrums indicating the attribute of hydrogen bonded hydroxyl (—OH) group presents in the CELC. For the three spectrums obtained, strong absorption was found at wavenumbers 2916 and 2850 cm^{-1} corresponding to the C—H stretch. The carbonyl group (C=O) correspond to lipid ester was found at 1737 cm^{-1}. The absorption shown at 1465 and 1375 cm^{-1} are peaks of C=C

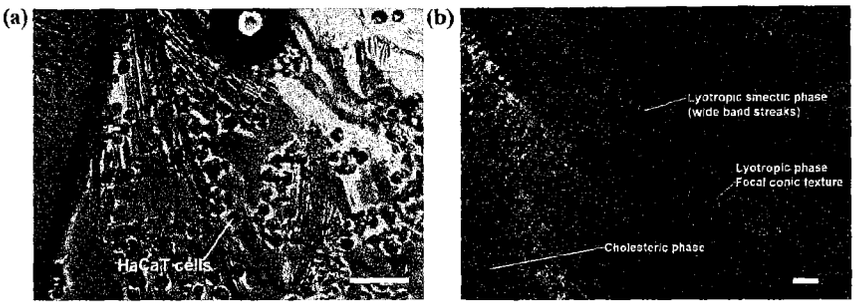

Fig. 3. (a) A phase contrast and (b) cross-polarizing micrographs of cholesteric based lyotropic liquid crystals. The translucent thin film with attachment of cells is flipping over the bulk liquid crystals in (a).

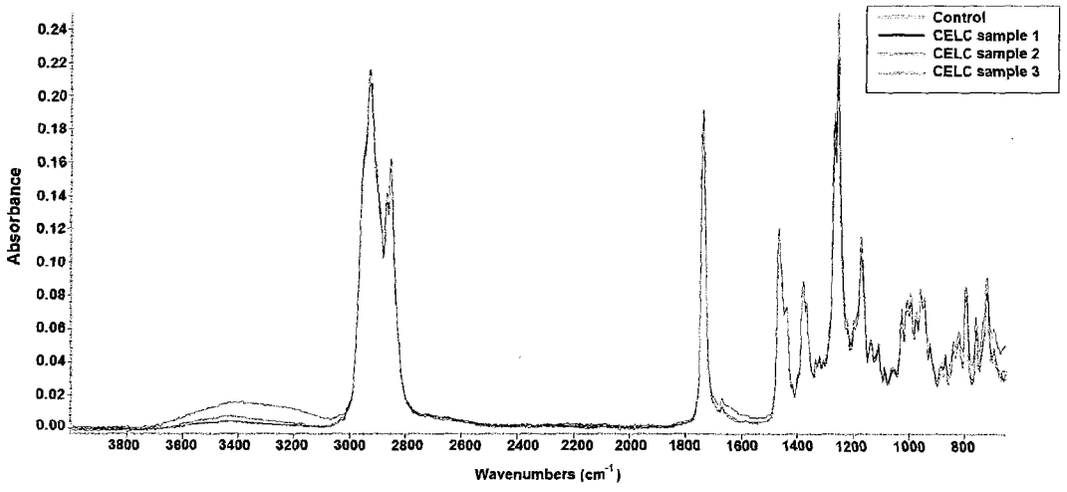

Fig. 4. The FTIR spectrum of cholesteryl ester liquid crystals.

stretches of aromatic ring. The absorption band below 1300 cm^{-1} consisting of high oscillation of spectrum is the typical fingerprint region for the CELC. Cholesteryl ester liquid crystal characterized by the cholesterol and lipid ester moieties may be simulating closely the situation in vivo. In the biological system, cholesteryl ester is a long-chain fatty acids derived from the tissue phospholipids (Ziboh and Dreize, 1975) that is predominantly found in stratum corneum (Unna and Goldsdetz, 1910). Cholesteryl esters are usually contained in the intracellular lipid droplets. When cholesterol is required by the cell membrane and lipoprotein formation, cholesterol ester can be hydrolysed in cells to liberate cholesterol. This cholesterol is essential for cell membrane formation and stabilizing bilayer lipid membrane (Feingold, 2007). In the skin, cholesteryl esters occupy up to 20 wt% of the extracellular space of the stratum corneum (Norlen et al., 2007). Cholesterol esters function as enhancers for transdermal delivery as demonstrated in (Kravchenko et al., 2011). Esterification of cholesterol is also known to be associated with the keratinization of epidermis (Unna and Goldsdetz, 1910). The culturing of human keratinocytes cells on the liquid crystals dramatically increased the lipid moieties in the culture and thus, creating an adhesion layer preferred by cell adhesion. Swelling of the lipid bilayers which is a transition into a liquid crystalline structure or lamellar matrix could occur at the stratum corneum due to chemical gradients of water (Norlen et al., 2007). These lamellar structures described for cholesteryl ester (Engelman and Humlan, 1976) are very similar to our observation for the CELC presented in Fig. 3b.

As indicated in the AFM micrographs (Fig. 5), the cells cultured on plain glass expressed flattened and extended lamellipodia with a thickness of 38 ± 8 μm ($p = 0$, $N = 30$) which is significantly different from the cells cultured on the liquid crystals. These cells expressed retracted cell membranes with a thickness of 99 ± 21 μm ($p = 0$, $N = 30$). The AFM images (Fig. 5a and b) indicate that the adherent cells on the glass were characterized with smoother surface as compared with the high roughness over the entire surface of lamellipodia cultured on the liquid crystals. For the cells culture on plain glass, only a small region of cell surface adjacent to the cell nucleus was relative uneven (Fig. 5a). In the line profiles obtained from the images of the cell membrane (Fig. 5c), larger oscillations were observed for thus, elucidating the high surface roughness of cells cultured on liquid crystals as compared to those cultured on plain glass. The high surface roughness seemed to be an effect of cell membrane retraction and having a strong relationship with the microenvironment changes. Adherent keratinocytes cultured on the plain glass usually take on a broadly spread morphology. Contrarily, a glass surface coated with liquid crystals could stimulate the cells to reduce their adhesion area while retaining adhesion to the liquid crystal substrate (Soon et al., 2013b). Our previous studies (Soon et al., 2013a,b) showed that cell adhered to liquid crystals were characterized by diffused actin filament, organized focal adhesion and well-structured interfacial topology of cell adhesion. The current study with AFM indicates that cells reduced their adhesion area by contracting the cell membrane leading to the increased of cell surface roughness which is analogous to a window

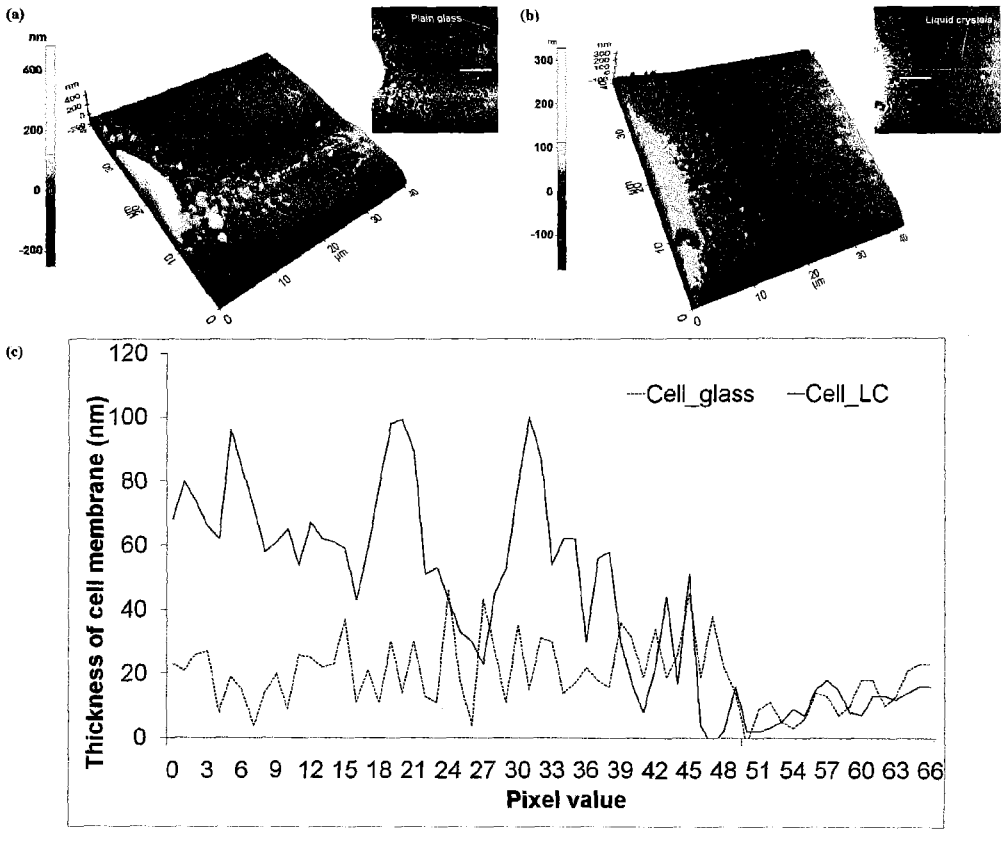

Fig. 5. AFM images for keratinocytes cultured on a (a) plain glass and (b) liquid crystal substrates. (c) Line profiles of cells cultured on plain glass and liquid crystal substrate. These profiles were taken from the yellow lines as indicated in the insets of (a) and (b), respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

blind being folded and pulled back. The stiffness of liquid crystals seemed to play a role in regulating the cell membrane spread and this indicates that cell spreading is coordinated by mechanosensing and mechano-transduction system involving the integrins and cell surface adhesion proteins.

4. Conclusions

This study proved the adhesion of cells to the liquid crystals was mediated by the binding of cell surface integrins to the extracellular matrix proteins that are self-derived by the cells. The lyotropic phase and cholesteryl ester compound of the liquid crystals mimics the in vivo system may be the factor attracting the affinity of cells. The surface roughness of the cells increased and this occurred concurrently with the retraction of cell membrane when the cells were cultured on the liquid crystals. The biophysical behaviour of cells cultured on the liquid crystals without the need of pre-coating with ligands showed that cholesteryl ester liquid crystals could be a new class of biological relevant material to functionalize a surface.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.micron.2013.10.011.

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