Photonic artificial muscles: from micro robots to tissue engineering

Daniele Martella, a,b Sara Nocentini, a,c Camilla Parmeggiani a,c and Diederik S. Wiersma a,b,c,*

Light responsive shape-changing polymers are able to mimic the function of biological muscles accomplishing mechanical work in response to selected stimuli. A variety of manufacturing techniques and chemical processes can be employed to shape these materials on different length scales, from centimeter fibers and films to 3D printed micrometric objects trying to replicate biological functions and operations for different applications. Controlled deformations are shown towards mimicking basic animal operations as walking, swimming or grabbing objects, while controlling also the refractive index and the geometry of photonic devices open to the implementation of tunable optical properties. Another possibility is that of combining artificial polymers with cells or biological tissue (such as intact cardiac trabeculae) aiming to improve tissue formation in vitro or to support the mechanical function of biological muscles when damaged. Such versatility is afforded by chemistry. New customized liquid crystalline monomers are here presented for the modulation of material properties in different applications. The role of synthetic material composition is highlighted demonstrating as, using apparently similar molecular formulations, liquid crystalline polymers can be adapted to different technological and medical challenges.

A Introduction

The natural world is governed by fascinating phenomena that humans tried to understand since prehistoric cultures. At the same time, it represents a source of inspiration to replicate biological mechanisms that have already been optimized by evolution. At different length scales different physical laws rule complexity of nature: from large scale where mechanics acts in the kilometre range down to molecular interaction below the nanometre scale. Some functions are common to different organisms and one of the great challenges of science is focused on their replication for biological and technological applications.1 Even more ambitious is the introduction of novel and combined functionalities that would bring together biological mechanisms proper of different systems by mechanical engineering as well as biological and chemical approaches.2

Herein, we would highlight the role of elastic responsive materials for applications in optics, robotics and biology especially focusing on the role of their chemical design that reflects in physical and chemical properties. Preparation of new monomers and their formulation is presented to approach specific needs of each application. Polymers can be manufactured using different lithographic techniques thus controlling their morphology in a 2D or 3D fashion.1 The choice of the geometry determines the mechanical behaviour and, when the typical length scale becomes comparable with the visible light wavelength, it rules the optical appearance as in the case of structural coloration. Combination of geometrical design with the shape-changing properties of stimuli responsive materials allows to introduce time as a further degree of freedom in the movement that can be dynamically controlled by an external stimulus. This promising research field has been named 4D printing.4 Within this scenario, we will focus our attention on Liquid Crystal Elastomers (LCEs) and Networks (LCNs), rubber-like polymers constituted by chemically cross-linked liquid crystalline networks. These materials are of extreme interest because of their ability to undergo shape changes if properly triggered.5 Depending on their composition, the energy required for these movements can be entirely provided by light6, which makes such elastomers extremely interesting in micro robotics and photonics.7 Light provides undoubtedly advantages, such as high spatial resolution and real-time control on the material properties which can be designed to respond in a smart way, in response to different features (from the colour of the light, to its power or polarization).6

The excellent biocompatibility of certain elastomers9 triggered the idea to use them in a hybrid form, combined with living cells, towards tissue formation in vitro10 or as polymeric muscle. In this contribution, we will discuss the use of both elastomers, and combinations of elastomers with cells, for (micro) robotics (as photoresponsive actuators), for photonics, and for biomedical applications (as cell scaffold or actuators towards the realization of cardiac assist devices). For the different application fields, we will show recent examples from literature and how new polymeric formulations can be adopted as promising chemical solutions that hold specific material requirements towards further development.

B Elastomers, photonics and robotics

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a European Laboratory for Non-Linear Spectroscopy (LENS), University of Florence, via Nello Carrara 1, 50019 Sesto Fiorentino, Italy. E-mail: wiersma@lns.unifi.it
b Department of Physics and Astronomy, University of Florence, Via Sansone 1, 50019 Sesto Fiorentino, Italy.
c Istituto Nazionale di Ricerca Metrologica INRIM, Strada delle Cacce 91, 10135 Turin, Italy.
d Department of Chemistry “Ugo Schiff”, University of Florence, via della Lastruccia 3-13, 50019 Sesto Fiorentino, Italy.

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Liquid crystal elastomers are smart materials that combine the entropic elasticity of elastomers with the ability of self-organization into liquid-crystalline phases. Their deformation is anisotropic and can be very large (up to a factor of 2-3) upon illumination with light. With respect to shape-changing hydrogels, their use is not limited to liquid environments. At present, most of the activity on optical microrobots focuses on shape changing liquid-crystalline polymers, and only very recently biohybrid materials, integrating synthetic polymers with alive phototactic bacteria, algae, or muscle cells, has been described as actuators.

Liquid crystalline polymers are very versatile thanks to their ability to perform different movements (from contraction to bending and curling) without needing complex material preparation since their deformation can be ruled by controlling the mesogen alignment within the polymeric network. Engineering the LC alignment inside a microstructure is possible thanks to a variety of techniques (like micrograting templating and photoalignment) and provides complex movements. In contrast to other systems, no gradient compositions or the use of bilayer structures is needed for out of plane deformations. LC polymers can be divided in two main categories, namely liquid-crystalline elastomers (LCEs) or liquid-crystal networks (LCNs) depending on their glass transition temperature ($T_g$) and elastic modulus. LCEs typically exhibit a larger deformation but their backbone is quite soft, which means they produce small forces under actuation. LCNs, on the other hand, produce stronger forces that can be of the order of hundreds of mN mm$^{-2}$. The liquid-crystalline order at room temperature (typically resulting in a highly anisotropic medium) is lost upon stimulation, resulting in a controlled and fully reversible shape change. Their sensitivity to the environmental conditions is used to prepare structures that adapt themselves or that can exhibit some form of mechanical action when triggered. Preparation of materials that have a photochemical response involves the addition of covalently bonded mesogens or cross linkers that change their physical configuration upon absorption of a photon at a specific wavelength. For this purpose, azobenzenes are the most exploited units generally leading to a two-colour sensitive material (one colour to obtain the cis isomer and the material phase transition and another one to come back to the initial state). Another common mechanism is that of light absorption and energy dissipation into heat in the system. This photo-thermal process enables the LCE phase transition and deformation by an intermediate step where the dye works as a nanoscale heater.

Aiming at 3D printing of such materials at the nanoscale, our research group pioneered the combination of LCNs with Direct Laser Writing (DLW) for light driven photonic and microbotics. This lithographic technique takes advantage of a two-photon absorption process to achieve a 3D spatially confined material polymerization that results in a (quasi) free form fabrication with a resolution up to 100 nm for commercial materials. As polymer patterning by DLW is based on photoactivated processes, acrylate photopolymerization was selected also for LCN inks. This methodology was first introduced by Broer et al. and its use in LC polymer field has continuously grown up to the development of first examples of intelligent polymeric robots in the last years. Material precursors of photosensitive LCN are composed of mesogens functionalized by one or two polymerizable groups. Molecules presenting one polymerizable units (mesogens) can be inserted as side chain in the final material and their terminal group can be used to introduce further functionalities. In the next paragraphs, new monomers containing different functionalizations, from biomolecules (cholesterol) to hydrophilic oligoethylene moieties or plasticising alkyl chains are presented to tailor the polymeric properties. On the other hand, molecules with more than one polymerizable group represent the crosslinkers, which mostly affects the mechanical properties and ensures the reversibility of the shape-change. A photoinitiator is added to control the material preparation by irradiation while a photochromic unit (e.g. azobenzene) is used to dope the polymeric matrix when photoactuation is required. Playing on these components a wide variety of materials with specific properties has been described. Not only their mechanical properties, but also wettability or biocompatibility, can be modulated by mixing different monomers before the polymerization. This approach can replace more complex material post-processing as micro-patterning or plasma/chemical treatment of the surface. On the other hand, looking for mass scale production of functional polymers, synthetic pathways with a high yield and short reaction times are envisioned. Figure 1 shows a typical synthesis of liquid crystalline monomers.

![Figure 1. Synthesis of new LC monomers. General scheme for the preparation of new liquid crystalline molecules containing acrylate groups.](image)

Starting from 4-hydroxybenzoic acid (1), the LC derivative 3 is prepared without chromatographic processes. Its carboxylic groups can be differently functionalized, and was actually employed in different esterification, with commercial or customized phenol, to obtain new monomers. It is very important to design synthetic approaches to obtain mesogens, and their precursors, in gram scale and of a high purity level without the need of expensive and long purification processes.

The chemical formulation determines the key features of the material depending on the specific application: in robotics, real-time control on the actuator and compliant movements are desired; in photonics, high resolution and reproducible deformations of the printed devices are important; while, for tissue engineering, biocompatibility and cell adhesion are needed.

The first issue to be addressed when microscopic objects are the final goal, was the integration of LC inks with a DLW technique. A successful formulation is depicted in Figure 2a. After melting, the material can be easily aligned in standard LC cells and crystallization is not observed for several hours. Scanning the laser inside the nematic mixture allows printing point by point the 3D microstructures. After the removal of the unpolymerized resin, the resulting elastic element shows a contraction along the LC alignment.
direction under light exposure. The LC properties are very sensitive to small molecular changes that can result in different mesomorphic behaviours. A clear example is shown in Figure 2b, where molecule LC1 (the main component of a LC ink) is compared with molecule LC3. Even if they have a very similar structure (only the ester in the central aromatic core has a different orientation), DSC analysis shows a main difference. On cooling, the trace of LC3 presents an exothermic peak which indicates crystallization around 35°C. This means that at room temperature the molecule is not anymore LC and it can not be used as ink for DLW to satisfy our purposes.

When the materials are structured in films at the macroscale, the same molecular change leads to different shape changing behavior. In Figure 2c, two LCN films prepared by the two monomers are reported.

![Figure 2. Inside a LC ink for DLW.](image)

They are both curled at room temperature (for the effect of the strain formed during the macroscopic irradiation) but once activated, both by heating or irradiation, their bending behaviour is totally opposite. In LC1-containing film, the material first becomes flat and then inverts the starting curvature, while with monomer LC3 the material only increases its initial curvature. This different response to the same activation stimulus can be exploited when designing complex deformation, as for origami systems.

Once selected an appropriate ink, many other fabrication parameters have to be adjusted to obtain different mechanical responses. As already introduced, the LC alignments affect the deformation under an external stimulus. By varying the glass slide coating of the LC cell, different deformations can be obtained at the microscale as reported in Figure 3a. These shape-changing microstructures constitute the main toolbox for the application of LCNs in microrobotics and tunable photonics. The most common alignment – the planar homogeneous one - leads to contraction along the nematic director (with expansion in the perpendicular directions), while splayed and twisted organization result respectively in bending and twisting movements. The microstructures can be detached from the glass by the use of a micromanipulator and assembled together or used directly attached to a surface (to modify its wettability), still preserving their deformability under irradiation.

![Figure 3. LCN microstructure deformations as toolbox for microdevices.](image)

The extent of deformation (% of contraction or maximum degree of bending) and its dynamics can be modulated by adjusting the degree of crosslinking. Modifying the fabrication parameter, a good modulation on the actuation can be achieved as reported in Figure 3b-e. The actuation dynamics can be modulated using the same laser power but modifying the scanning velocity. Slower writing speeds lead to a higher energy dose absorbed by the material, resulting in an increased degree of crosslinking and material rigidity. Increasing the writing speed from 15 μm s⁻¹ to 90 μm s⁻¹, the maximum contraction decreases from 23 % to 6 % of the original length while the average contraction speed lowers from 1.2 to 0.3 μm s⁻¹. This approach has the huge advantage of using the same resist to fabricate structure with a different time and mechanical response in a single writing step. On the other hand, a similar effect can be obtained by changing the amount of crosslinker inside the ink without changing the polymerization parameter.

To further improve the material response, we recently tested also monomers with “side-on” architecture where the highest coupling in
between LC cores and polymer backbone leads to an improved deformation for macroscopic films (with maximum contraction from 10-20% in “end-on” polymers to 35-40%).\(^5\) However, the use of the ink containing the “side-on” monomer LC3 presented many challenges for DLW. Even if the monomeric formulation is nematic at room temperature, a typical polycrystal texture is present as shown from the POM images of Figure 4. The use of different annealing temperature/time and aligning coating was not sufficient for the monodomain formation. In this situation, writing microstructures is still possible but the resulting deformation is not predictable. In Figure 4c, the images of different cylinders within homeotropically aligned cell is shown. While for mixture containing LC1, the alignments and the deformation under light (isotropic expansion of the top) were both well predictable, in case of LC4, the movement is not predictable and consequently, more difficult to be implemented in material applications. Improvement of the alignment with LC3 based inks can be achieved by other techniques such as magnetic field application.

LCNs can be employed to modulate light with photonic devices from millimeter scale fiber\(^35\) to microscale 2D arrays or 3D structures. Deformable structures can be used as actuators to mechanically change the position of optical elements, e.g. to steer a beam position (by modulating a mirror position with a rotating fibre),\(^36\) or they can act themselves as photonic materials whose refractive indices and shape can be dynamically changed.\(^37\) Light-tuneable photonic elements have been realized by DLW and integrated on existing photonic circuits to locally control the optical properties by a wireless optical stimulus. Some examples are reported in Figure 5: a 2D diffraction grating changing its pitch under light activation can steer the diffracted beam at different angles directing light in adjacent waveguides,\(^38\) while a LCN cavity can be finely tuned by light to control the resonant frequency of a ring resonator.\(^39\) In the former example, only the LCE shape change rules the mechanical steering of the light beam while in the latter the refractive index change determines the blueshift of the cavity resonance. If both the refractive index change and the shape modulation are combined, more complex phenomena can be observed, such as a multi-channel polarization control enabled by light through a 1D nanograting made by LCN.\(^40\)

![Figure 4](image4.png)

**Figure 4. “End-on” versus “side-on” monomers for DLW ink.** Monomer structure (a), POM images of LC cell (b) and deformation of a micro cylinder (c, scale bar: 15 μm) for mixture containing the “end-on” monomer LC1 (on the lest) or the “side-on” monomer LC3 (on the right).

![Figure 5](image5.png)

**Figure 5. LCN microstructures for photonic application.** a) Scheme of the light control beam steering by a LCN diffraction grating. Adapted with permission.\(^38\) Copyright 2018, Wiley-VCH b) SEM (scanning electron microscope) image and schematic drawing of an Integrated photonic circuit made by a glassy polymeric waveguide vertically coupled with a LCN cavity. Using light the resonance of the cavity can be tuned. Adapted with permission from ref. 39. Copyright 2018 American Chemical Society; c) An optically tuned 2D grating made by LCNs that works as a multichannel polarization control over the diffracted light beams. Adapted from ref. 40, with the permission of AIP Publishing.

The versatility of LCNs in photonics is highlighted by the possibility to integrate them with different materials to fabricate tuneable photonic structures. A meaningful example is the combination of a synthetic photonic crystal (silica opal) and LCE to create tuneable colour filter by temperature control.\(^41\) On the other hand, the implementation of tuneable structural colours can be achieved by the integration of LCN with photonic structures optimized by nature. In collaboration with Dr. Li and Prof. Keller groups, we demonstrated as the *Morpho Menelaus* butterfly wing can be combined with a thermo-responsive elastomeric substrate towards a hybrid temperature responsive platform with different functionalities (figure 6).\(^42\) Structural colour tuning in *Morpho* butterfly wing was provided under heating. In addition, liquid droplet rolling out of the wing was achieved under the same stimulus, creating a responsive natural/synthetic assembly for self-cleaning applications.

![Figure 6](image6.png)

**Figure 6. LCE at the interface with biological photonic materials.** a) *Morpho Menelaus* blue butterfly. b) *Morpho* butterfly appearance observed using an optical microscope. c) SEM (scanning electron microscope) image of ground...
and cover scales composing the wing. d) Wing activation and colour tuning using LCEs. e) Illustration of the cross section scale deformation induced by LCE film contraction.

The same responsive properties can be exploited to mimic some fundamental actions of living systems towards soft robot development, including the ability to sense-and-act.29 The softness of these smart materials is particularly suitable for motion and manipulation mimicking adaptive mechanisms exploited by nature for locomotion in different environments (e.g., crawling and creeping of snails and worms or from slithering of snake).41 Assembly of LCN microstructures with other glassy polymeric elements allowed the fabrication of robots with micrometric dimensions entirely powered by light.8 For example, Figures 7a and 7b show microrobots able to walk on different surfaces or to manipulate objects.

Figure 7. Photonic microrobotics. a) SEM image of micro walker (scale bar: 20 μm) and frame of video demonstrating the walker movement of a microstructured surface under a chopped light (532 nm, 50 Hz). Adapted with permission.44 Copyright 2015, Wiley-VCH; b) A micro-hand in action: sequence of frame where the hand is placed closed to a micro cube hold on a glass tip and then closed by light irradiation to detach this block from its support. Adapted with permission.44 Copyright 2015, Wiley-VCH; c) Simulated deformation for a micro disk irradiated with a flat or a patterned light and frame of the video showing the disk swimming in a 2D square trajectory thanks to dynamic structure light. Adapted with permission.46 Copyright 2016, Springer Nature.

The micro walker is composed of a LCN body with 4 conical legs to reduce the adhesion with the surface. The use of a chopped light allowed the cyclic contraction/expansion of the body with the progressive advancing of the robot.44 In the second example, a cross-like shape microhand has been prepared taking advantage of different LC alignments to obtain the closure of the four fingers toward the same point (Figure 7b).45 Gripping action is controlled by varying the light intensity thus closing/opening the microhand to manipulate objects. In liquid environments, different microswimmers have been demonstrated as shown in Figure 7c,46 whose simple LCN bodies were prepared by manual fibre drawing or photolithography. Their actuation has been achieved with a structured light: using a dynamic illumination pattern, a travelling wave deformation is obtained to guide/steer the robot position in a liquid environment.46 An asymmetric deformation was thus obtained for swimming at the microscale.

C. Biomedical applications

Liquid Crystalline Elastomers use in biology is limited to few examples,47 but their employment as polymeric actuators to substitute or support biological muscle activity would open to new revolutionary medical applications. In particular, two different approaches are currently explored towards muscular tissue engineering by interfacing LCNs at different “biological” levels: LCNs can be employed as cell scaffold to promote the tissue formation or they can be used to directly replaced damaged tissue portions. Regarding culturing tissues in vitro (e.g. for transplantation or pharmacological essays), simple techniques to manipulate the cell organization during their proliferation are needed.

First in 2015, Verduzco et al. exploited the reversible contraction of polysiloxane based LCEs to create a dynamic cell scaffold.48 The cyclic substrates mechanical deformation guides the growth of cardiomyocytes with a certain degree of unidirectional alignment.49 When plated on standard support (as Petri Dish), the same cells proliferate giving rise to totally disorganized patterns thus far from reproducing their organization in the native tissue.

More recently, we reported how cells can “feel” the molecular anisotropy of LCNs directly influencing cell alignment without the need of external stimulation.50 Initially, biocompatibility and cell adhesion on LCNs prepared by acrylate chemistry need to be assessed. Also in this case, the choice of the LC formulation is crucial. Different monomers have been prepared and tested for culture of different cell lines (e.g. dermal fibroblasts and murine myoblasts). Besides the use of LC1, we tested the new mesogens shown in Figure 8a: LC5 contains cholesterol (a typical biological LC), and LC6 presenting oligoethylene glycol chains (the main constituent of gels used as cell scaffold with hydrophilic properties). Polymers containing LC5 present a chiral nematic phase, while using LC6 standard nematic LCNs are prepared. The three sets of materials (containing respectively only LC1, LC5 and a mixture of LC1 and LC6) have similar wettability (Figure 6b): all films are hydrophilic with water contact angles that decrease from 70° (for LC1) to 60° (for LC5 and LC6). On the other hand, cell adhesion dramatically changes on the different substrates. Only for LCNs containing exclusively LC1, cells can adhere (Figure 8c) while, introduction of the other compounds results in a totally ineffective scaffolds. Such interesting preliminary results, although negative, represent an important starting point towards optimized substrates. Applying the synthetic approach presented in Figure 1, different monomers, presenting modulated hydrophobicity, can be prepared to achieve a selective adhesion of specific cell lines, but also to test the bacterial adhesive properties.

Both dermal and muscular cells seeded on LC1 containing scaffolds demonstrate spontaneous unidirectional alignment, which was coincident with the nematic director. Only in case of planar homogenous LC scaffold, the cell alignment is formed while exploiting other molecular arrangements (e.g. isotropic polymers of LC ones but presenting homotropic alignment) a standard chaotic organization was observed.50 Statistical analysis on the nuclei orientation highlights as the quality of the cell alignment increases with the crossinglink degree (increasing the material rigidity).50 The formulation resulted fundamental also for cell differentiation: the formation of myotubes occurs only for a specific crosslinker...
concentration, while in other cases myoblast fusion was not observed even if the nuclei were well aligned on the substrate. This evidence makes clear the strong interplay in between the synthetic substrate with highly tailored chemistry and the living cell growth.

Interestingly, similar LCN based scaffolds were also demonstrated able to promote maturation of cardiomyocytes (CM) derived from human induced pluripotent stem cell (hiPSC) in a shorter period of culture than standard support. Such improved maturation results of particular interest since to date the use of hiPSC-CM in regenerative medicine is mainly limited by the difficult obtainment of mature and adult-like cells.

A second approach for tissue engineering is to replace a damaged biological tissue with a functional synthetic equivalent. For instance, LCNs, hybrid materials of elastomer and cells or biological tissue as cell scaffolds, should be used to light activate the cardiac tissue in vivo.

Figure 8: LCNs as cell scaffolds. a) monomers tested for cell culture; b) water contact angle of different LCNs (all contain 20% mol/mol of crosslinker LC2): from left to right, materials prepared by LC1, LC5 and a mixture of LC6 and LC1 (ratio 1:1 mol/mol); c) optical image of a cell adhesive LCN containing LC1 after 24 h from seeding of dermal fibroblast; d) optical image of myoblast culture on LCN with homogeneous planar alignment and the relative directionality histogram. The histogram reports the probability density function to find a cell tilted by a certain angle θ with respect to the nematic director (white arrow). Scale bar: 100 µm.

D. Role of composition towards a new material generation

Many applications of smart actuators, from microfluidics to robotics (e.g. swimming and gripping actions in water) and tissue engineering, required a particular focus on the material response in biomimetic underwater environment.

A recent paper describes as the addition of a low molecular weight LCs (SCB) inside a standard acrylate based LCN leads to formation of a gel with improved light deformability under water. The effect of SCB is that of plasticizing the network and lowering the phase transition temperature which can be driven by a photothermal effect also in water, where heat dissipation is higher than in air (thus limiting the approach of this effect). Using stripes with 1-2 cm length, different movements have been achieved in water.

We moved our attention on how to obtain a similar effect by bonding dopants to the network. This new approach should be very useful to extend this result to 3D printing at the millimetric and micrometric scale. Using covalently bonded additives will provide a stable crosslinked plasticized network that does not suffer from additive deswelling over time. This chemical design will be then applicable to multi step DLW processes for the integration of different photoresists (that would require developing bath in proper solvents after the printing process).

Plasticizing chains can be adopted to modify the mechanical properties of LCNs as reported in Figure 10 using 2-ethyl-hexyl chains. DSC traces show the glass transition of polymers prepared with LC1 adding two different plasticizing agents (PL): the commercial 2-ethyl hexyl acrylate (PL1) and the custom-made PL2, where the alkyl chain is bonded to a mesogenic core. In the first case, PL1 is not able to efficiently modulate the thermal properties and it

Figure 9. Artificial muscles based on photoactivated LCNs. The polymeric actuator is able to support a mouse right ventricle trabecula contraction: scheme of the experiment (a) and examples of force trace from the LCN and the muscle when allowed to synchronously contract (b). Adapted with permission. Copyright 2019, Wolters Kluwer Health.
This journal is characterized by a $T_g$ very similar to the LC1 one (the $T_g$ decreases from 26 to 22 °C). Moreover, the high volatility of the compound limits the reproducibility of the procedure and the scalability of material processing. Motivated by this results, we prepared the new compound, PL2, finally obtaining a $T_g$ of 15°C and solving the volatility issue.

![Figure 10. Effect of plasticizer chains on LCN.](Image)

This survey on LCE properties and applications highlights as tailoring the material composition replies to the open demands of different research areas. The material development starts from the molecule synthesis as the fundamental building blocks for functional devices that takes their first inspiration by nature. In this respect, new synthetic routes have been proposed and analysed with their pros and cons.

The monomer architecture (from “side-on” to “end-on”) strongly affects the LC alignment in standard cell, while playing on different functionalizations both material elasticity and cell adhesion can be modulated. The use of pendant mesogens with methoxide terminal group supports adhesion of different cell lines, but this property is lost for material containing cholesterol or oligo ethylene moiety with little effect on the surface wettability. Exploitation of other chemical groups on the mesogens will be explored towards selective cell adhesion or antibacterial properties. Moreover, the insertion of plasticizing chains into the mesogen structure of this class of polymers allows an improved control on the glass transition behaviour with respect to commercial plasticizer.

It is interesting to notice as the complex biological functions can be mimicked in a simplified way by modulating the intrinsic properties of smart polymers. Following this route, customized optical and mechanical properties could establish elastic responsive polymers as promising materials for large scale tunable photonics, microrobots and synthetic/biologic systems in biomedical applications. Looking for energy saving applications, a further challenging inspiration is provided by nature: responsive devices powered by sunlight.

### Conflicts of interest

There are no conflicts to declare.

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### Notes and references

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