Materials Science and Engineering C 65 (2016) 369–378

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

# Magneto-responsive liquid crystalline elastomer nanocomposites as potential candidates for dynamic cell culture substrates

Stephany Herrera-Posada [a], Camilo Mora-Navarro [a], Patricia Ortiz-Bermudez [a], Madeline Torres-Lugo [a], Kyle M. McElhinny [c], Paul G. Evans [c], Barbara O. Calcagno [b], Aldo Acevedo [a,*]

[a] Department of Chemical Engineering, Call Box 9000, University of Puerto Rico, Mayagüez PR 00681, Puerto Rico
[b] Department of General Engineering, Call Box 9000, University of Puerto Rico, Mayagüez PR 00681, Puerto Rico
[c] Department of Materials Science and Engineering, 1509 University Avenue, University of Wisconsin-Madison, WI 53706, USA

ARTICLE INFO

*Article history:*
Received 28 December 2015
Received in revised form 31 March 2016
Accepted 18 April 2016
Available online 20 April 2016

*Keywords:*
Liquid crystal elastomers
Actuation
Magnetic composites
Dynamic cell culture

ABSTRACT

Recently, liquid crystalline elastomers (LCEs) have been proposed as active substrates for cell culture due to their potential to attach and orient cells, and impose dynamic mechanical signals through the application of external stimuli. In this report, the preparation of anisotropic and oriented nematic magnetic-sensitized LCEs with iron oxide nanoparticles, and the evaluation of the effect of particle addition at low concentrations on the resultant structural, thermal, thermo-mechanical, and mechanical properties is presented. Phase transformations produced by heating in alternating magnetic fields were investigated in LCEs in contact with air, water, and a common liquid cell culture medium was also evaluated. The inclusion of nanoparticles into the elastomers displaced the nematic-to-isotropic phase transition, without affecting the nematic structure as evidenced by similar values of the order parameter, while reducing the maximum thermomechanical deformations. Remote and reversible deformations of the magnetic LCEs were achieved through the application of alternating magnetic fields, which induces the nematic–isotropic phase transition through nanoparticle heat generation. Formulation parameters can be modified to allow for remote actuation at values closer to the human physiological temperature range and within the range of deformations that can affect the cellular behavior of fibroblasts. Finally, a collagen surface treatment was performed to improve compatibility with NIH-3T3 fibroblast cultures, which enabled the attachment and proliferation of fibroblasts on substrates with and without magnetic particles under quiescent conditions. The LCEs developed in this work, which are able to deform and experience stress changes by remote contact-less magnetic stimulation, may allow for further studies on the effect of substrate morphology changes and dynamic mechanical properties during *in vitro* cell culture.



## 1. Introduction

Cells in living organisms experience multiple forces in the form of shear and normal stresses. For example, cells from the vascular system are constantly exposed to both shear and normal stresses from blood flow and cyclic pulsatile pumping from the heart, whereas chondrocyte cells from the knee cartilage experience compression during normal human motion [1]. Forces can actuate on cells and alter their extracellular matrix stiffness, which regulates cell functions such as proliferation, adhesion, and differentiation, amongst others [2,3]. Even though cell–matrix interactions play an important role on cell fate, only a few studies on the conversion of mechanical signals into biochemical and functional responses focusing on dynamic responses of cells have been reported. Conventional materials for cell culture do not provide experimental conditions that allow for the dynamic application of strains or stresses. Conventional cell culture substrates are passive with properties that can only be modified during fabrication. Focus has been directed towards developing active soft substrates for *in vitro* cell culture and mechanobiology studies that closely resemble the dynamics of *in vivo* cell environments [4–9].

Proposed biocompatible materials and systems for dynamic cell culture are limited by their high activation temperatures and irreversible shape changes [4,10], or require complex instrumentation to impose the deformation or stresses [1,7,11,12]. Liquid crystalline (LC) materials have been recently proposed as promising candidates for tissue engineering applications since their mechanical properties can match those of soft tissues, their inherent anisotropy can align and direct cell growth, and their sensitivity to external stimuli can address the limitations of conventional methods and materials to develop dynamic substrates. Kirkwood and Fuller demonstrated that deposited collagen films assembled into cholesteric liquid crystalline structures, with periodic banding undulations of approximately 150 nm, induced the contact guidance of adult human fibroblasts [13]. Moreover, it was also demonstrated that extruded small cylindrical scaffolds of anisotropic collagen

* Corresponding author.
*E-mail address:* aldo.acevedo@upr.edu (A. Acevedo).

http://dx.doi.org/10.1016/j.msec.2016.04.063
0928-4931/

also dictate the directional growth of the fibroblasts [14]. Meanwhile, Agarwal et al. were able to fabricate colloid-in-liquid crystals gels coated with thin gelatin films with mechanical properties suitable for the adhesion, spreading, and proliferation of mammalian cells [15]. While these reports demonstrate that the mechanical properties of LC-based materials can match those required for the attachment and proliferation of cells their lack of responsiveness to external stimuli, such as light, magnetic fields or electric fields, limits their applicability as dynamic substrates.

Applications of liquid crystalline elastomers (LCEs) as sensor and micromechanical actuators have been extensively explored due to their ability to undergo large reversible shape changes when the material transitions from the liquid crystal to the isotropic phase (or *vice versa*) [16]. These transitions can be manipulated through external stimuli. Thus, LCE systems have been proposed as an alternative for cell culture and tissue engineering applications [5,17,18]. Bera and coworkers reported the fabrication of porous scaffolds from a simultaneous microemulsion photopolymerization and crosslinking of nematic microspheres, and the successful attachment and growth of myoblasts cells [5]. The acrylate-based chemistry of these materials may allow for their modification with light responsive *azo* groups [19], however, this was not evaluated by Bera and coworkers. Agrawal and coworkers demonstrated that cardiomyocytes attached to nematic LCE substrates and under dynamic conditions were preferentially oriented along the direction of elongation and contraction [17]. The LCE substrates were subjected to cyclic heating which produced uniaxial strains of up to 5% in contact with water. This is the first report with direct evidence of the attachment and orientation of cells on LCEs under dynamic conditions. Nevertheless, the proposed device requires physical contact of the heating element with the substrate. Inclusion of nanoparticles can provide the means to obtain additional stimuli-responses in LCEs as they have been shown to allow for remote and contactless activation either by applying external alternating magnetic fields [20], or by irradiation with light at different wavelengths if carbon nanotubes are used [21,22].

In this work, we report the preparation of magnetic-sensitized LCEs with iron oxide nanoparticles, and the evaluation of the effect of particle addition at low concentrations, on the resultant structural, thermal, thermo-mechanical, and mechanical properties in the context of applications in dynamically varied cell culture substrates. Additionally, the performance of magnetic LCEs in the presence of alternating magnetic fields in contact with air, water and a common cell culture medium is evaluated. We demonstrate that the addition of nanoparticles reduces the nematic-to-isotropic phase transition temperature, which allows for the remote actuation of the LCEs at values closer to the human physiological temperature range. In addition, a collagen functionalization of the LCEs surface was performed to improve their compatibility with NIH-3T3 fibroblast cultures, and demonstrate the ability of our system to promote cell attachment and cell viability.

## 2. Experimental

### 2.1. Synthesis of magnetic composites

Liquid crystalline elastomer films were prepared following the two steps crosslinking reaction proposed by Finkelmann [23] using 10% (41.38 mg, 0.1 mmol) of the crosslinker 1,4-bis(undecenyloxy)benzene (11UB), synthesized according to reported methods [24], and 80% (238.16 mg, 0.80 mmol) of the mesogenic monomer 4-(3-butylenoxy)benzoic acid methyl ester (MBB). The latter was purchased from TCI America. The polymer backbone (60 mg, 1 mmol), poly(methylhydrosiloxane) (PHMS Mn 1700–3200, Sigma-Aldrich) was simultaneously mixed with the mesogenic monomer and the crosslinker followed by the addition of 1 mL of the reaction solvent (toluene, HPLC grade, Sigma-Aldrich) and 20 to 50 μL of a 1 wt% Pt catalyst (1,5-cyclooctadienyl platinum(II) dichloride, Sigma-Aldrich) solution prepared in dichloromethane (Sigma-Aldrich, used as received). A schematic representation of the preparation procedure, as well as the chemical structure for the polymer backbone, crosslinker and mesogenic monomer, are shown in Scheme 1. The mixture was transferred to a rectangular PTFE mold, sealed and left to react at 65 °C. After 1 h, the gel-like material was exposed to the atmosphere under an ice water bath in order to allow for some of the solvent to evaporate. Once the elastomer was strong enough to be handled, it was removed from the mold and subjected to a constant load to align the sample while the crosslinking reaction was still progressing at room temperature. After the films developed optical transparency, an indication of alignment, they were left standing at 40 °C for at least 48 h to ensure completion of the reaction. Finally, the films were rinsed with toluene in order to remove any unreacted material.

A similar procedure as described above was followed to prepare the magnetic LCEs with the exception of using solutions of iron oxide nanoparticles in toluene as the reaction solvent. Commercial 20 nm diameter oleic acid-coated iron oxide nanoparticles (Sigma-Aldrich) were used in all cases. The hydrodynamic particle diameter, $d_H$, in toluene was confirmed by dynamic light scattering as 17.53 ± 0.26 nm in a Brookhaven Instruments BI 90 Plus Particle Size Analyzer. Particle solutions of 0.07, 0.17 and 0.25 wt/v% were used resulting in concentration of 0.2, 0.5 and 0.7 wt%, respectively, of nanoparticles in the final LCE matrices. TEM micrographs were obtained in a Tecnai T-12 Cryo TEM at −80 °C with film samples prepared with a Leica EM VC7 Ultramicrotome. Particle size distributions within the films were determined from these micrographs using ImageJ software.

### 2.2. Materials characterization

#### 2.2.1. Order parameter

The nematic order parameter, $S$, was determined from wide angle X-ray scattering measurements for each specimen at temperatures above and below the nematic-to-isotropic transition in a Bruker D8 Discover diffractometer operating with a CuKα radiation ($\lambda_{CuK\alpha} = 1.5418$ Å) and equipped with a Vantec 500 multiwire area detector. The incident beam was collimated with a diameter of 0.3 mm collimation and incident along the surface normal of the elastomer film. The sample-to-detector distance was set to 200 mm at a detector angle of 20° with respect to the incident beam direction. $S$ was calculated from the azimuthal intensity distribution, $I(\chi)$, obtained following the method of Davidson et al. [25] and using Eq. (1).

$$S = \frac{1}{2}\frac{\int_0^{\pi/2} I(\chi)\langle 3\cos^2\chi - 1\rangle \sin\chi \, d\chi}{\int_0^{\pi/2} I(\chi)\sin\chi \, d\chi}. \quad (1)$$

Here $\chi$ corresponds to the azimuthal angle in the X-ray scattering patterns.

#### 2.2.2. Thermal, mechanical and magneto-mechanical properties

Thermal transitions of the composites were probed using differential scanning calorimetry DSC thermograms obtained on a TA Instruments Q2000 under a nitrogen flow of 50 mL/min in heating–cooling–heating cycles at rates of 10 °C/min. The reported values for the glass transition correspond to the midpoint of the step change in the thermograms, while the temperature $T_{ni}$ of the nematic-to-isotropic transition was identified as the maximum of the endothermic peak obtained on the second heating cycle.

Thermomechanical expansion–contraction experiments were conducted by manually recording the film length at 5 °C intervals between 90 and 20 °C during successive cooling and heating cycles. The temperature was held fixed until stabilized in an INSTEC HSC302-mK1000A thermal stage and was independently verified with a surface thermocouple connected to the aluminum stage. The reported values correspond to