

Influence of Time Dependent Factors To The Phases and Poisson's Ratio of Cholesteryl Ester liquid crystals

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Abstract

Liquid crystal based biosensors detect physical restructuring of cells via changes in the birefringence property or orientation order of the liquid crystal molecules. The strain induced in the liquid crystal molecules is closely associated with the liquid crystalline phases and Poisson's ratio of the liquid crystals. Cholesteryl ester liquid crystals (CELC) and CELC based lyotropic liquid crystals (LLC) that were regarded as viscoelastic materials are subjected to time-dependent factors (a fluidic environment, incubation time, and temperature). The effects of these factors, qualitatively and quantitatively, were investigated by using cross-polarising microscopy and a uniaxial micro-tensile technique. More lyotropic phases were observed in the liquid crystals as the incubation time in cell culture media was increased. Poisson's ratio of approximately 0.58 and 0.6 were determined for CELC and LLC, respectively, at room (25 °C) and incubation (37 °C) temperatures over a period of 3 days.

Keywords: Time dependent, Poisson's ratio, liquid crystalline phases, strain

1. INTRODUCTION

Emerging research involving liquid crystals as a label-free biosensor are increasing because of their potentials in bio-chemical analysis [1-4]. The liquid crystal based biosensors that have been developed to date respond to cell proliferation, adhesion and differentiation via changes in birefringence is an indication of the deformation or displacements of the liquid crystal molecules [3, 5]. Recently, the biocompatible cholesteryl ester based lyotropic liquid crystal (LLC) [6] has been shown to possess the flexibility and elasticity to enable the sensing of cell derived dynamic mechanical activities via the generation of localised deformation lines [7]. Similar soft substrate techniques based on deformations formed in the polymers that were induced by cells have provided a means in studying cell adhesion, contraction, cytokinesis and cell signaling [8-11]. However, semi-solid liquid crystals based cell force transducer has shown a higher spatial resolution and flexibility in sensing cell mechano-physiological changes than previously available systems [12]. Based on the deformation lines induced by single cells, the exerted cell forces were quantitatively modelled by using finite element method with an assumption that the Poisson's ratio of viscoelastic CELC is close to that of the rubber at 0.5 [13].

As one of the elastic constant, Poisson's ratio, ν , is defined as negative ratio between the longitudinal strain to the transverse strain under a tensile or compressive loading [14]. An isotropic material has Poisson's ratio bounded by $-1 < \nu < 0.5$ but may not be relevant to anisotropic material [15]. Cholesteryl ester liquid crystal which exhibits cholesteric phase is anisotropic [16-18] and the normal definition of Poisson's ratio for isotropic material may not be suitable. In distinct contrast to the isotropic material, anisotropic solids such as cubic solids could demonstrate Poisson's ratios with arbitrary large negative and positives values [19-21]. Nonetheless, the infinitesimal deformations in mutual directions for the viscoelastic material are probably related to the time based functions [22], and therefore the Poisson's ratio is defined by [23] as

$$\nu_{lt}(n, t) = -\frac{\varepsilon_{ll}(n, t)}{\varepsilon_{tt}(n, t)} \quad \text{for } tt \neq ll \quad (1)$$

where $\nu_{lt}(n, t)$ is the time dependent (t) Poisson's ratio in a Cartesian coordinate system, n. $\varepsilon_{ll}(n, t)$ and $\varepsilon_{tt}(n, t)$ is the time dependent lateral strain

and the transverse strain in a constant magnitude, respectively. A more comprehensive and detail considerations for Poisson's ratio of viscoelastic materials proposed by [23, 24] suggest that the Poisson's ratio for viscoelastic materials is not only dependent on the compliance of the material but also on the time, time history and thermal expansion. Our rheological result showed that viscoelastic properties of the lyotropic liquid crystals gradually decreased as the incubation time increased from 24 to 72 hours [25]. These studies suggested that the time dependent factors such as cell culture media, culture incubation time and temperature may affect the Poisson's ratio and the phases of the liquid crystals. Therefore, the liquid crystal phases of the liquid crystals after immersion in cell culture media for a period of 3 days will be studied by using cross-polarising microscopy, in which, the phases displayed may indicate the changes in the compliance of the liquid crystals. To study the influences of the time dependent factors to the Poisson's ratio, a custom built micro-tensile measurement instrument incorporated with a microscopic technique will be described and used in determining the Poisson's ratio of CELC. Polydimethylsiloxane (PDMS) with a well-established Poisson's ratio will be used to calibrate the system developed. Similar tensile test techniques have been used in determining the Poisson's ratio of liquid crystal elastomers [26, 27] and Polyacrylamide gel [28]. However, these studies did not include the time dependent factors. In our uniaxial tensile tests by using a custom made and bespoke micro-tensile tester, the Poisson's ratio of the CELC and LLC at room and incubation temperatures under isothermal conditions will be considered because a report by [23] suggested that isothermal uniaxial stress leads to constant measurements of Poisson's ratio.

2. MATERIALS AND METHODS

2.1 Preparation of cholesteryl esters and lyotropic liquid crystals

Ester based cholesteryl liquid crystal compounds were mixed to synthesise a shear sensitive cholesteryl liquid crystal (CELC) which has provided stability below the melting temperature [29]. The cholesteryl ester liquid crystals used consisted of Cholesteryl Chloride $C_{27}H_{45}Cl$, Cholesteryl Perlagonate $C_{36}H_{62}O_2$ and Cholesteryl Oleyl Carbonate $C_{46}H_{80}O_3$ (Sigma Aldrich, UK). The mixtures termed CELC were formulated with 25 % of $C_{27}H_{45}Cl$, 38 % of $C_{36}H_{62}O_2$ and 38 % of $C_{46}H_{80}O_3$. One gram of these mixed compounds was melted to their isotropic phase at a temperature range of 55 oC to 75 oC in a glass vial [6]. Using a cell scraper (Corning Incorporation), 10 μ l of the liquid crystal gel in isotropic phase was spread on a glass cover slip at a thickness of about 100 μ m, re-melted on a hot stage and spin-coated at 1200 rpm. Five liquid crystal (LC)

substrates were prepared using similar procedures and placed separately in five petri dishes. They were cooled to their cholesteric phase at room temperature (25 °C). Subsequently, 6 ml of Rosewell Park Memorial Institute (RPMI)-1640 cell culture media (Sigma Aldrich, UK) was added to four of the petri dishes containing the liquid crystal substrates (LLC) and one (CELC) was left without an addition of cell culture media. A petri dish containing substrates with CELC were incubated with and without culture media at room temperature (25 °C) for 24 hours. The other three liquid crystal samples containing in the cell culture media (LLC) were incubated at 37 °C for 24 hours, 48 hours and 72 hours, respectively.

2.2 Cross-polarising microscopy

After the designated incubation periods, the samples were removed from the petri dishes and placed on a Linkam THM600 hot stage, in which, the hot stage was controlled by Linkam control plug-in in AxioVision software. This hot stage was synchronised with a Zeiss AxioCam MRc5 camera fitted to an AxioPlan2 polarising optical microscope, in which, the liquid crystalline phases of the liquid crystal samples were studied. Whilst the LC samples were under the illumination of the cross-polarising microscopy at 40x magnification, the temperature of the hot stage was increased at a rate of 1 °C/min and maintained at the set temperature (25 °C or 37 °C). The liquid crystalline phases of the samples were captured using AxioVision version 4.6 software bundled to the camera.

2.3 The calibration and measurements for the Poisson's ratio of PDMS and cholesteryl esters liquid crystals

For measuring the Poisson's ratio of liquid crystals at micrometer scale, the tensile tester for a solid material was found lacking the sensitivity. Therefore, a custom-built microscopy based tensile testing technique was developed to measure the in-plane uniaxial tensile Poisson's ratio in a temperature controlled environment. The measurement system was designed according to the method discussed in Stannarius et al. 2004 [26]. In the system as shown in Figure 1a, one fixed and movable glass displacer was placed on a gridded glass slide (25 µm grids spacing), the movable displacer was attached to a three-axis micrometer and the temperatures of the sandwiched glass slides was regulated by a Proportional Integral Derivative (PID) feedback temperature controller

system. This temperature controller consists of a thermistor attached adjacent to the fixed plate and a heating pad placed under the glass slides as show in Fig. 1a-c.

Calibration of the system was first achieved by measuring the Poisson's ratio of Polydimethylsiloxane (PDMS). The PDMS was prepared at 1 : 10 (curing agent : elastomeric gel, Sylgard 184, Corning Incorporation) mixing ratio. Before the PDMS was cured, 10 nl of the PDMS gel was placed in between the edge of the fixed glass slide and movable displacer by using a glass microneedle. The gel contacting the two plates at an initial distance of 10 μm was left to be cured over night before the measurements was performed. With the gridded glass slide as a guide, the separation distance between the fixed plate and displacer was finely tuned by using a 3-axis micrometer. Subsequently, the uni-axial deformation in longitudinal direction (y) of the sample was measured by displacing the movable plate in x direction at a rate of 25 $\mu\text{m}/\text{min}$, and the deformations in bi-axial directions were captured by using a digital camera mounted on a phase-contrast microscope. The deformation of viscoelastic material is a time-dependent quantity, and hence a relaxation time of 2 minutes was considered before each measurement was taken. The Poisson's ratio of the PDMS was determined at room temperature only. As shown in Figure 1b, x_0 and y_0 are the initial transverse and longitudinal lengths of the sample under test, while x and y are the same defined parameters after the uni-axial stretching, respectively.

In subsequent experiments, similar procedures used in determining the Poisson's ratio of PDMS were used for determining the Poisson' ratio of the liquid crystals. The uniaxial stretching of LC was performed by first putting approximately 10 nl of LC gel at the edge of a fixed glass slide by using the glass microneedle and steadily bringing the LC gel into close contact with the edge of another piece of movable glass slide as shown in Figure 1b-c. Extraction of the lyotropic liquid crystals was performed by scraping the transparent membrane resided on top of the CELC, in which, this membrane was formed from lyotropic liquid crystals [30, 31]. The temperature of the test sample was constantly checked with an infrared thermometer at close proximity. With the gridded glass slide as a guide, the space distance between the

displacers was finely tuned by a 3-axis micrometer. Subsequently, the tensile properties of the samples were monitored by displacements of the movable plate in the x direction at a rate of 25 $\mu\text{m}/\text{step}$. A digital camera mounted on the phase-contrast microscope facilitated the imaging of the LC gel stretching. The initial slides separation, x_0 for LC was set at 50 μm and Δx was increased at a rate of 25 $\mu\text{m}/\text{step}$.

For CELC and LLC specimens incubated at 25 $^{\circ}\text{C}$, three LC samples were randomly picked from each of the specimens, and the Poisson's ratios of these samples were determined in isolated experiments at 25 $^{\circ}\text{C}$. For LLC incubated at 37 $^{\circ}\text{C}$, the Poisson's ratio was determined at 37 $^{\circ}\text{C}$. Image-J software was used for the measurements of the strain of the deformed LC in x and y directions. All experiments were repeated three times. Subsequently, equations (2-3) were used to determine the in-plan deformation in and Poisson's ratio was subsequently calculated [32]. The strain of the material in uni-axial direction was determined by using,

$$\varepsilon_1 = \frac{x}{x_0} \quad (2)$$

$$\text{and} \quad \varepsilon_2 = \frac{y}{y_0} \quad (3)$$

where x_0 and y_0 are the initial dimensions. The parameters, ε_1 and ε_2 are the transverse and longitudinal strains. Whereas, x and y are the dimensions after deformation in bi-axial directions (Figure 1a-c). For a material which exhibits non-linear strain such as the PDMS [28], the generalised solution for Poisson's ratio, ν was given by [32],

$$\varepsilon_2 = \varepsilon_1^{-\nu} \quad (4)$$

$$\nu = -\frac{\log \varepsilon_2}{\log \varepsilon_1} \quad (5)$$

These equations are also applicable for determining the Poisson's ratio of the liquid crystals.

3. RESULT AND DISCUSSIONS

3.1 Effects of the Time Dependent Factors to the Liquid Crystalline Phase

In the cross-polarising microscopy, cholesteryl ester liquid crystal which exhibits cholesteric phase in homogeneous textures is as shown in Figure 2a. The arrangement of the mesogens in helical twist induces different reflectivity of incident light is as implied by the colourful structures. After immersion in cell culture media, the surface of the cholesteric liquid crystals transformed into the lyotropic liquid crystals phase (LLC) which was visualised as a thin film on the surface of the cholesteric liquid crystals as reported in [13]. Similarly, for the cholesteryl ester liquid crystals incubated in the cell culture media at 25 °C and 37 °C for 24 hours, translucent films was also seen overlying the surface of the cholesteryl ester liquid crystals. In cross-polarising microscopy, the transparent films were observed with a number of focal conic textures and stratification of white bands co-existing with the cholesteric phase indicating the occurrence of isotropic and smectic liquid crystalline phases (Figure 2b). This is a dramatic change from the cholesteric phase which displayed patterns of multiple reflectivities. The focal conic texture found is a unique optical microstructure which is usually found in the defective smectic or lyotropic lamellar mesophases [33-35]. Defects could occur when the liquid crystal substrates were removed from the petri dishes containing the cell culture media. For the LLCs that have been incubated at 37 °C for 24, 48 and 72 hours (Figure 2d-f), a mixture of lyotropic smectic film in association with the cholesteric phase were observed. As the incubation time in the cell culture media increased, more focal conic textures and stratification of dark and white streaky bands were formed in comparison with the samples that have only incubated at 25 °C and 37 °C for 24 hours (Figure 2b-c). The streaky bands indicate that the uniform and homeotropically aligned lipid in lyotropic phase have their directors normal to the glass surfaces. These well-aligned molecules appeared uniformly dark when viewed through the cross-polariser because their alignments do not polarised the incident light but allow lights to passage through the first polariser and blocked by the second polariser [34]. The defects in the LC alignments created a plane of polarisation to the incident light which is neither parallel nor perpendicular to the tilt direction, in which, the light will be elliptically polarised through the pair of crossed polarisers [34, 36] and thus, formed the white bands. Therefore, an unaligned lipid-water layer appears to be non-uniform and highly reflective in high variations of tilt layers (Figure 2e). When the uni-axial layered lyotropic liquid crystals flow or rolled up in defects, they form the concentric ringed defect structures or focal conic structures [34]. With underlying cholesteric liquid crystals in cell

culture, any distortion to the uni-axial lyotropic liquid crystals could induce some focal conic textures when being viewed down the optical axis as seen in Figure 2f. The existence of multiple optical structures is usually found in severely distorted lyotropic liquid crystals (Figure 2d-f).

This study shows that cholesteryl ester liquid crystals when immersed in a solvent could form lyotropic layers associated with the amphiphilic molecules re-orientation, such that the hydrophilic head (ester bond) orientated towards the water and hydrophobic hydrocarbon tail (fatty acids) orientated to the bulk layer of cholesteric liquid crystals. It is these layers that form the actual interface layer between the cells and a liquid crystal in culture media [7]. Clearly, the cell culture media has transformed the structure of the cholesteric liquid crystals, in which, the amphiphilic liquid crystal molecules have self-assembled into bilayers of lipid molecules that are interlaced by water molecules in a lyotropic or lamellar system [34]. However, differences in incubation temperature (25 °C and 37 °C) induced little difference in the liquid crystalline phase of the cholesteric based lyotropic liquid crystals which is consistent with our thermal analysis of this cholesteric liquid crystal [6]. However, the incubation period and the fluidic environment collectively, transformed the cholesteric phase of the liquid crystals to lyotropic phase under the same concentration of solute (cell culture media).

3.2 Effects of time dependent factors to the Poisson's ratio of liquid crystals

The Poisson's ratio was determined by using a micro-tensile test technique. The test system was initially calibrated by using cured polydimethylsiloxane (PDMS) which has a well-established Poisson's ratio [37]. Figure 3a shows the phase contrast images of the PDMS located in a small initial space of 10 μm (x_0) between the stationary and movable glass slides after the PDMS has been cured for 24 hours. The deformation length, y of the liquid crystals in longitudinal direction increased gradually and was inversely proportional to the transverse deformation length of the liquid crystals, x . This yielded a Poisson ratio of 0.49 ± 0.0067 (mean \pm SD) as determined by using least square method (Figure 3b). This figure seemed to be in good agreement with the Poisson ratio of PDMS which was approximately 0.5 as reported in [37]. Our measurement results showed that the custom-built system is reliable and can be used for determining the Poisson's ratio of LC.

Figure 4a shows an example of longitudinal strain measurements at different transverse strains (ϵ_1) for the cholesteryl esters liquid crystals

captured in the phase contrast microscope. A necking effect was observed at the centre of the liquid crystal slab where the light penetrated through the thinning region. By applying the least-squares method, the Poisson's ratio of the CELC and LLC (25 °C) was characterised by a linearised regime up to 160 % of transverse strain ($\log \varepsilon_1 = 0.2$), and were both determined at 0.5854 ± 0.0192 (mean \pm SD), respectively (Figure 4b and Figure 5). In this context, the longitudinal deformation of LC is linearly dependent on the transverse strain. This result also shows that the Poisson's ratio of LLC has not changed much within a short incubation time in cell culture media. As the transverse strain exceeding 160 % ($\log \varepsilon_1 > 0.2$) and increased to 600 % ($\log \varepsilon_1 = 0.8$), both liquid crystals began to show non-linear viscoelastic behaviour and small differences of strains were identified (Figure 4b). At a maximum transverse strain of 800 %, the gel reached its ultimate tensile strength and snapped.

The relationships for the normal pair of strains in the measurements of LLC at 37 °C for 24, 48 and 72 hours are as shown in Figure 5a. Similar to the samples incubated at 25 °C, the normal pair of strains was linearly proportional to each for $\varepsilon_1 < 160$ % ($\log \varepsilon_1 = 0.2$) but they exhibited non-linearity for $\varepsilon_1 > 160$ % (result not shown). In both measurements, the LLC was subjected to a wide uni-axial strain (160 % of strain). Poisson's ratio of LLC incubated for 24, 48 and 72 hours measured at 37 °C were yielded at 0.58 ± 0.0586 , 0.58 ± 0.0345 , 0.60 ± 0.0254 , respectively (Figure 5b). Under iso-thermic condition, the immersion in cell culture media for first 48 hours showed small differences in the Poisson's ratio which was approximately 0.58. The Poisson's ratio of the LLC increased to 0.6 after 72 hours of incubation which seems to be associated with the liquid crystalline phases displayed by the LLC after 72 hours of incubation. For LLC after prolong incubation in cell culture media, the increment in irregular lyotropic structures suggested that the strength of the lyotropic liquid crystals might have been weakened by the infusion of water molecules. Consistently, the viscoelasticity of the LLC at 37 °C after 72 hours of incubation was greatly reduced and this is consistent with the rheological studies of the cholesteryl ester based lyotropic liquid crystals [25]. It seemed that the Poisson's ratio of LLC is a function of temperature and immersion time in culture media. According to [23, 24], a higher value of Poisson's ratio may yield higher compliance of viscoelastic material and this seems to have occurred in the LLC. However, temperature may not be the major factor as indicated by a similar Poisson's ratio of 0.58 regardless whether the LC substrates were incubated at 25 °C or 37 °C. This is supported by a Differential Scanning Calorimetry (DSC) study of LLC which showed that cholesteryl ester liquid crystal was thermally stable at room and incubation temperatures [6].

These results also show that Poisson's ratio of the liquid crystals is a strain dependent function. This applies to viscoelastic material regardless whether they are isotropic or anisotropic [23]. In our study, the Poisson's ratio measured for LLC is slightly greater than normal isotropic material which is greater 0.5. Poisson's ratio measured for human nucleus pulposus [38] and polyvinyl alcohol [39] was similarly greater than 0.5. This is a typical characteristic of anisotropic material as suggested by [15]. In cholesteric liquid crystals, mesogens of the same director are arranged in layers of helical twist. The cohesion forces binding the mesogens of the same director are different from the forces linking the helical layers. Therefore, strain on the cholesteric liquid crystal overcoming the molecules binding forces in a plane is different from the strain required to deform molecules lying in another plane or vice-versa. Due to the anisotropy, liquid crystals may have more than one Poisson's ratio which depends on the plane and direction of the stress [19] and it is technically challenging to determine the Poisson's ratio of other planes. A higher Poisson's ratio could suggest weaker layer binding forces in the liquid crystal structure and could be attributed to the small increment of water solubilisation in the liquid crystal caused by the weak surfactant such as sodium hydrogen carbonate (2.0 g/l of NaHCO_3) [24] contained in the RPMI-1640 media. With the infinitesimal deformation, the Poisson's ratio of the liquid crystals was clearly affected by the immersion time in a fluidic environment. Over a period of incubation in cell culture media at 37 °C for 3 days, Poisson's ratio of approximately 0.58 was suggested for cholesteryl ester based lyotropic liquid crystals.

4. CONCLUSIONS

In a fluidic environment, cholesteryl ester liquid crystals have transformed into a lyotropic phase which coexisted with the cholesteric phase. Increased irregular structures in the lyotropic phase suggested the gradual infusion of the cell culture media which weakened the compliance of the liquid crystals. The ratio between the longitudinal and transverse uniaxial strain of liquid crystal in a plane demonstrated a linear constitutive relationship at lower strain and behaved non-linearly after exceeding 160 % of strain. Due to the viscoelastic property, Poisson's ratio of lyotropic liquid crystals was determined at 0.58 which is literally not delimited by the Poisson's ratio for isotropic material at 0.5. Among the time dependent factors, prolonged incubation in a fluidic environment is the major contributing factors to increase the increase of the Poisson's ratio and the induction of lyotropic liquid crystalline structures. A

combination of time, temperature and hydration could cause infinitesimal deformation of the cholesteryl ester liquid crystals. Our experimental results also suggest that if liquid crystals were to be used in the application as a biosensor, the strength and compliance of the liquid crystals are collectively impacted by the fluidic environment, incubation temperature and incubation period.

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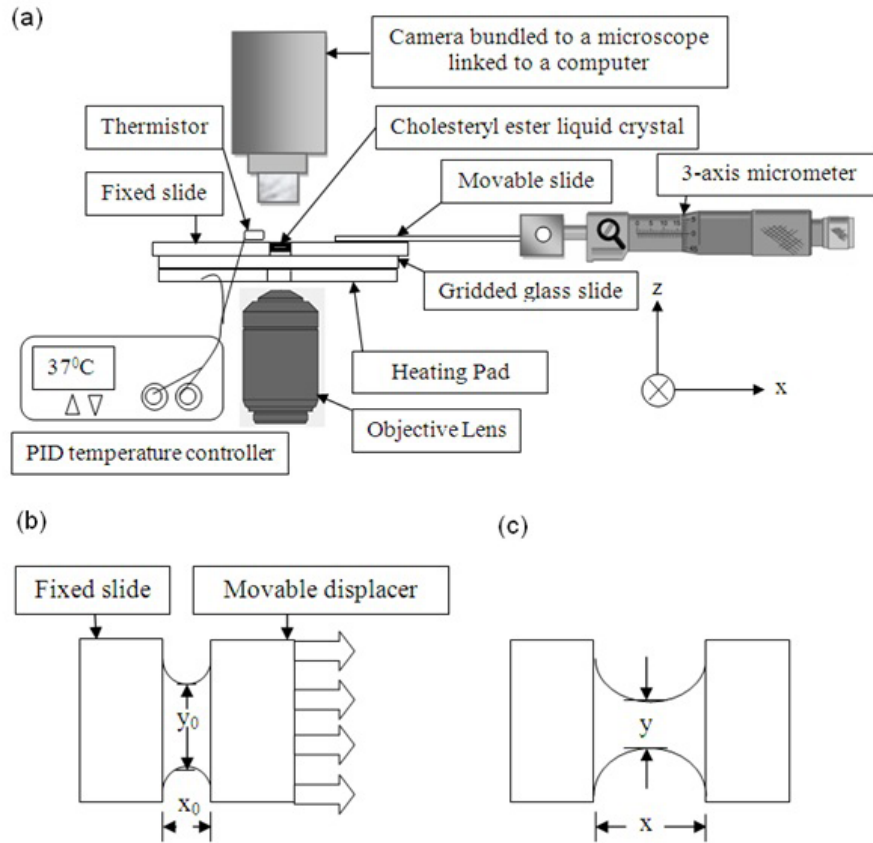


Figure 1. (a) A schematic diagram showing a setup a setup of a micro-tensile tester for measuring Poisson's ratio of cholesteral ester liquid crystal. A schematic drawing showing (a) uni-axial tensile strain of a sample at initial state with normal pairs of deformation x_0 and y_0 , and (b) strain of the sample in transverse direction. The parameters, x and y represent the deformations in transverse and longitudinal directions.

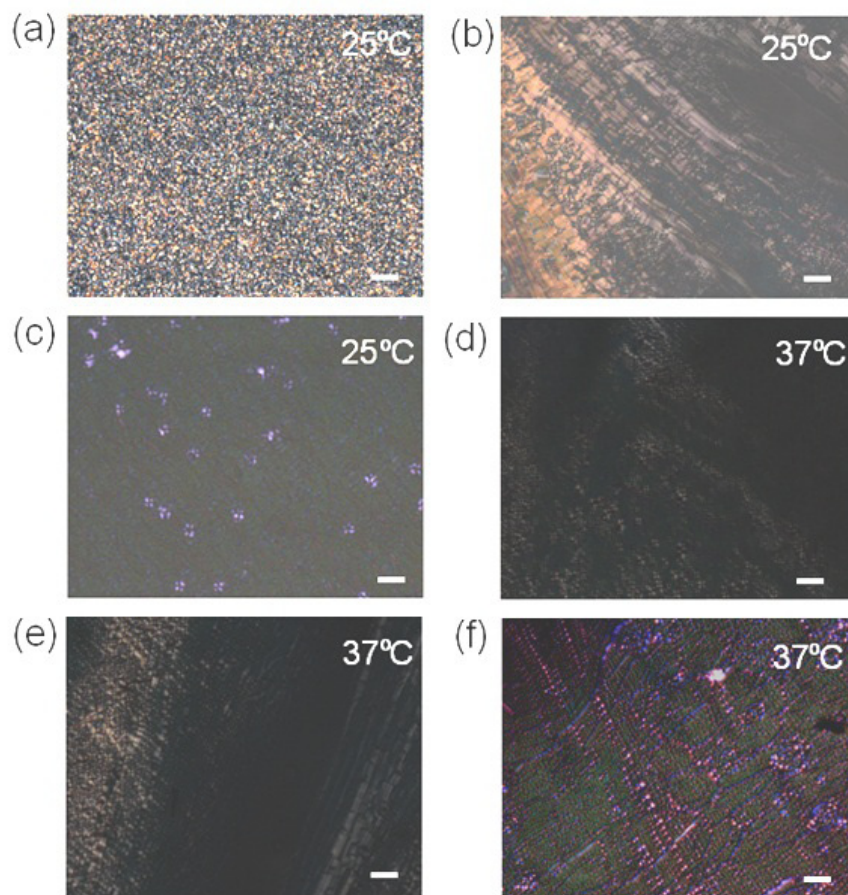


Figure 2. Cross-polarising micrographs of the (a) cholesteric liquid crystals, (b-c) cholesteric based lyotropic liquid crystals incubated at 25 °C and the lyotropic liquid crystals incubated in the RPMI-1640 cell culture media at 37 °C for (d) 24, (e) 48, and (f) 72 hours. (Scale bar: 20 μm)

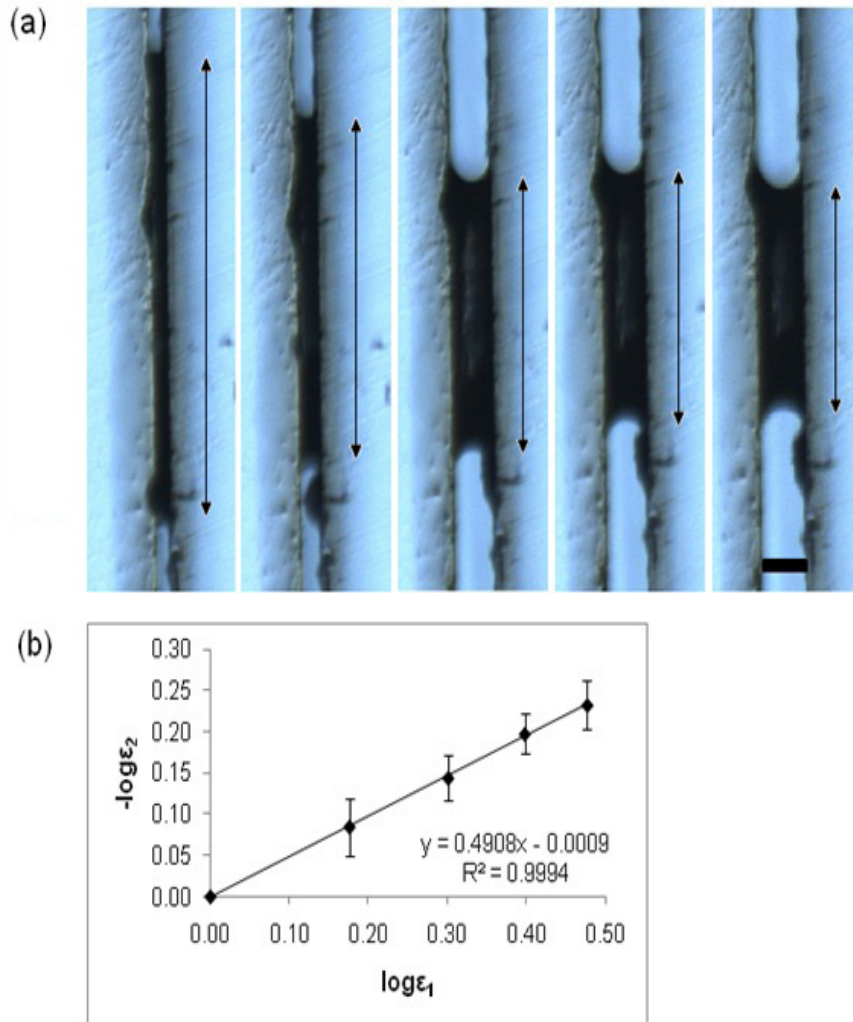


Figure 3. The uni-axial deformations of PDMS. The bi-headed arrows show the deformation in longitudinal direction (y) of PDMS at 380 μm , 276 μm , 220 μm , 192 μm and 178 μm that are corresponding to the deformation in transverse direction (x) at 10, 20, 30, 40 and 50 μm , respectively (image from left to right). The first image from the left shows the measurement for x_o and y_o . (Scale bar: 50 μm) (b) Plots of logarithmic longitudinal strain (ϵ_2) versus transverse strain (ϵ_1) or the Poisson's ratio of PDMS at room temperature 25 °C for N = 10 in three repeat of experiments.

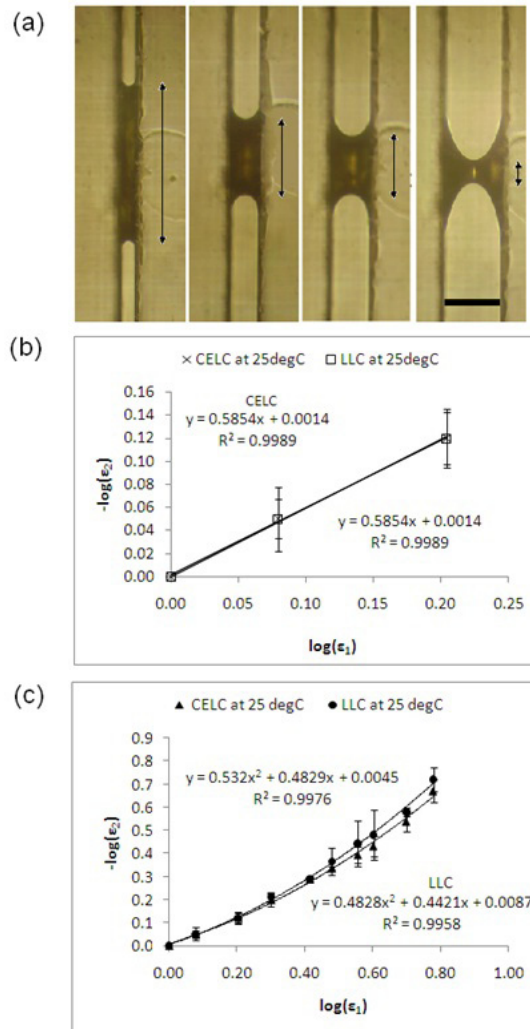


Figure 4. The uni-axial deformations of CELC. The bi-headed arrows show deformation in longitudinal direction (y) of LLC at $490\ \mu\text{m}$, $260\ \mu\text{m}$, $190\ \mu\text{m}$ and $90\ \mu\text{m}$ that are corresponding to transverse deformation (x) at 50 , 100 , 150 and $200\ \mu\text{m}$, respectively (image from left to right). The first image from the left shows the measurement for x_0 and y_0 . (Scale bar: $200\ \mu\text{m}$). Plots of logarithmic longitudinal strain (ϵ_2) versus transverse strain (ϵ_1) or the Poisson's ratios for CELC and LLC incubated at $25\ ^\circ\text{C}$ up to (b) $\log \epsilon_1 = 0.2$ (160% of strain) and (c) $\log \epsilon_1 = 0.8$ (600% of strain).

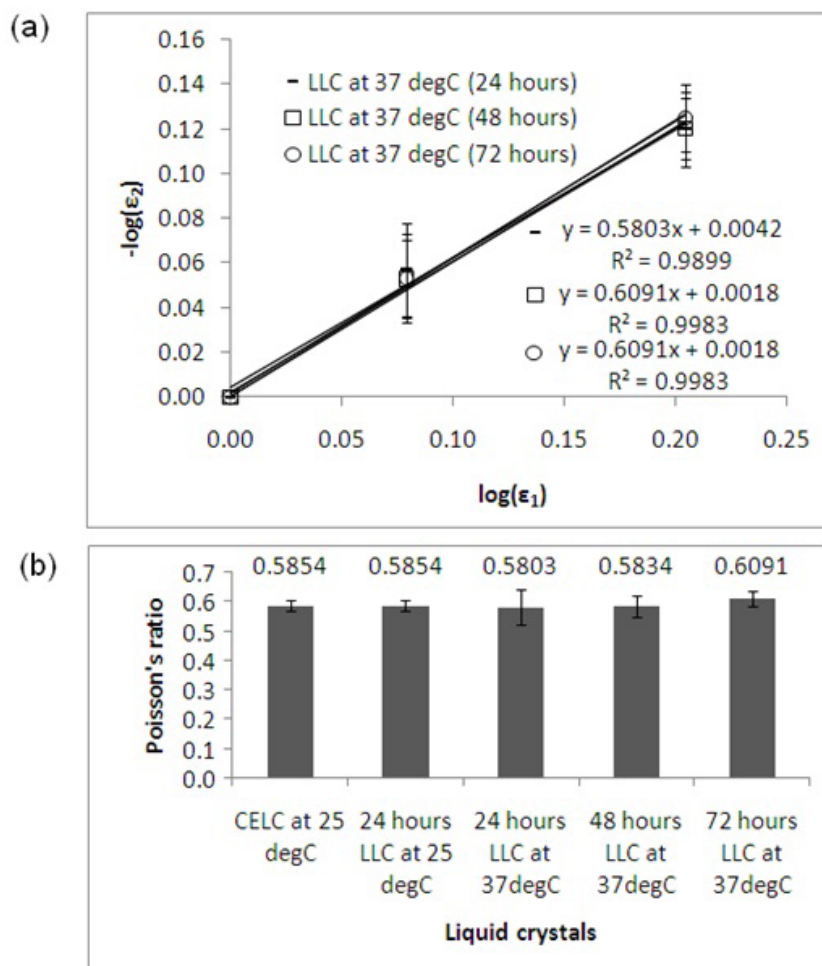


Figure 5. (a) Plots of logarithmic longitudinal strain (ϵ_2) versus transverse strain (ϵ_1) for CELC and LLC incubated at 37 °C for 24, 48, and 72 hours. (b) A comparison of Poisson's ratio for CELC and LLC at room (25 °C) and incubation temperature (37 °C).

The error bars represent the standard deviation (SD) of the means.