Development of Light-Responsive Liquid Crystalline Elastomers to Assist Cardiac Contraction

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Rationale: **Despite major advances in cardiovascular medicine, heart disease remains a leading cause of death worldwide. However, the field of tissue engineering has been growing exponentially in the last decade and restoring heart functionality is now an affordable target; yet, new materials are still needed for effectively provide rapid and long-lasting interventions. Liquid crystalline elastomers (LCEs) are biocompatible polymers able to reversibly change shape in response to a given stimulus and generate movement. Once stimulated, LCEs can produce tension or movement like a muscle. However, so far their application in biology was limited by slow response times and a modest possibility to modulate tension levels during activation.**

Objective: **To develop suitable LCE-based materials to assist cardiac contraction.**

Methods and Results: **Thanks to a quick, simple, and versatile synthetic approach, a palette of biocompatible acrylate-based light-responsive LCEs with different molecular composition was prepared and mechanically characterized. Out of this, the more compliant one was selected. This material was able to contract for some weeks when activated with very low light intensity within a physiological environment. Its contraction was modulated in terms of light intensity, stimulation frequency, and t_{on}/t_{off} ratio to fit different contraction amplitude/time courses, including those of the human heart. Finally, LCE strips were mounted in parallel with cardiac trabeculae, and we demonstrated their ability to improve muscular systolic function, with no impact on diastolic properties.**

Conclusions: **Our results indicated LCEs are promising in assisting cardiac mechanical function and developing a new generation of contraction assist devices. (*Circ Res*. 2019;124:e44-e54. DOI: 10.1161/CIRCRESAHA.118.313889.)**

Key Words: elastomers ■ mechanics ■ muscle contraction ■ physiology ■ tissue engineering

Despite major advances in cardiovascular medicine, end-stage heart failure remains a leading cause of morbidity and death worldwide.[1] Therefore, in recent years, many researches were focused on building functional tissues to restore heart mechanical functionality. A challenge in the clinical arena is to exploit smart materials to support or restore the cardiac mechanical function. Ideally, materials should be able to modulate their strength, kinetics, and stiffness to fit the features of striated muscles, paving the way for different treatments. For example, they may serve as cardiac assist devices, to assist the ventricular systolic function of a failing heart.[2]

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So far, many attempts to create materials or devices have failed in reproducing the natural muscle function and regulation. In this regard, biocompatible polymers, able to work as actuators, are gaining interest. Among smart materials, liquid crystalline elastomers (LCEs) are able to respond to external stimuli in a reversible manner to generate movement or tension.[3] This unique behavior is because of the combination of the main features of liquid crystals with those of elastomers; in fact, LCEs conserve the orientational order and sensitivity to different external stimuli (such as temperature, pH variations, light, electric, or magnetic fields) from the former, and elasticity and resilience to mechanical stresses from the latter.[4,5] However, even if LCEs are commonly called artificial muscles,[6] their application in biology is still limited to few examples.[7–10] Major drawbacks of such elastomers as mechanoactive materials in

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Novelty and Significance

What Is Known?

- Cardiac injury, as a consequence of acute or chronic ischemia, hypertension, infection or inflammation, results in irreversible loss of cardiac cells, which are incapable of clinically significant regeneration in adults.
- To date, left ventricular assist devices or inotropic drugs are not feasible for long-term support of cardiac contraction and are only used as a bridge-to-transplantation in failing patients.
- Strategies for myocardial regeneration based on stem cell approaches have proved ineffective so far and are a long way from effective clinical applications.

What New Information Does This Article Contribute?

- A palette of biocompatible light-responsive materials capable of producing tension or movement like a muscle was developed and mechanically characterized.
- Light-responsive materials can be continuously activated for a very long period (weeks) with very low light intensity within a physiological environment.
- The mechanical properties of these artificial muscles can be modulated in terms of light intensity, stimulation frequency, and duty cycle to fit different contraction amplitude/time courses of human muscle.
- Artificial muscles mounted in parallel with cardiac trabeculae are capable to improve muscular systolic function, with no impact on diastolic properties.

Cardiac muscle repair represents one of the major medical challenge. Here, we develop a palette of biocompatible liquid crystalline elastomer-based materials capable to assist cardiac contraction. We demonstrate that tension level and time course of these responsive materials can be light- and chemical-modulated to reproduce the twitch contractions of cardiac samples from patients affected by specific diseases and that it is possible to develop concentric contraction, as in cardiac chambers. Finally, we show that liquid crystalline elastomer is able to improve the contractile force of a muscle without affecting the systolic and diastolic dynamic.

Nonstandard Abbreviations and Acronyms	
LCE	liquid crystalline elastomers

cardiovascular applications, so far, were related to high transition temperatures and tedious synthetic procedures.[11]

Thus, to develop LCEs with myocardial-like features, a quick and versatile synthetic approach and the understanding of how mechanical properties depend on material composition and stimulus characteristics were needed. Here, we report the preparation and complete analysis, in terms of passive and active mechanical properties, of a palette of biocompatible LCEs. Because light provides certain ideal features (it is noninvasive, allows energy transfer through thin wires or even without a physical connection between source and object, remote control, and selective activation), we focused on light-responsive materials demonstrating their ability to work synchronously with intact cardiac muscles. Their ability to enhance systolic tension without affecting diastolic properties set the stage for a new generation of self-contracting cardiac assist devices.

Methods

The authors declare that all data supporting the findings of this study are available within the article or from the corresponding author on reasonable request.

General Procedure and Materials

Molecules M1, M3, CL1, and CL3 were purchased by Synthon Chemical; 6-(acryloyloxy)hexyl acrylate (CL2) and Irgacure 369 were purchased from Sigma Aldrich. Molecules M4 and D1 were prepared as previously reported,[6,12] while synthesis of CL4 and M3 is described in the Online Data Supplement (Online Scheme I and II and Online Figures I and II). Complete characterization of the mixtures is also reported in the Online Data Supplement (Online Table I and Figure III).

LCE Film Preparation

Film preparation followed a procedure already reported[13] and briefly described in the Online Data Supplement.

Biological Essays

M1CL1_20 films were preliminary assessed as cell scaffold for C2C12 cells as already reported.[14] C2C12 cells (from ATCC American Type Culture Collection; 10801 University Boulevard Manassas (VA) 20110–2209 used at 10th passage) were seeded directly on M1CL1_20 films surface and growth in DMEM F-12 supplemented with 10% FBS, 1% penicillin/streptomycin solution, in a humidified chamber at 5% CO_2 and 37°C. After 24 hours, M1CL1_20 films were transferred in new dishes, washed with PBS, and fixed using solution number one of the Diff-Quik Kit. Fixed cells were fluorescently labeled after simultaneous incubation for 15 minutes with Hoechst 33342 (Thermofisher) at a concentration of 1 µg/mL, and with Wheat Germ Agglutinin 488 (WGA488, Thermofisher), at 5 µg/mL. Imaging was performed with a confocal microscope (Nikon Eclipse TE300), equipped with the Nikon C2 scanning head Coherent CUBE (diode 405 nm), Melles Griot (Argon 488 nm), and Coherent Sapphire (Sapphire 561 nm) lasers. Emission filters for imaging were 452/45, 514/30, and 595/60 nm.

Mechanical Measurements on LCE Strips

The passive tension of artificial muscle samples was measured by applying controlled elongation steps (see Online Data Supplement for detailed methods and Online Figure IV). Maximal active tension was evaluated when the tension reached a steady state plateau level. Force kinetics was assessed by measuring the half time of force rise and decay. The complete description of the experimental procedures is reported in the Online Data Supplement.

A 514.8±0.3 nm fixed-wavelength continuous-wave diode pumped laser (beam diameter: 900±100 µm) was used as light source for LCE strip activation, providing a confined illumination with an intensity range between 0 and 200 mW/mm^2 (measured on the strip). Alternatively, we used a 525±32 nm LED source with a beam of ≈5 cm in diameter providing a wide illumination with an intensity between 0 and 1.5 mW/mm^2, measured on the LCE strip.

Statistics

In the Table and Figures 3 through 5, data from LCE are expressed as mean±SEM, and the number of samples (ie, the number of LCE strips that underwent a specific measurement) are indicated in the respective legends. For each LCE strip, 10 experimental replicates (ie, raw data

Table. Composition and Mechanical Analysis of the Palette of LCE*

Name	Mesogen	%	Crosslinker	%	n†	Active Tension (mN/mm^2)	Passive Tension (mN/mm2, µm)
M1CL1	M1	88	CL1	10	6	387±45	1.83±0.15
M1CL1_15	M1	83	CL1	15	4	393±82	2.46±0.64
M1CL1_20	M1	78	CL1	20	4	423±17	3.21±0.47
ANOVA‡						*P*=0.883	*P*=0.077
M1CL2	M1	88	CL2	10	5	134±43§	1.12±0.35
M1CL3	M1	88	CL3	10	4	194±62	0.56±0.14§
M1CL4	M1	88	CL4	10	4	69±38§	0.39±0.05§
ANOVA‡						*P*=0.001	*P*=0.001
M2CL1	M2	88	CL1	10	5	179±45	1.45±0.38
M3CL1	M3	88	CL1	10	5	87±62§	1.68±0.33
M4CL1	M4	88	CL1	10	3	285±54	1.23±0.39
ANOVA‡						*P*=0.004	*P*=0.129

LCE indicates liquid crystalline elastomer.

*All samples contain Irgacure 369 in 1% mol/mol and azobenzene D1 in 1% mol/mol. Mean passive and active tension, as well as activation and relaxation kinetics, were obtained by illuminating the samples at the maximal power actuation (>100 mW/mm^2) in air at room temperature.

†No. of LCE strips used for the mechanical measurements.

‡The statistical test used to calculate *P* values for each data set was a 1-way ANOVA.

§*P*<0.01 with the Dunnett test used to compare each LCE with the reference material M1CL1.

acquired in a specific condition) were averaged to obtain representative values of different parameters (eg, active tension and passive tension in Table, tension and kinetic parameters in Figures 2 through 5). Data reported in Figure 2C and 2D are obtained from a single strip in each condition (confined or wide illumination). In this experiment, mean data are obtained by averaging the tension levels during the day as specified in the figure. Statistical analysis was performed using OriginPro 2018. In brief, all sets of variables were checked for normality (Shapiro-Wilk test) and for homogeneity of variances among groups (Levene Test). The *t* test was used to compare 2 data sets while 1-way ANOVA and Dunnett tests were used for multiple comparisons. In each figure legend, the statistical tests used were reported.

Results

Preliminary Assessment

Development of a Palette of LCE and Biocompatibility Assessment

Acrylate-based LCEs were selected for this work, given the recently demonstrated biocompatibility[10,14] and the easy and quick preparation by photopolymerization (Figure 1).[15] Compounds indicated as M1-4 (mesogens, Figure 1D) and CL1-3 (crosslinkers, Figure 1E) are liquid crystalline molecules. CL4 does not present liquid crystalline behavior but still acts as a crosslinker, leading to the formation of a structured network during polymerization. This complex polymeric structure is responsible for the mechanical properties of the LCEs and for their nonsolubility in common solvents. Mesogens M1 and M2 differ only for the terminal alkyl group (R) on the aromatic core, while M3 has one additional aromatic ring. These 3 mesogens are responsible for a side-chain end-on LCE architecture (Figure 1B), while M4 leads to side-chain side-on (Figure 1B) LCE architecture.[16] The materials prepared were indicated as MxCLy (where x and y indicate the monomer and the cross-linker used, respectively) and their compositions are reported in the Table.

All mixtures contained Irgacure 369 in 1% mol/mol to induce the polymerization process by UV light, and the organic dye azobenzene D1 in 1% mol/mol to induce the response[17,18] of the material upon illumination with a green light (Figure 1C).[12] The use of this specific dye, with a short half time of the cis state, producing a fast and short-lasting photoinduced thermal effect, allowed us to generate LCEs with sub-millisecond dynamics of light-driven actuation. Using D1 in such a small amount resulted in a concomitant isomerization of the dye and material heating during illumination. Both effects contribute to the phase change of the material and thus to its activation.[19] The temperature variation caused by light exposure[20] is local and immediately dissipated when the illumination is switched off.

A full chemical and physical workout has been performed on the above-mentioned molecules, and details on their synthesis together with a complete characterization of the mixtures are reported in the Online Data Supplement (Online Table I and Figure III).

All films were prepared with a homogeneous planar alignment (Figure 1F), resulting in a contraction along the direction of the molecular alignment and an expansion in the perpendicular direction when the shape-change is triggered by light.[21,22] Moreover, we chose a fixed thickness of 20 µm for all tested LCE strips, for the following reasons: (1) while producing LCE strips, 20 µm is the ideal thickness to obtain a good compromise between resistance and uniformity of molecule alignment (thinner strips would have a low tear resistance while alignment uniformity would be compromised in thicker strips); (2) 20 µm thickness provides a sufficient light penetration across the whole film thickness, allowing the simultaneous activation of the entire film; and (3) 20 µm is the average transversal dimension of ventricular cardiomyocytes.

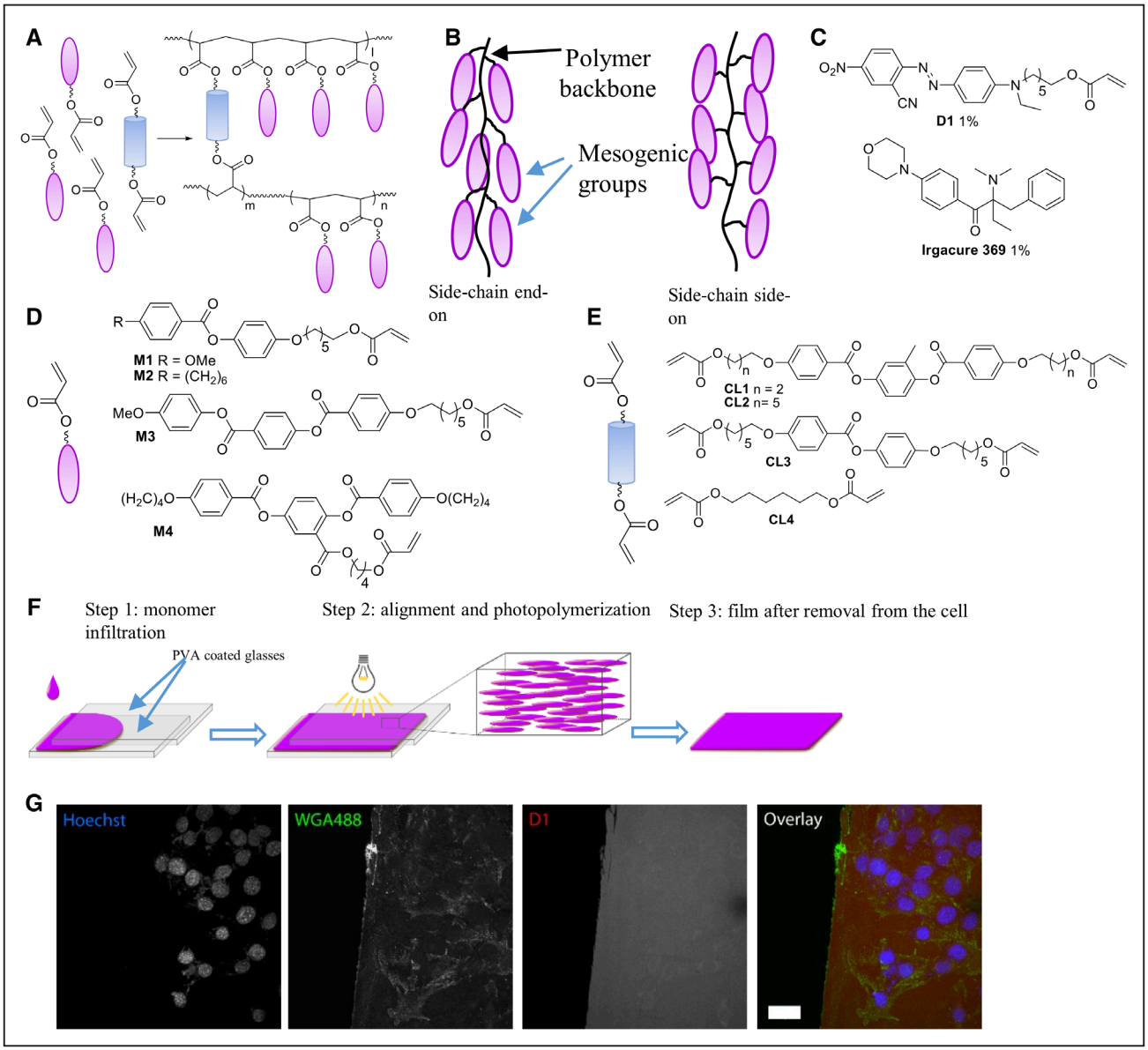

Figure 1. Liquid crystalline elastomer (LCE) film components, preparation, and biocompatibility assessment. **A**, Acrylate-based synthetic strategy; **B**, Scheme representing the end-one and the side-on side-chain material architectures; **C**, Common components to all mixtures; **D**, Mesogens and **E**, crosslinkers used; **F**, Scheme are representing the different steps needed for the preparation of LCE with homogeneous planar alignment. **G**, Confocal microscopy images of C2C12 seeded on M1CL1_20 containing D1 dye (red channel) after 24 h incubation in standard conditions. Cells were labeled with the fluorescent Hoechst (blue channel) and WGA488 (green channel) dyes, which specifically target DNA and sialic acid—a component of the plasma membrane—respectively. The healthy condition of the cells clearly demonstrates that the D1 dye does not perturb cellular attachment and growth on M1CL1_20. Scale bar, 30 µm.

The majority of the samples was prepared with 10% of the crosslinking agent, except for M1CL1_15 and M1CL1_20, which contain 15% and 20% of crosslinker, respectively. We prepared and evaluated such samples because we had previously demonstrated that different amounts of crosslinker affected the material elastic modulus, as well as the actuation time.[13,23]

Biocompatibility of LCE materials was previously tested by us using strips not containing the azobenzene dye.[14] To prove that azobenzene does not affect biocompatibility of the films, C2C12 cells were seeded on M1CL1_20 and subsequently imaged with a confocal microscope. As shown in Figure 1G, C2C12 cells adhered on films containing the D1 dye, exhibiting the standard shape and dimension that we previously observed in films not containing the dye.[14]

Choice of the Material

LCE films were cut into strips whose shape and dimension were similar to those of thin cardiac trabeculae (200–400 µm width, 1–4 mm length). LCE strips were mounted isometrically between a force transducer and a linear motor, both connected to micromanipulators (Figure 2A).[24] Contraction of the LCE strips was activated with a confined illumination provided by a green light laser (0–200 mW/mm^2, see Methods section); the strips were then relaxed by switching the light source off.

We first studied the mechanical behavior of LCE strips by measuring passive tension and active tension in the air on stimulation with the green laser light source at its maximal intensity. Examples of the full light–induced activation-relaxation

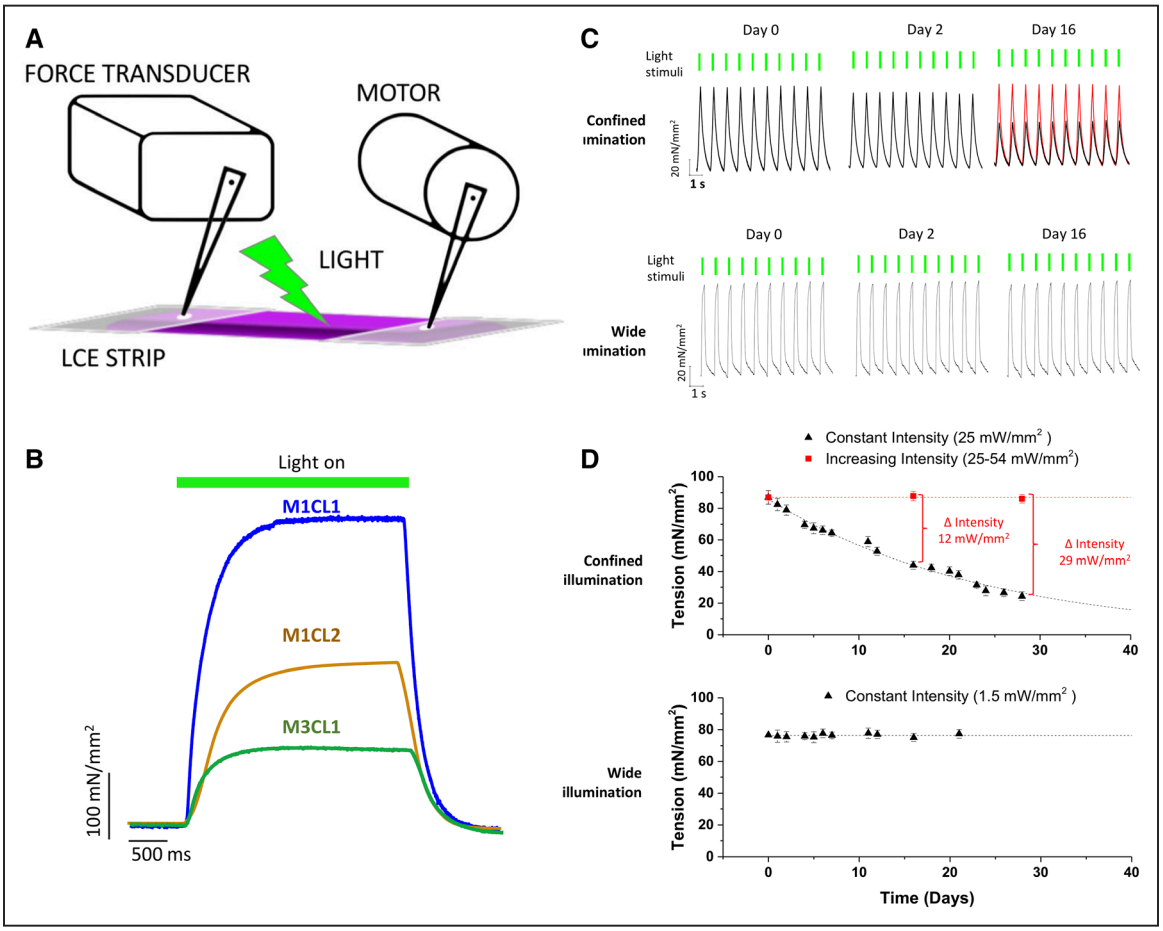

Figure 2. Choice and longevity of the material. **A**, Liquid crystalline elastomer (LCE) films were cut into strips, mounted isometrically between a force transducer and a linear actuator and activated with a green light provided by a laser (confined illumination: air environment) or a LED lamp (wide illumination: water environment). **B**, Representative force measurements for selected LCE strips (M1CL1, M1CL2, and M3CL1): active tension is dependent on the chemical composition. **C**, Long-term experiments performed with a confined (**top**) or wide (**bottom**) illumination for 24 and 21 d, respectively. **D**, Confined illumination (**top**) led to a decay of 68% tension of twitch amplitude after 24 d ($P<0.001$ vs day 0) restored by progressive increase of the light power over time. Wide illumination (**bottom**) produced no tension reduction in the long-term period. Twitches are acquired for 30 s once every 15 min and mean data are obtained by averaging the tension levels during the day. One-way repeated measurements ANOVA was used to test the significance of tension variations over time.

cycle of 3 different LCE materials (ie, M1CL1, M1CL2, and M3CL1) are reported in Figure 2B. Both passive tension and the maximal level of generated force depend on the composition of each material (Figure 2B; Table).

Specifically, passive tension was found to be strongly dependent on the amount of crosslinker and, as expected,[13] it increased with the percentage of crosslinker in the mixture. Interestingly, the amount of crosslinker did not significantly affect active tension (Table). In contrast, we found that active tension was modulated by the chemical structure of the crosslinker (Table). In particular, the increase in the spacer length or the decrease in the number of aromatic rings in the rigid core depressed both active and passive tension generated by the analyzed materials. This effect was more evident for passive tension and consistent with a reduction of the interactions between the liquid crystalline components and the polymer backbone, which caused an increase of the material flexibility. A different relationship between molecular structure and tension was observed for changes of the mesogen series: the active tension was strongly influenced by the mesogen structure, while the passive tension was unaffected. The decrease of the active tension can be most likely attributed to the molecular structure containing extended flexible chains or a higher number of aromatic rings in the rigid core. An interesting point was raised by the analysis of the mechanical properties of 2 different monomers, both having 3 aromatic rings and similar flexible chains (M3 and M4, Figure 1) but characterized by dissimilar side-chain material architectures, that are, end-on and side-on, respectively. In particular, when we switched from the end-on to the side-on architecture, we observed an increase of the active isometric force developed by the LCE, with no significant change in passive tension (Table).

As expected, active tension can be directly modulated by light intensity (Online Figure VA) in a nearly linear dose/response manner.

We also observed that the initial strip length affected the maximal active tension developed by the LCE (M1CL1, Online Figure VB and VC). Indeed, maximal tensions were reached when the LCE strips were stretched by >2% of their slack length; however, LCE strip stretching led to a significant increase of passive tension. Nonetheless, our strips already developed a relatively high tension (≈250 mN/mm²) when they

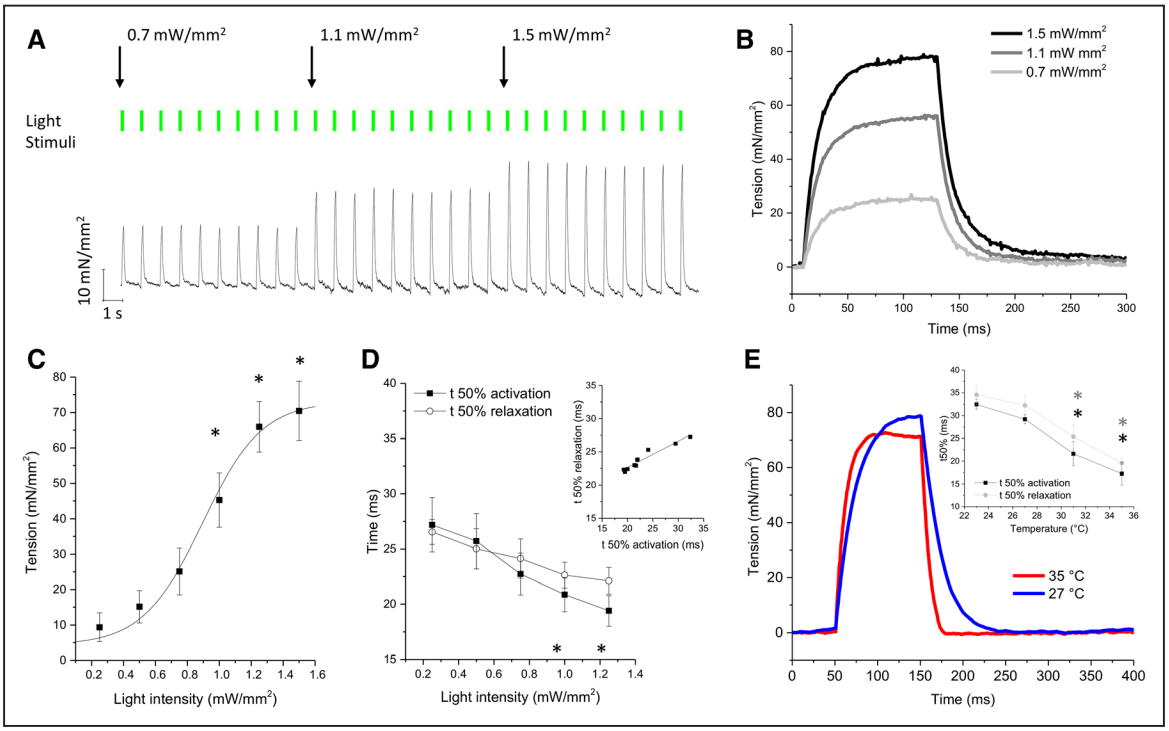

Figure 3. Liquid crystalline elastomer (LCE) mechanical performance modulated by LED stimulus in water environment. **A** and **B**, Representative traces of LCE activation by increasing light intensities (from 0 to 1.5 mW/mm²) resulted in (**C**) progressively higher twitch tension production (*P<0.01 for light intensities ≥1 vs 0.25 mW/mm²) with (**D**) progressively increased kinetics of activation (*P<0.01 for light intensities ≥1 vs 0.25 mW/mm²) but no changes in relaxation times. **E**, Effect of higher (35°C) vs lower temperature (27°C) on LCE showed faster activation and relaxation kinetics with the rise in temperature (*P<0.01 for temperature above 30°C vs 23°C). Mean data are from 6 LCE strips. One-way repeated measurements ANOVA was used to test the significance of tension and kinetic variations on increasing light intensity and temperature.

were activated at their slack length, where passive tension was negligible (Online Figure VC). Therefore, it was possible to identify a range of lengths where passive tension is rather low while active tension is largely above that developed by intact cardiac muscle (>100 mN/mm²).[25]

To assess the dependence of the mechanical properties of LCE strips on film thickness, we also prepared and characterized M1CL1 films with 30 and 50 µm thickness (Online Figure VI). Film thickness was linearly and positively related to the active isometric force. When the force was normalized to the cross-sectional area, mean tension values were comparable and independent from the film thickness, in line with the behavior of striated muscle[25,26] (Online Figure VI).

On the basis of this mechanical analysis, we selected M1CL1 as the best compromise between (low) passive and (high) active tension values. The high maximum tension of M1CL1 allowed us to use low light intensities to reach an effective force generation. For instance, with an intensity of 25 mW/mm² we obtained active tensions of ≈100 mN/mm², a value that is 3 to 4 times higher than that measured in the human healthy cardiac muscle[24,25] under basal conditions, and comparable to the maximal twitch amplitude measured during positive inotropic stimulation (eg, during β-adrenergic stimulation or postrest potentiation).

Longevity of the Material

In the beating cardiac muscle, every contraction can be described as a twitch, with a period of force development (from resting to peak tension) followed by a relaxation phase (from peak to resting tension). To mimic cardiac twitches, M1CL1 was activated by properly setting both the duration (ie, the t_{on} and the t_{off} of the light source) and the power of the applied light stimulus (Figure 2C).

To test the longevity of our materials, twitch contraction parameters were analyzed for a period of about one month (Figure 2D). Isometrically mounted LCE strips were activated at 1 Hz stimulation frequency, with an intensity of 25 mW/mm² and a t_{on} and t_{off} of 250 and 750 ms, respectively. With this illumination energy, at day 0, the LCE produced a baseline active tension of ≈90 mN/mm². When the illumination power was kept constant, a slow exponential decline of twitch amplitude occurred (time constant equal to ≈24 days), with no changes in contraction kinetics. However, by progressively increasing the light power over time (of just a few mW/mm² over a month), we were able to maintain the tension constant for the whole 30-day period, again with no changes in contraction kinetics. This slow loss of functionality could be ascribed to the degradation of the dye by photobleaching.[27]

With the aim of reproducing working conditions of cardiac muscle, we performed the longevity assessment of our material in a water environment, that is, with LCE strips completely immersed in an experimental water bath. Furthermore, for these experiments, we used a green LED lamp (525±32 nm) as light source, with an illumination beam of about 5 cm in diameter, providing a wide illumination. The peak light intensity provided by the LED is much lower (1.5 mW/mm²) if compared with the laser, but it proved to be sufficient to fully activate the material in solution (Figure 2C). LCE strips were

activated with a 1 Hz repetition frequency, with a t_{on} and t_{off} of 250 and 750 ms, respectively (Figure 2C). In these conditions, when the illumination power was kept constant, no decay of twitch amplitude and no changes in contraction kinetics occurred over a period of 20 days (Figure 2D), suggesting that the photobleaching of the organic dye under these conditions (simulating an in vivo use) is undetectable over such a period of time.

LCE Functional Analysis

A series of experiments were designed to assess the modulation of LCE twitch contraction by a wide activating LED-light beam in a water environment.

Active tension can be modulated easily by changing the intensity of the activation stimulus, as evident for M1CL1 strips in Figure 3. Activation of the LCE strips in solution at increasing light intensities (from 0 to 1.5 mW/mm^2) resulted in a progressively greater twitch tension (Figure 3A; Online Movie I). Changes in light intensity produced only a slight change in twitch activation times (Figure 3B through 3D) with no variation on relaxation kinetics. Interestingly, twitch kinetics were symmetrical and extremely fast with half time of force development and relaxation both in the order of 20 to 30 ms (inset in Figure 3D).

A quick evaluation of the effects of temperature on LCE activation was performed by activating and relaxing the same LCE strip at different temperatures, showing that higher operating temperatures led to faster contraction kinetics (Figure 3E).

To assess if a rate-modulation occurred in our materials (as in native cardiac muscle), the LCE strips were stimulated with pulses at various frequencies (0.5–3 Hz) while keeping the light intensity (1.50 mW/mm^2) and the stimulus duration (t_{on} =150 ms) constant. Representative traces are reported in Figure 4A, and single twitches are shown normalized and superimposed in Figure 4B. The mean data (Figure 4E) show that, in these conditions, neither the amplitude nor the time evolution of the twitches was frequency dependent. However, it was possible to mimic the physiological force-frequency dependency and rate-adaptation of twitch duration by modulating both light intensity and t_{on} (Figure 4C and 4D). Figure 4F shows how a slight increase in light intensity and a small reduction of t_{on} could be used to mimic frequency-dependent adaptation of twitch amplitude and duration, respectively. Instead, the kinetics of force development cannot be modulated by the sole variation of the t_{on}, as it is already extremely fast even at low pacing rates (see below).

LCE to Assist Cardiac Contraction

As a proof of concept, intact cardiac muscles and LCE strips were allowed to work in parallel. In this experiment (Figure 5A) intact ventricular trabeculae dissected from the right ventricle of mouse hearts were mounted isometrically, stabilized in a Krebs physiological solution and paced at 1 Hz through external electrodes. After some baseline recordings from the intact trabecula, LCE strips of various thickness (20 and 50 µm) were mounted above the muscle and were connected to the same force transducer and motor lever arm. If no illumination was provided (LCE-off), the presence of the LCE strip did not affect the mechanical performance of the muscle (Figure 5B and 5C), in line with the negligible passive tension of LCE strips at slack length. When the illumination was turned on (LCE-on, activated by a LED source with an intensity of 1.5 mW/mm^2) and synchronized with the electrical stimulation of the muscle, the additional mechanical work done by the LCE enhanced maximal twitch tension levels to 100 mN/mm^2 or above (Figure 5B and 5C). The LCE strip thickness positively correlated with the additional tension provided. The contraction time course in the LCE-on and LCE-off configurations was similar also at high pacing rate (Figure 5D), suggesting that the contraction kinetics of LCE strips are sufficiently fast not to hamper force development and relaxation of the native muscle.

Discussion

Summary

We identified biocompatible LCEs as candidate materials to develop novel cardiac contraction assist devices. The most performing material was demonstrated to support long trains of light pulses (>20 days total duration, 86.400 beats per day) in water, with no rundown of contraction parameters. The same material allowed us to modulate twitch contraction parameters by properly selecting the light stimulus in terms of intensity, stimulation frequency and t_{on}/t_{off} ratio. Force development and relaxation kinetics during twitch contractions were extremely fast, half times of both phases being ≈20 to 30 ms, that is, similar to those of human atrium and twice faster than those measured in the human ventricle. Finally, relevant to the practical application of our material, LCE strips proved to be capable of working in parallel and simultaneously with cardiac trabeculae. We found a positive effect of the material on systolic function, with no impact on diastolic properties.

Chemistry as a Determinant of Passive and Active Tension

The LCEs described here presented passive and active tension levels in line with data reported in the literature for similar materials[6,11,28] and higher than those reported for human cardiac samples.[29] Both parameters could be finely tailored by modifying the material components (crosslinkers and mesogens) and, as expected,[13] passive tension increased with the crosslinker percentage (Online Figure VIIA). We also noted that higher initial lengths resulted in higher active tension levels, resembling the ascending limb of the active tension/length relationship of cardiac muscles.[30] However, while intact muscle presents an optimal length, that is, ≈10% longer than the slack length, our LCEs developed high forces without being elongated, that is, in a range of lengths where passive tensions are negligible (Online Figure VC). LCE samples having different crosslinker percentage (M1CL1 and M1CL1_15, Online Figure VIIB and VIIC), chosen because of the direct correlation between crosslinker amount and passive tension, behave similarly, that is, showed a direct relationship between the length of the LCE strip and active tension. This preliminary assessment determined M1CL1 as the LCE of choice because it offers the best compromise in terms of mechanical properties (passive versus active tension).

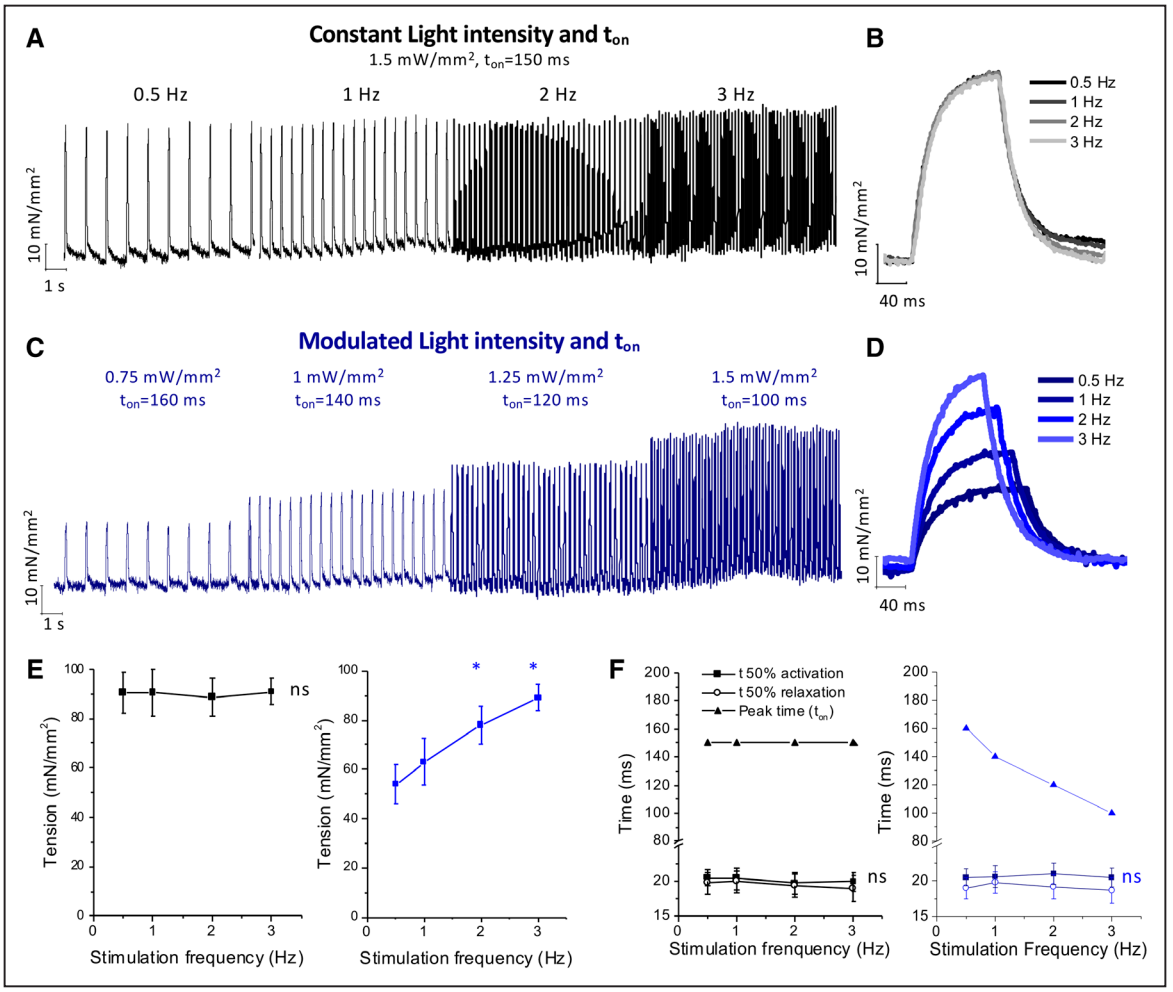

Figure 4. Force-frequency relationship and rate-adaptation of liquid crystalline elastomer (LCE) twitch duration in water environment. **A**, Representative traces of LCE twitches stimulated at progressively increasing stimulation frequency with a constant wide illumination intensity (1.5 mW/mm^2) and time of activation, t_{on} (150 ms), or (**C**) by modulation of both light intensity (0.75–1.5 mW/mm^2) and t_{on}(160–100 ms). The traces obtained at the various frequencies in **A** and **C** are shown superimposed in **B** and **D**, respectively. **E**, Mean±SEM data from 4 LCE strips of peak tension at progressively increasing stimulation frequency. The positive force-frequency dependence was reproduced by modulation of light intensity (*P<0.001 at 2 and 3 Hz vs 1 Hz). **F**, Rate adaptation of twitch duration is reproduced by changes in t_{on} which directly affect peak times. Peak times values are dictated by the illumination time (peak time =t_{on}) and no experimental variability is reported. The kinetics of force development (t 50% activation) and relaxation (t 50% relaxation) do not vary with the t_{on}/t_{off} ratio. One-way repeated measurements ANOVA was used to test the significance of tension and kinetic variations on changes in stimulation frequency at constant and modulated light intensities.

Light Sources and Material Longevity

During long-term experiments performed with a confined laser illumination at constant intensity in air, twitch amplitude declined by 68% after 24 days (Figure 2C and 2D). Such decay could be overcome by progressively increasing the light power over time (of a few mW/mm^2 over a month), maintaining the tension levels constant for the entire 30-day period, with no changes in contraction kinetics (Figure 2C and 2D). These results suggest that the observed tension decay was likely related to the photobleaching of the organic dye. Notably, dye concentration in our mixture (currently 1%) could be easily increased to further improve the material lifetime. An additional evidence of the durability and stability of our materials comes from a specific subset of experiments using a 10 mm long LCE strip: during the month-long experiment at constant laser power, tension could be immediately restored to 100% by moving the laser beam from the central illumination spot (≈1 mm^2) to a neighbor region of the LCE strip where the dye molecule is unbleached. These results confirmed that a dye photobleaching phenomenon rather than a deterioration of the LCE mechanical properties was responsible for tension rundown over time (Online Figure VIII).

Such photobleaching-associated mechanical rundown of the material was overcome by the use of a wide illumination in water environment (Figure 2C and 2D) allowing the LCE to beat for a remarkable number of cycles (about 2 million, to date) without any loss of mechanical performance. The absence of tension decay in a long-term experiment suggests that the LCE could efficiently beat for very long times (possibly years) in physiological conditions and thus such materials could be considered for a long-term in vivo implantation as cardiac support devices. Longer duration experiments are needed to estimate the actual lifetime of our LCE.

Kinetic Modulation

Kinetic parameters were evaluated by using a wide illumination allowing the simultaneous and complete activation of the

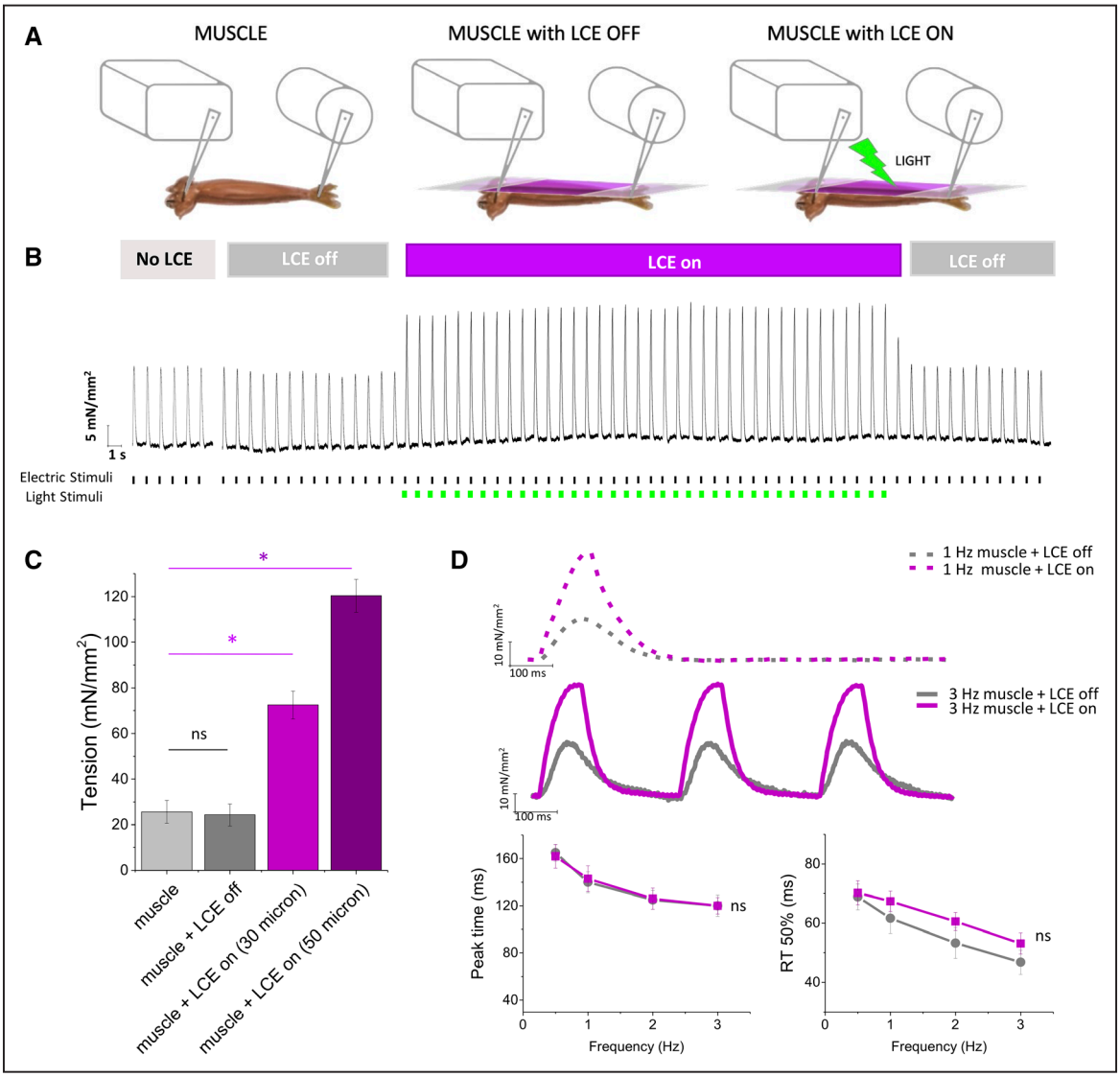

Figure 5. Liquid crystalline elastomer (LCE) to assist cardiac contraction. A, Scheme of the experiment performed with a mouse right ventricle trabecula mounted in parallel with a LCE film of similar dimension between the same force transducer and length control motor lever arms (25°C, Krebs solution). **B**, Examples of force recordings from the muscle and the LCE allowed to synchronously contract. **C**, When the LED is switch-on (LCE-on), the global force is the sum of the individual forces generated by the muscle and the LCE. The additional tension provided depends on the LCE thickness (30 or 50 μm). **D**, Representative traces and mean data at various frequencies (0.5–3 Hz) showing that the time course of assisted contractions reproduce that of the muscle alone. Mean data are from 5 muscle+LCE preparations. Data were tested for significance by applying paired *t* tests (muscle+LCE-off and muscle+LCE-on conditions vs muscle alone).

whole LCE strip because of the large beam (≈5 cm). Previous data on LCEs demonstrated how their application in biology was limited by a slow response time[11] that was only partially circumvented by the preparation of LCE nanocomposites (responding in the range of hundreds of milliseconds[28,31]). In this respect, our material represents a good improvement over current literature, with contraction and relaxation kinetics one order of magnitude faster than those previously reported. Indeed, our LCE strips responded in the range of 10s of milliseconds (Figure 3D and 3E). Moreover, the higher the light intensity, the faster the kinetics of contraction and relaxation phases (Figure 3D). Kinetics of activation and relaxation were also affected by the temperature (Figure 3E). Higher temperatures of the water bath allowed our materials to work above their glass transition temperature, thus making them more flexible[13] and leading to a lower passive tension. Overall, because of the very fast kinetics, the effects of light intensity and temperature on contractile kinetics were rather small (in the order of few milliseconds) and the most important parameter affecting the contraction time course was the duration of the light illumination (t_{on}) during simulated twitches. This property allowed us to reproduce the rate adaptation of twitch duration (typical of cardiac muscle) that the material does not intrinsically exhibit (Figure 4). The kinetics of LCE contraction could be too fast to couple with the activity of the human myocardium, especially in pathological conditions when the cardiac contraction is often prolonged.[32–34] However, contraction kinetics could be slowed down by increasing the series compliance of the artificial muscle. To test this possibility, long strips (2–8 mm) were activated by a small laser beam (≈1 mm²) focused to the central region of the strip, with the 2 nonactivated ends behaving like additional series compliance (Online Figure IXA

and IXB). Interestingly, the half time of activation and relaxation progressively rose with the increase in strip length, thus confirming that a larger series compliance slows contraction kinetics. Using partial material illumination, we were then able to mimic the contractile behavior of specific ventricular samples from failing patients, as reported in Online Figure IXC and IXD.

LCE to Assist Cardiac Contraction

The final goal of this study was to follow the route of recently proposed epicardial contraction assist devices.[35] Because external ventricular compression is the most physiological way to improve pump function,[36,37] as proposed by recent preclinical investigations,[38] we tested our fittest material, M1CL1, in an experimental setting where cardiac muscle contraction was supported by the LCE working in parallel with a mouse cardiac trabecula (Figure 5). The sum of the parallel forces compared with that developed by the muscle alone demonstrated that our LCE strips provided a significant systolic assistance. The time course of the contraction of the entire system (muscle+LCE) remained comparable to that of the muscle alone also at high pacing rates (Figure 5D), thus demonstrating that the LCE behaves as an effective contraction assist device without affecting diastolic compliance.

Furthermore, we found that this material could mimic the work-loop of the heart contraction by adjusting the length of the preparation, as described in Figure 2A (Online Figure XA and XB). Force was recorded while controlled motor movement reproduced the 4 phases of in vivo cardiac contraction. In a 2-dimensional linear system, the phases of the cardiac cycle could be reproduced as follows: isometric contraction (phase 1, Online Figure XA), quasi-isotonic shortening (phase 2), isometric relaxation (phase 3), and quasi-isotonic lengthening (phase 4). This force/length relationship showed that the LCE produced external mechanical work and further confirmed the LCE as a promising material for the cardiac contraction assistance. As a proof of concept, to move from the linear tension-length study to the working myocardium, we tested the capability of a simple LCE-based contraction assist device to develop concentric pressure, as in the chambers of the heart (Online Figure XC and XD). We proved that our materials developed significant concentric pressure, 3× higher than that of human ventricular myocardium (Online Figure XD).

Conclusions and Perspectives

Present results demonstrate that LCEs can be suitable to assist cardiac muscle contraction; they exhibit fast kinetics and adjustable systolic tension without impairing relaxation. Together with the demonstrated in vitro biocompatibility of LCE films, these results pave the way to a new generation of LCE-based contraction assist devices through the development of more complex prototypes such as additional artificial chambers, resembling currently used left ventricular assist device. Alternatively, the described materials could allow external dynamic compression of the failing heart (the so-called artificial cardiomyoplasty) by bladder-shaped (for left ventricular global failure) or patch-shaped (for necrotic areas) LCE-based epicardial contraction assist devices. Atrial function could be also assisted by atrial-specific epicardial devices.[2] Finally, thanks to the patient-designed 3-dimensional printing, our material could advance cardiac surgery, for example, to replace damaged papillary muscles or to seal large ventricular or septal defects.[39]

The demonstrated versatility in the modulation of LCE mechanical properties obtained by changing the molecular parameters and the actuation stimuli may also allow us to match the features of skeletal or smooth muscles, thus extending this technology to noncardiac applications. Examples in this sense would be the design of artificial smooth muscle sphincters or gastrointestinal tracts, as well as artificial skeletal muscles.

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Disclosures

None.

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