Thermal induced reversible deformations

Potential candidates for dynamic cell culture

Magnetic induced reversible deformation

***Magneto-Responsive Liquid Crystalline Elastomer
Nanocomposites as Potential Candidates for Dynamic Cell
Culture Substrates***

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27 **ABSTRACT**

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

47 **Keywords:** liquid crystal elastomers, actuation, magnetic composites, dynamic cell culture.

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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 (2009) 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 also dictate the directional growth of the fibroblasts [14]. Meanwhile, Agarwal *et al.* (2008) were able to fabricate colloid-in-liquid crystals gels coated with thin gelatin films with mechanical properties suitable for the

76 adhesion, spreading, and proliferation of mammalian cells [15]. While these reports demonstrate that the
77 mechanical properties of LC-based materials can match those required for the attachment and
78 proliferation of cells their lack of responsiveness to external stimuli, such as light, magnetic fields or
79 electric fields, limits their applicability as dynamic substrates.

80 Applications of liquid crystalline elastomers (LCEs) as sensor and micromechanical actuators have
81 been extensively explored due to their ability to undergo large reversible shape changes when the material
82 transitions from the liquid crystal to the isotropic phase (or vice versa) [16]. These transitions can be
83 manipulated through external stimuli. Thus, LCE systems have been proposed as an alternative for cell
84 culture and tissue engineering applications [5,17,18]. Bera and coworkers (2015) reported the fabrication
85 of porous scaffolds from a simultaneous microemulsion photopolymerization and crosslinking of nematic
86 microspheres, and the successful attachment and growth of myoblasts cells [5]. The acrylate-based
87 chemistry of these materials may allow for their modification with light responsive *azo* groups [19],
88 however, this was not evaluated by Bera and coworkers. Agrawal and coworkers (2015) demonstrated
89 that cardiomyocytes attached to nematic LCE substrates and under dynamic conditions were
90 preferentially oriented along the direction of elongation and contraction [17]. The LCE substrates were
91 subjected to cyclic heating which produced uniaxial strains of up to 5% in contact with water. This is the
92 first report with direct evidence of the attachment and orientation of cells on LCEs under dynamic
93 conditions. Nevertheless, the proposed device requires physical contact of the heating element with the
94 substrate. Inclusion of nanoparticles can provide the means to obtain additional stimuli-responses in LCEs
95 as they have been shown to allow for remote and contactless activation either by applying external
96 alternating magnetic fields [20], or by irradiation with light at different wavelengths if carbon nanotubes
97 are used [21,22].

98 In this work, we report the preparation of magnetic-sensitized LCEs with iron oxide nanoparticles,
99 and the evaluation of the effect of particle addition at low concentrations, on the resultant structural,
100 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 μ L 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 one hour, 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.

Scheme 1. Representation of the preparation procedure and chemical structures of the liquid crystalline elastomer precursors.

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 $\text{CuK}\alpha$ radiation ($\lambda_{\text{CuK}\alpha} = 1.5418 \text{ \AA}$) 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 Equation 1.

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

Here χ 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 $10^\circ\text{C}/\text{min}$. 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°C and 20°C during successive cooling and heating cycles. The temperature was held fixed until stabilized in an INSTRON HSC302-mK1000A thermal stage and was independently verified with a surface thermocouple connected to the aluminum stage. The reported values correspond to averages of at least three measurements and are reported with their corresponding standard deviations.

Tensile tests were performed parallel and perpendicular to the director (stretching direction) using a stress-controlled Anton Paar MCR 302 rheometer equipped with CTD450 convection oven and an X-pansion Instruments SER2 extensional test fixture. The tests were performed at a constant strain rate of $2 \times 10^{-4} \text{ s}^{-1}$ and temperatures corresponding to 0.7 times the nematic-to-isotropic transition temperature, i.e. $T_{\text{test}} = 0.7T_{ni}$, to obtain comparable nematic order in each of the magnetic composites. The data obtained for the stress-strain behavior was corrected for the slack and toe following the method proposed by Finkelmann *et al.* [26]. Reported values of Young's modulus (E) and ultimate tensile strength (UTS) correspond to the average and standard deviation of three measurements. The Young's moduli were calculated from the slope of the low strain linear regime, typically from 0 to 10% strain, while values for the UTS were obtained from the maxima of the stress-strain curves. Measurements of the tensile properties were performed at room temperature and at a strain rate of 0.017 s^{-1} for the neat and 0.5 wt% elastomers before and after surface treatment and collagen functionalization, using an Instron ElectroPuls model E3000 tensile testing instrument.

Magneto-mechanical experiments were performed in an Ambrell EASYHEAT LI (RHS) induction heater apparatus. Samples of 2.6 cm in length were placed vertically in the center of an induction coil (four turns, 3.16 cm ID, 2.97 cm height) and clamped at the upper end. The deformation of the films was determined by monitoring the change in position of the suspended end by recording digital images with a camera every 20 seconds and analyzed using ImageJ software. The temperature profiles of the elastomers were monitored using a FLIR Thermacam A20 Researcher infrared camera, which was pointed at a fixed angle from the top of the coil. An alternating magnetic field of 48 kA/m and a frequency of 298 kHz was applied on successive on-off cycles. The effect of magnetic field was evaluated by varying the field strength from 29 to 48 kA/m during one cycle experiments for each MLCE. Magneto-mechanical experiments were also conducted for the LCE with 0.5 wt% of magnetic nanoparticles submerged in either deionized water or cell growth medium (Dulbecco's modified Eagle's medium, DMEM, from Sigma-Aldrich) and an applied field of 35 kA/m at 298 kHz. A liquid volume of approximately 32 mL

was used. Small weights were attached to the lower end of the elastomer to compensate for buoyancy. The temperature was monitored near the surface of the elastomer and the bulk of the fluid using Teflon coated thermocouples, while deformation was monitored by recording digital images every 20 seconds.

2.2.3. Cell viability and proliferation

Cell culture experiments were performed over collagen functionalized LCEs. Collagen was bound to the LCE surfaces using reported methods for the immobilization of collagen onto polysiloxane substrates [27,28]. Once the material was functionalized and all impurities removed, 5 mm × 5 mm samples were placed inside the wells of a clear flat-bottom 96-well plate with dark walls. Mouse embryonic fibroblast cells NIH3T3 from a 70-80% confluent culture were trypsinized and 2500 cells were seeded per well. The plate was incubated for four days to allow adhesion and cell proliferation. NIH3T3 cells were chosen since fibroblasts, which form connective tissue, are known to be mechano-responsive cells [29] and as such are suitable as model cells for mechano-biology studies.

Cell proliferation was verified by direct visualization of the cell cultures on an Olympus IX81 confocal microscope equipped with a spinning disk 3i System, a Xenon Fl source, and Rolera EM-C2 camera. After incubation, cell cultures were stained with dye solutions of acridine orange and CellMask™ Deep Red Cell Membrane, both purchased from Life Technologies. To determine cell viability the culture medium was replaced with fresh media containing PrestoBlue® (Life Technologies), a cell viability reagent. The viability determination protocol was performed following the instructions given by the manufacturer with five hours of exposure to the reagent. Cells that metabolize PrestoBlue (rezazurin-based method) generate a fluorescent signal which is proportional to the number of viable cells over the elastomeric substrates. The fluorescence intensity (560Ex/590Em) was measured using a Spectra Max Gemini EM spectrofluorometer. Reported cell viability ratios were calculated as the fluorescence intensity of each sample over that of neat elastomer, chosen as the control for this experiment. Values correspond to the average of at least three independent measurements with their corresponding standard

deviations. Detailed procedures for the collagen immobilization and characterization, as well as sample preparations for biological tests are included on the Supplementary Information S3 section.

3. RESULTS AND DISCUSSION

3.1. Morphology and Structure

After synthesis, homogeneous films with no indication of phase separation were obtained. Films changed from transparent with a yellowish tinge for the neat elastomers to opaque and brownish as the concentration of nanoparticles increased as shown in Figure 1a-d. The yellowish tinge is caused by the diffraction of light by the liquid crystalline arrangement of the microstructure, while the brown color is due to the iron content of the nanoparticles. The average particle diameter determined from TEM micrographs, shown in Figure 1b and 1c, was 15.3 ± 3.2 nm and 16.1 ± 4.3 nm, which is similar to the hydrodynamic diameter of the suspended particles in toluene. Nanoparticles were well-dispersed within the elastomeric matrices, as evidenced by the nearest neighbor analysis from different images of the elastomers (See Supplementary Information S1 Section). In addition, the synthesis method effectively produced anisotropic elastomers with LC molecules oriented in average along the stretching direction during the crosslinking step, as demonstrated by the diffuse arcs in the wide angle x-ray 2D patterns (See Supplementary Information S2 Section).

Figure 1. Images of the (a) neat, (b) 0.2, (c) 0.5 and (d) 0.7 wt% LCEs, and TEM images for the elastomers with nanoparticle loadings of (e) 0.5 and (f) 0.7 wt%. Scale bar 100 nm.

The phase behavior of the elastomers was identified from DSC thermograms from which glass transition temperatures (T_g) and nematic-to-isotropic phase transition temperatures (T_{ni}) were reported previously elsewhere [30] and are summarized in Table 1. Both T_g and T_{ni} decreased with increasing nanoparticle concentration. Liquid crystalline elastomers composites described to date, have shown slight shifts of the glass and nematic-to-isotropic transition temperatures to lower values after incorporating nanoparticles [20,22,31]. In some cases, these changes have been attributed to poor dispersion [31] and phase separation of the nano-fillers within the polymer matrix [22]. As previously discussed, well dispersed non-aggregated particles within the elastomeric matrix were observed in our system. Alternatively, the presence of well-dispersed spherical nanoparticles in low molar mass liquid crystals and liquid crystalline polymers has been reported to reduce the transition temperature which is attributed to a decrease of the average strength of the LCs intermolecular interactions, a phenomenon that is known as the “dilution” effect [32–34]. Since the 15 nm diameter nanoparticles used in this work are larger than the molecular size of the mesogens, ~4 nm [35], a dilution effect is expected. Additional information of

the effect of particle inclusions and concentration on the microstructure was obtained from evaluation of the liquid crystalline order parameter as a function of temperature.

Table 1. Summary of thermal, thermo-mechanical and extensional mechanical properties of the LCEs as a function of magnetic content.

Particle Loading (wt %)	T_g (°C)	T_{ni} (°C)	Mechanical Properties					
			Extension $\parallel$ to the director			Extension $\perp$ to the director		
			E (kPa) ¹	UTS (kPa) ²	Elongation at Break	E(kPa)	UTS (kPa)	Elongation at Break
0	-4.5 ± 1.2	63.3 ± 0.6	191 ± 38	73 ± 24	0.29 ± 0.01	40 ± 14	177 ± 100	0.98 ± 0.25
0.2	-11.3 ± 0.9	52.7 ± 1.4	569 ± 132	195 ± 32	0.28 ± 0.02	579 ± 51	240 ± 107	0.39 ± 0.14
0.5	-16.8 ± 1.3	38.2 ± 1.1	895 ± 119	119 ± 10	0.15 ± 0.02	527 ± 43	186 ± 18	0.44 ± 0.04
0.7	-17.7 ± 1.2	34.3 ± 0.8	821 ± 75	158 ± 5	0.19 ± 0.01	437 ± 89	167 ± 35	0.41 ± 0.02

¹Young's Moduli measured at $T_{test} = 0.7 T_{ni}$

²Ultimate Tensile Strength

Nematic LC phases can be described by the director (n), which denotes the average direction of the oriented molecules, and the order parameter (S), which is a measure of the degree of orientational order or the spread of the molecules around the director. The scalar orientational order parameter, S , was determined from x-ray diffraction experiments as a function of temperature and nanoparticle loading as shown in Figure 2. Temperature was scaled as a function of the nematic-to-isotropic phase transition temperature for each sample. Elastomers, with and without magnetic inclusions, have S values between 0.5 and 0.6 at low temperatures, $T/T_{ni} < 0.5$. The high value of S in the ordered phase indicates that the degree of order in the low-temperature phase is not significantly reduced by the addition of the nanoparticles. As the temperature increases the order parameter slightly decreases for all samples, dropping to values between 0.2 and 0.3 as it approaches the phase transition. The transitions into the isotropic phase for the neat elastomer and the 0.2 wt% of nanoparticles are sharper and reach slightly lower values of S at the phase transition temperature. Smoother transitions are observed in Fig. 2 for higher particle concentrations, suggesting that a higher degree of orientation is retained in the isotropic phase for these samples.

Figure 2. Order parameter, S , as a function of reduced temperature (T/T_{ni}) of the liquid crystalline elastomers at different particle loadings neat (●), 0.2 (□), 0.5 (△) and 0.7 wt% (◆).

3.2. Mechanical and Thermomechanical Properties

The mechanical properties of the composites were evaluated at a similar liquid crystalline state corresponding to $0.7T_{ni}$ (or equivalently S of approximately 0.54) in extensional experiments performed along both parallel and perpendicular to the stretching direction during synthesis (taken as the direction of the director). The stress-strain behavior during extensional experiments is depicted in Figure 3, while the Young's modulus and the Ultimate Tensile Strength values are summarized in Table 1. The stress-strain behavior was anisotropic. In the parallel case, the stress-strain dependence remained linear across the evaluated strain range, whereas for the perpendicular case three distinct regions were observed. For the neat elastomer, when stretched in the perpendicular direction, the stress increased proportional to strain at low strains and was followed by an intermediate-strain regime characterized by a pronounced plateau, which then turned into a third region at higher strains where the stress increased proportional to the strain again. The intermediate plateau has been denoted in the literature as a soft or semi-soft elastic region, where deformations take place with little or no resistance [16,36]. The inclusion of the nanoparticles

greatly influences this soft or semi-soft elasticity by reducing the range of the plateau to the point where it is mostly imperceptible when 0.7 wt% of nanoparticles were added to the elastomers. In this case, nanoparticles are imposing an additional resistance that the polymer chains and mesogenic building blocks have to overcome in order to reorient to the direction of the applied force. The Young's modulus increased significantly in both parallel and perpendicular directions with the addition and increase of particles, while the ultimate tensile strength only showed a statistical significant difference in the parallel case. The elongation at break decreased with the addition and increase of particles for the parallel deformation, while the elongation at break for the perpendicular case decreased with the addition of particles, but was statistically independent of particle concentration. The increase of the modulus can be related to a mechanical reinforcement promoted by the addition of the nanoparticles, an effect that has been reported for many polymer composites whose inorganic fillers are often stiffer than the matrix [37].

Figure 3. Stress-strain behavior of the neat (●), 0.2 (□), 0.5 (△) and 0.7 wt% (◆) elastomers under a strain rate of $2 \times 10^{-4} \text{ s}^{-1}$ at extensions (a) parallel and (b) perpendicular to the orientation axis (director).

The thermomechanical expansion-contraction capacity of the elastomers was reduced with addition of particles, as shown in Figure 4. The relative sample length corresponds to the length at the specified temperature normalized using as a reference the constant length of the films well within the isotropic

state. The maximum thermally-induced deformation was observed at the lowest studied temperature. At that point a decrease of the relative sample length from 1.52 for the neat elastomer to 1.32 for the 0.2 wt% of iron oxide nanoparticles was measured. Upon further increase of the particle content the relative sample length was observed to remain unchanged. The thermo-mechanical behavior was fully reversible, since no hysteresis between the cooling and heating cycles was observed. The reversibility was not affected by the inclusion of the nanoparticles. The deformations across the nematic-to-isotropic transition and vice versa (i.e. $T/T_{ni} = 1$) follow similar trends as those observed for the order parameter. A sharp transition is observed for the neat elastomers, while the composites show smoother transitions.

Figure 4. Relative sample length, λ/λ_0 , of the liquid crystalline elastomers as a function of reduced temperature for the (a) neat elastomer and with loadings of (b) 0.2 wt%, (c) 0.5 wt% and (d) 0.7 wt% during cooling (closed symbols) and heating (open symbols). λ_0 corresponds to the film length at the isotropic phase.

3.3. Magneto-mechanical Response

Figure 5 shows the dynamic contraction of the elastomers surrounded by air in response to a 48 kA/m and 298 kHz oscillating magnetic field over five successive on-off cycles of 480 seconds each. The composites responded immediately to the magnetic field, however, a steady value was reached approximately after four (4) minutes for all the composite elastomers during each cycle. After the field was turned off during each cycle, the contraction was observed to return to zero. Thus, the magnetically

induced contraction was completely reversible within the experimental time frame. In addition, the maximum contraction increased with increasing particle loading and did not show variations over time. The response of the elastomers with 0.5 and 0.7 wt % particle loadings was similar and accordingly reached comparable values of maximum contraction around 24%. The latter suggests a saturation of the response to the magnetic field.

Figure 5. Magneto-mechanical behavior over five eight-minutes on/off cycles under an alternating magnetic field of 48.24 kA/m and frequency of 298 kHz for the neat (●), 0.2 (□), 0.5 (△) and 0.7 wt% (◆) elastomers. Contraction % was calculated as $\Delta L/L_0$ where L_0 is the initial length at $t=0$.

The temperature evolution of the elastomers was directly determined from thermal images, shown in Figure 6a-d, obtained with an infrared camera. From the images the temperature variations of the films over time was determined. Reported temperatures correspond to a single point within the elastomer, but no color variations in the thermal images were observed over the elastomer. Figure 6e shows the superposition of the temperature and contraction profiles of the 0.5 and 0.7 wt% magnetic elastomers obtained during one magnetic actuation cycle with an applied alternating field of 48.24 kA/m and oscillating frequency of 298 kHz. The profiles parallel each other which indicates that the deformations are directly related to the thermal fluctuations caused by the magnetic field. In fact, magnetic nanoparticles can experience relaxation and hysteresis phenomena in the presence of oscillating magnetic

fields which leads to energy dissipation or heat loss [38]. This heat loss is sufficient to increase the LCE temperature above the nematic-to-isotropic transition causing the deformation. Thus, these experiments confirm that the magnetic actuation of these LCE composites is a consequence of the thermally-driven phase changes from the heat generated by the nanoparticles.

Figure 6. Thermal images of the 0.5 wt% magnetic elastomer when subjected to an alternating magnetic field of 48.24 kA/m and 298 kHz oscillating frequency at (a) 0, (b) 27, (c) 159 and (d) 926 s and (e) superposition of the temperature (open symbols) and contraction (closed symbols) profiles of the 0.5 (●) and 0.7 wt.% (■) elastomers obtained in a magnetic actuation experiment. Crosses in thermal images represent the reference point where the temperature data was collected using the infrared camera software.

Figure 7 shows the maximum contraction and temperature for various magnetic field strengths as a function of particle loading. Temperature increases gradually up to 41 °C by increasing both magnetic content and field strength. On the other hand, the contraction % at the bottom section on Figure 7 showed an abrupt increase above 0.2 wt%. Afterwards, the contraction remains constant or slightly decreases without a discernible pattern as a function of magnetic field strength. Contractions in the presence of alternating magnetic fields of about 26% have been previously reported for magnetic LCE composites with nanoparticle loadings of up to 1.64 v% [39]. However, these deformations were triggered approximately at 80 °C, which corresponded to their phase transition temperature. The magnetic LCEs presented in this work displayed comparable magnetomechanical responses, yet as discussed in previous

section variations of the nanoparticle concentration decreased the thermal transitions. Therefore, thermal and magnetic actuation can be achieved at temperatures below 40 °C. This temperature range is representative of biological systems providing an added benefit to the potential use of these elastomers as biomaterials.

Figure 7. Steady state temperature (top) and magnetic-driven contraction (bottom) of the LCE nanocomposites as a function of magnetic particle loading under different applied magnetic fields of 29.30 (●), 34.73 (□), 41.78 (△), and 48.24 (◆) kA/m. The frequency of the alternating field was held constant at 298 kHz and the surrounding fluid was air at room temperature.

Since the application of the elastomers in biological systems requires mechanical functionality in wet environments the magnetomechanical response was evaluated in deionized water and DMEM, a model cell growth medium. A composite elastomer with 0.5 wt% of nanoparticles was submerged on each fluid

and an oscillating magnetic field of 35 kA/m and 298 kHz was applied during successive on-off cycles. The time profiles for the contraction and fluid temperature close to the films and at the bottom of the container are shown in Figure 8. When the film was surrounded by deionized water the maximum contraction observed was 3% while the temperature close to the film increased by 6 °C with respect to its initial value. The ambient temperature in the liquid, measured at the bottom of the container, increased by only 2 °C. The thermally induced contraction of the elastomer was fully reversible and similar during each cycle. The contractions in water were much lower than the 14% maximum contraction displayed under similar conditions when surrounded by air because the surrounding water acts as a heat sink dissipating the heat generated by the nanoparticles in the film. Thus, the temperature within the films did not increase enough to cause changes in the liquid crystalline order of the elastomer. When DMEM was used the magnetomechanical response increased during each successive cycle. The maximum contraction increased from 6% during the first cycle to 21% in the fifth cycle. However, the film did not regain its original shape after the field was turned off. The temperature close to the film showed a similar behavior: did not return to its initial value after turning off the field and the temperature increment ($T - T_0$, where T_0 is the temperature at $t = 0$ seconds) went from 15 to 25 °C from the first to the fifth cycle, respectively. Furthermore, the temperature of the fluid measured at the bottom of the container increased about 7 °C during the experiment. Since the DMEM medium is comprised of a mixture of salts and electrolytes, the alternating magnetic field can cause inductive heating over time [40]. Thus, heat is generated in both the fluid and the elastomer. The temperature gradient in the elastomer is higher, which will cause heat dissipation to the fluid. In addition, the fluid is not able to transfer heat to its surroundings within the timeframe of a cycle. This caused the bulk temperature of the fluid to increase over time to the point where it reached values surpassing the nematic-to-isotropic transition temperature and displaying similar maximum contractions as those observed in air. In general, under similar experimental conditions, the magnetically-induced contractions in the evaluated liquids were lower than in air, nevertheless, values of up to 20%, similar to the mechanical strains that have been reported to affect fibroblasts cellular behavior [1], were achieved at temperatures below 41 °C.

Figure 8. Mechanical deformation (top) and temperature increments (bottom) of a liquid crystalline elastomer loaded with 0.5 wt% iron oxide nanoparticles in response to an applied alternating magnetic field of 34.73 kA/m at 298 kHz over five eight-minutes on/off cycles submerged in water (■) and DMEM growth medium (●). Closed and open symbols in the bottom figure correspond to temperatures measured close to the surface of the elastomer and at the bottom of the reservoir, respectively. T_0 corresponds to the initial temperature at $t=0$.

3.4. Magnetic LCEs as substrates for fibroblast NIH3T3 cell culture

Most of the substrates used today for cell culture are passive with properties that are set during fabrication [41]. An active substrate may allow for the study of the effect of dynamic mechanical stresses and deformations on cells and tissues resembling *in vivo* environments. Thus, the effect of the elastomers on cell viability and proliferation was studied. The elastomers were functionalized with collagen to improve cell-surface interactions, and the successful immobilization of the protein on the surface was confirmed with infrared spectroscopy and contact angle measurements with water (See Supplementary Information Figures S5 and S6). The water contact angle was found to decrease from $75 \pm 2^\circ$ to $57 \pm 6^\circ$ for the neat elastomer and from $73 \pm 2^\circ$ to $40 \pm 10^\circ$ for the 0.5 wt% elastomer, indicating a reduction of

the hydrophobicity characteristic of polysiloxane materials. Mechanical properties determined after the collagen functionalization were found to be similar to those of unfunctionalized films. The Young's modulus measured at room temperature and a strain rate of 0.017 s^{-1} was $157 \pm 20 \text{ kPa}$ for the neat elastomer with collagen coating and $156 \pm 15 \text{ kPa}$ without, while it was $504 \pm 33 \text{ kPa}$ and $425 \pm 62 \text{ kPa}$ for the 0.5 wt% elastomer with and without coating, respectively. Similarly, the maximum relative thermally-induced deformation for the functionalized neat and 0.5 wt% composite elastomer was 1.53 and 1.38, respectively. The NIH3T3 fibroblast cells were cultured directly on top of the functionalized elastomers. After 72 hours of incubation, cell cultures were stained with fluorescent dyes and confocal images of the cell cultures were obtained. Figure 9 illustrates confocal micrographs of the 0.2 wt% magnetic LCE at the top and 0.5 wt% magnetic LCE at the bottom. The columns, from left to right, correspond to the green, red, and collated fluorescent emission, which allowed for the visualization of the cell nuclei, membranes, and both, respectively. The images show that the magnetic LCEs may support fibroblast cell attachment and proliferation. Additionally, the metabolic rates of the NIH3T3 cell cultures were correlated to their fluorescence intensity following standard biological assays in order to determine the viability ratio taking the neat elastomer as the reference. Cell viability ratios for the 0.2 wt% and 0.5 wt% magnetic LCEs were determined as 1.26 ± 0.36 and 1.17 ± 0.30 , respectively and summarized on Figure 9g. These values are statistically similar to unity, which indicates that the embedded magnetic particles did not have a negative effect on the survival of the cells. These results suggest that if adequately functionalized, the LCEs may be used openly as substrates for cell culture without affecting cell adhesion and proliferation. The direct contact of the LCEs with cells is advantageous since it potentially decreases the number of materials and processes that are typically used to separate the cultures from harmful layers.

Figure 9. Confocal images of the magnetic LCEs with (a-c) 0.2 wt% and (e-f) 0.5 wt% particle content and (g) viability ratio of NIH3T3 fibroblasts growing on collagen coated liquid crystalline elastomers. Values correspond to the average of three independent experiments with the error bars corresponding to the standard deviations. Scale bar is 200 μm

4. CONCLUSIONS

Anisotropic and oriented nematic LCEs with magnetic response were successfully synthesized through the addition of low concentrations of iron oxide nanoparticles. Magnetic particles were homogeneously distributed within the elastomeric matrix. Inclusion of nanoparticles into the elastomers displaced the liquid crystalline range to lower temperatures, but the microstructure of the system was conserved as demonstrated by similar values of the nematic order parameter. Further increment of the nanoparticles content allowed for the nematic-to-isotropic transition temperature to approximate the human physiological temperature range. An increase of the Young's modulus with particle inclusion was observed, while the maximum thermally-induced deformations were completely reversible but diminished with the presence of the particles. This was attributed to the additional resistance of the nanoparticles to the stretching of the polymer network and reorientation of the mesogenic building blocks in the direction of the applied force.

Remote and reversible deformations of the magnetic LCEs were achieved through the application of alternating magnetic fields which promoted the phase transition through the heat generated by the nanoparticles. The magnetically-induced deformations can be controlled through the modification of formulation parameters, such as the particle concentration, and experimental conditions, such as the magnetic field strength or the surrounding fluid.

A suitable collagen surface treatment was applied to the elastomers which allowed for the effective attachment and proliferation of NIH3T3 fibroblasts on elastomers with and without magnetic particles under quiescent conditions. The collagen-treatment was effective in overcoming the limitations due to the inherent hydrophobicity of polysiloxanes. The LCEs developed in this work are capable to deform and experience stress changes by remote contact-less magnetic stimulation within the human physiological temperature range and surpass the typical limitations of potential candidates for dynamic cell culture. Further studies on the effect of substrate morphology and mechanical properties on cell adhesion, proliferation and differentiation during *in vitro* culture may be achieved through the use of these stimuli-responsive materials.

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