

REVIEW ARTICLE

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Bioactuators based on stimulus-responsive hydrogels and their emerging biomedical applications

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Abstract

The increasingly intimate bond connecting soft actuation devices and emerging biomedical applications is triggering the development of novel materials with superb biocompatibility and a sensitive actuation capability that can reliably function as bio-use-oriented actuators in a human-friendly manner. Stimulus-responsive hydrogels are biocompatible with human tissues/organs, have sufficient water content, are similar to extracellular matrices in structure and chemophysical properties, and are responsive to external environmental stimuli, and these materials have recently attracted massive research interest for fabricating bioactuators. The great potential of employing such hydrogels that respond to various stimuli (e.g., pH, temperature, light, electricity, and magnetic fields) for actuation purposes has been revealed by their performances in real-time biosensing systems, targeted drug delivery, artificial muscle reconstruction, and cell microenvironment engineering. In this review, the material selection of hydrogels with multiple stimulus-responsive mechanisms for actuator fabrication is first introduced, followed by a detailed introduction to and discussion of the most recent progress in emerging biomedical applications of hydrogel-based bioactuators. Final conclusions, existing challenges, and upcoming development prospects are noted in light of the status quo of bioactuators based on stimulus-responsive hydrogels.

Introduction

Soft actuators, composed of compliant materials and mechanically deformable constructs, exhibit prominent advantages, including being lightweight, having continuous deformability, and exhibiting body compliance^{1–4}. Such features in softness and shape adaptability to ambient environments have triggered the rapid research development of soft actuators in various areas, such as soft robotic locomotors^{5,6} and manipulation grippers^{7–9}. The most recent advances have witnessed the application of soft actuators for biomedical uses; these bioapplication-oriented actuation machines can be referred to as

bioactuators. Owing to their small sizes, large degrees of movement freedom, and capability to work in confined spaces and on rough terrains, bioactuators are promising for solving conundrums for some in vivo and in vitro bio-related uses. These bioapplications involve biochemical sensors for physiological monitoring^{10–12}, artificial muscles for sports-injury rehabilitation^{13–16}, and drug delivery carriers for disease therapeutics^{17–19}. However, it is worth noting that to realize these biomedical functionalities, actuators are typically required to be compatible with the internal environment and constituents (e.g., cells, tissues, and organs) of the human body. Therefore, soft materials with superb biocompatibility are desirable for fabricating bioactuators.

Over a long period of exploration for suitable materials targeted at bioactuator applications, the brightest spotlight has been gradually shone on stimulus-responsive hydrogels, owing to their high water content, sensitivity to ambient environment stimuli, superior biocompatibility,

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and tunable structural and physiochemical properties^{20–22}. Hydrogels with high water content (more than 90 wt%) can switch their sizes and shapes via swelling/deswelling with the change in water content in the polymer network, which can be induced by various external stimuli (e.g., pH value, temperature, humidity, light, electricity, and magnetic field) from the ambient environment^{23–28}. In this way, hydrogel-based actuators can be realized by using stimulus-responsive hydrogels and are capable of performing deformation and movement in response to various environmental cues. The superb biocompatibility is a major advantage of hydrogels, enabling them to outperform their counterparts (e.g., silicone elastomer) in implementing actuation for bio-related applications. Benefiting from this advantageous feature, hydrogels can interact with the internal constituents of the human body in a safe and human-friendly manner, and actuators based on hydrogels are hence able to function without adverse impacts on human physiological activities. Meanwhile, hydrogel materials possess structures similar to those of native extracellular matrices (ECMs), which further enhances their feasibility in bioactuators for implantable applications even down to the cellular level^{29,30}. In addition, the biophysical and biochemical properties of hydrogels can be easily tuned by different fabrication scenarios and composition proportions. This feature endows hydrogels with user-defined properties to meet varying demands of practical bioactuation tasks and further expands the applicability of hydrogel-based bioactuators.

Bioactuators have attracted tremendous research and practical interest and have undergone unprecedented development in the past decade. Hydrogels with sensitivity to external environmental stimuli are considered ideal materials in this field, and cutting-edge research has introduced hydrogel-based bioactuators into biomedical applications, including biosensing, targeted drug delivery, artificial muscle regeneration, and cell manipulation. In 2015, Peppas et al. extensively reviewed the basic theories and practical applications of stimulus-responsive hydrogels³¹. Based on their long-term research interest and outstanding contributions in the design and application of biomaterials, they presented a comprehensive identification of and discussion on multiple hydrogel-response mechanisms and the corresponding uses in medical and industrial fields, which are of crucial significance to driving the development of stimulus-responsive hydrogels. After this publication, some new material systems and applications emerged and propelled the rapid development of this field. One example involves encapsulating micro-/nanosize magnetic particles into hydrogel matrices to fabricate magnetically responsive hydrogels, which were recently employed for biomedical actuator applications such as artificial muscles and cell manipulation^{32,33}. Moreover, the addition of a novel research field, called cell

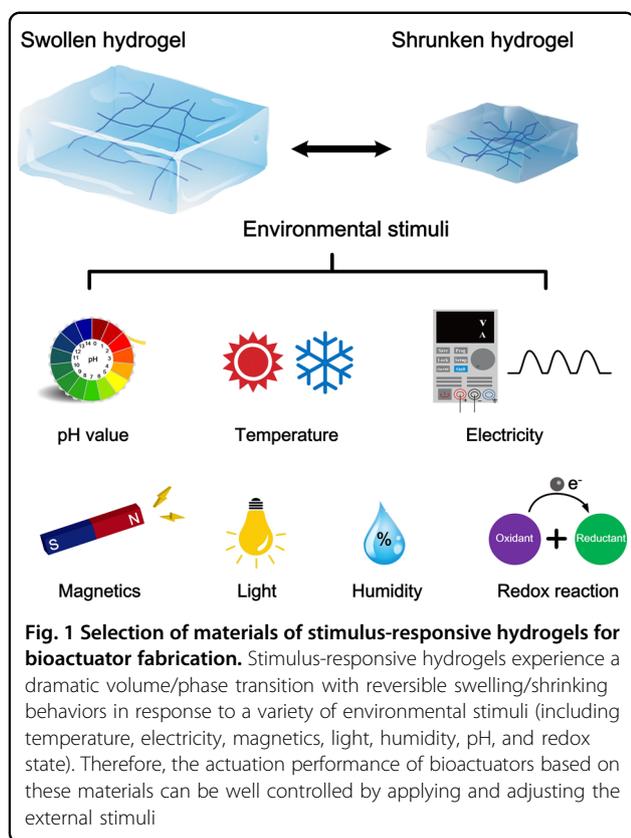
microenvironment engineering, has further expanded the application spectrum of stimulus-responsive hydrogels into the study of stimuli-induced cell behavior and the related disease mechanisms^{21,34}. Hence, this burgeoning field is still urgently demanding a review paper reflecting the most recent advances to provide comprehensive overviews and guidelines to researchers committed to developing biomedical-oriented actuators based on stimulus-responsive hydrogels. In this article, we summarize the material selection of hydrogels with multiple stimulus-responsive mechanisms for actuator fabrication, present the state-of-the-art advances in hydrogel-based bioactuators aimed at emerging biomedical applications, and finally note existing challenges to and future prospects of the development trends of hydrogel-based bioactuators.

Various stimulus-responsive hydrogels for actuator fabrication

Choosing hydrogel materials with appropriate stimulus-responsive properties is strategically important for bioactuator designs, as the actuation performance of a hydrogel is based on its specific response to physical or chemical signals, and various stimulus-responsive mechanisms can find a perfect match with demands from different practical applications. In this section, we summarize the underlying actuation mechanism of hydrogels with diverse stimulus-responsive characteristics, the stimulating factors of which include but are not restricted to pH, temperature, and electric and magnetic fields (Fig. 1). In addition, a concise illustration of potential biomedical applications for each stimulus-responsive hydrogel material is presented.

pH-responsive hydrogels

pH-responsive hydrogels constitute a major branch of the stimulus-responsive hydrogel family. They sensitively respond to pH changes in the ambient environment with deformation behaviors in terms of swelling and shrinking. The underlying mechanism is associated with the functional pendant group in the hydrogel polymer backbone, which is ionized and enables charge-density redistribution of the hydrogel when experiencing pH variation in the surrounding medium³⁵. In this way, electrostatic repulsion is generated between adjacent polymeric chains with similar charges (both positively or negatively charged), which induces the absorption of water inside the whole polymer network of the hydrogel, making the hydrogel swell and change its shape (Fig. 2a)³¹. According to the property of the pendant group in the hydrogel network, pH-responsive hydrogels can be classified into two major categories, i.e., anionic hydrogels and cationic hydrogels. The swelling/deswelling behavior of anionic hydrogels depends on the dissociation of anionic pendant groups on the hydrogel, which dissociate only when the pH of the



surrounding aqueous solution exceeds the acid dissociation constant (pK_a) of the hydrogel, losing protons and making the intrinsically neutralized hydrogel negatively charged. Then, swelling of the charged hydrogel is triggered by the osmotic pressure induced by electrostatic repulsion. In contrast, the cationic hydrogel functionalized with cationic groups (e.g., amine groups) behaves in the opposite way, becoming protonated and swelling at a lower pH ($<pK_a$).

Among various pH-responsive hydrogels, poly(acrylic acid) (PAAc) has received the most attention owing to its low cost and ease of fabrication. The functional pendant group on PAAc is a carboxylic group that is protonated at low pH and undergoes dissociation by releasing protons when the pH exceeds the pK_a of the hydrogel. Accordingly, the polymeric network retains its original size through chain–chain hydrogen bonding below the pK_a of PAAc and swells above this pK_a due to electrostatic repulsion among the anionic polymer chains. Benefiting from its pH-responsive property bringing about a significant size change ascribed to the subtle pH variation, PAAc-based hydrogels have found extensive applications as bioactuators for various applications^{36–40}. For instance, 2-hydroxyethyl methacrylate (HEMA) could be adopted for the PAAc architecture to produce a pH-responsive bioactuator based on copolymer p(AAc-co-HEMA) as a

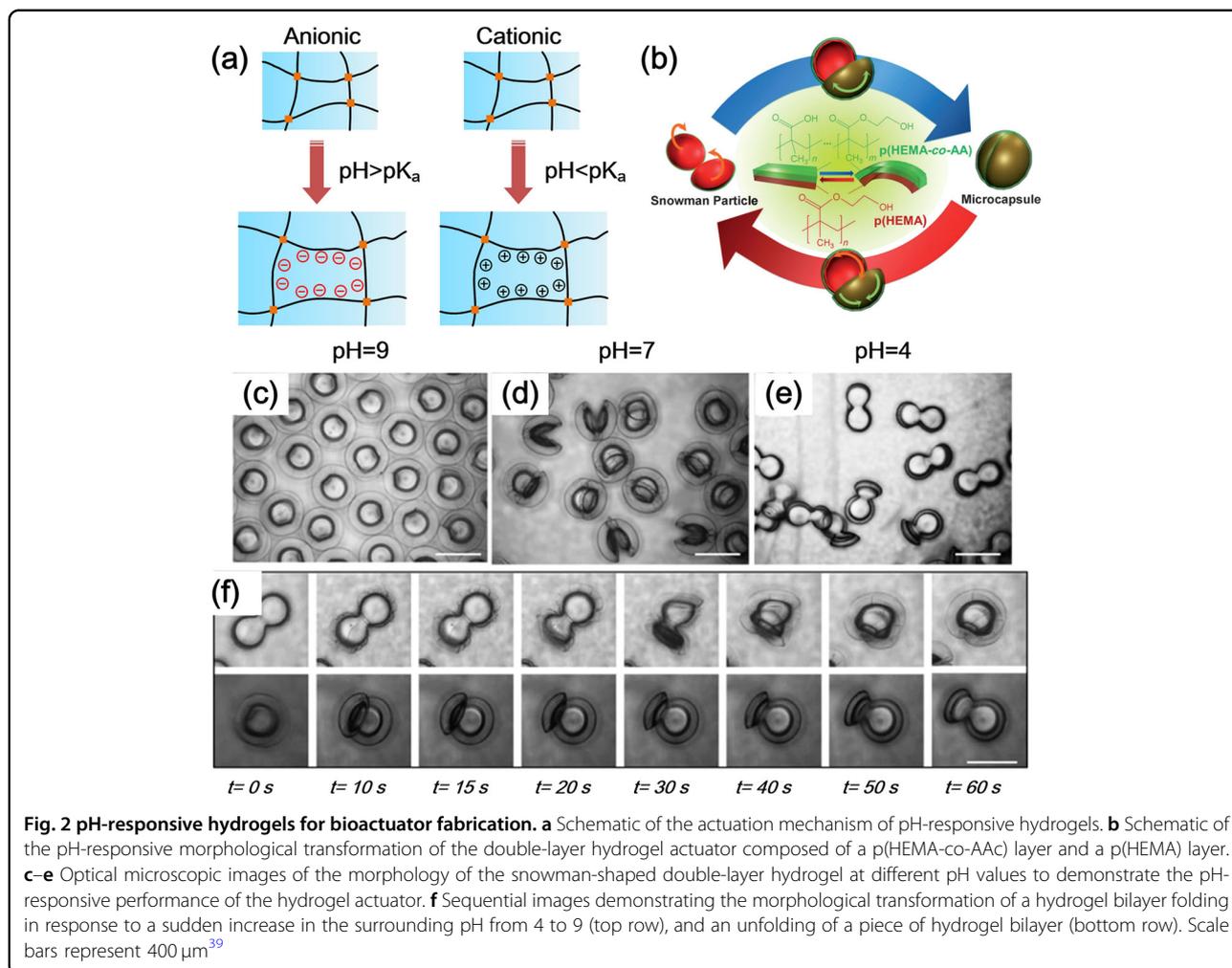
valve for autonomous flow control in microfluidics^{37,38}. The hydrogel valve, with a tailorable size and pattern, could be directly obtained through photopolymerizing precursor inside microfluidic channels with the help of custom-made photomasks³⁷. The fabricated smart valve regulated the flow rate and direction inside the microfluidic system according to ambient pH changes with a short response time of 8 s and generated a swelling pressure of ~ 5.5 kPa, showcasing the potential for self-regulated biosensors and drug delivery systems. By integrating a layer of pH-responsive p(AAc-co-HEMA) with another layer of non-pH-responsive p(HEMA), a bilayer hydrogel actuator that folds/unfolds in response to pH changes can be realized (Fig. 2b)³⁹. The pH-responsive layer can swell to 10 times its initial volume; hence, via swelling/deswelling of the bilayer when the pH is varied, the microscale hydrogel actuator with a snowman shape can present reversible transformation in shape and dimensions between 2D flat microparticles and 3D spherical microcapsules in 1 min (Fig. 2c–f).

Other pH-responsive hydrogels have also shared the stage for bioactuator fabrication, such as poly(acrylamide) (PAAm)-based hydrogels^{41–44}, poly(methacrylic acid) (PMAA)-based hydrogels^{45–47}, and poly(vinyl alcohol) (PVA)-based hydrogels^{48–50}. By taking the neutral PAAm hydrogel as an example, it shrank when placed in an aqueous solution with a pH either at a relatively high ($pH > 11$) or low ($pH < 3.5$) level, and a larger degree of hydrogel shrinking occurred in an acidic environment than that in alkaline media⁴¹. This interesting phenomenon can be explained by the size difference between different ions that diffuse out of the hydrogel when it is placed in different chemical environments. Diffusion of the hydroxide ion occurs under acidic conditions, while the hydronium ion leaves the hydrogel under alkaline conditions. Since the hydroxide ion is smaller than the hydronium ion, it passes through the membrane more easily and quickly, making the hydrogel shrinkage more intense at a low pH. In addition, one issue worth noting is that the actuation process is limited to a certain period before the ion diffusion reaches thermodynamic equilibrium, when ions are restricted from moving out of hydrogels into the bulk solution.

In all, pH-responsive hydrogels have presented advantages in easy accessibility in implementation and high specificity in sensing and responding to external pH changes; hence, they have been extensively used as biosensors and drug-delivery carriers. However, their inferiority in terms of the uncontrollable on/off state of actuation must be overcome for practical applications.

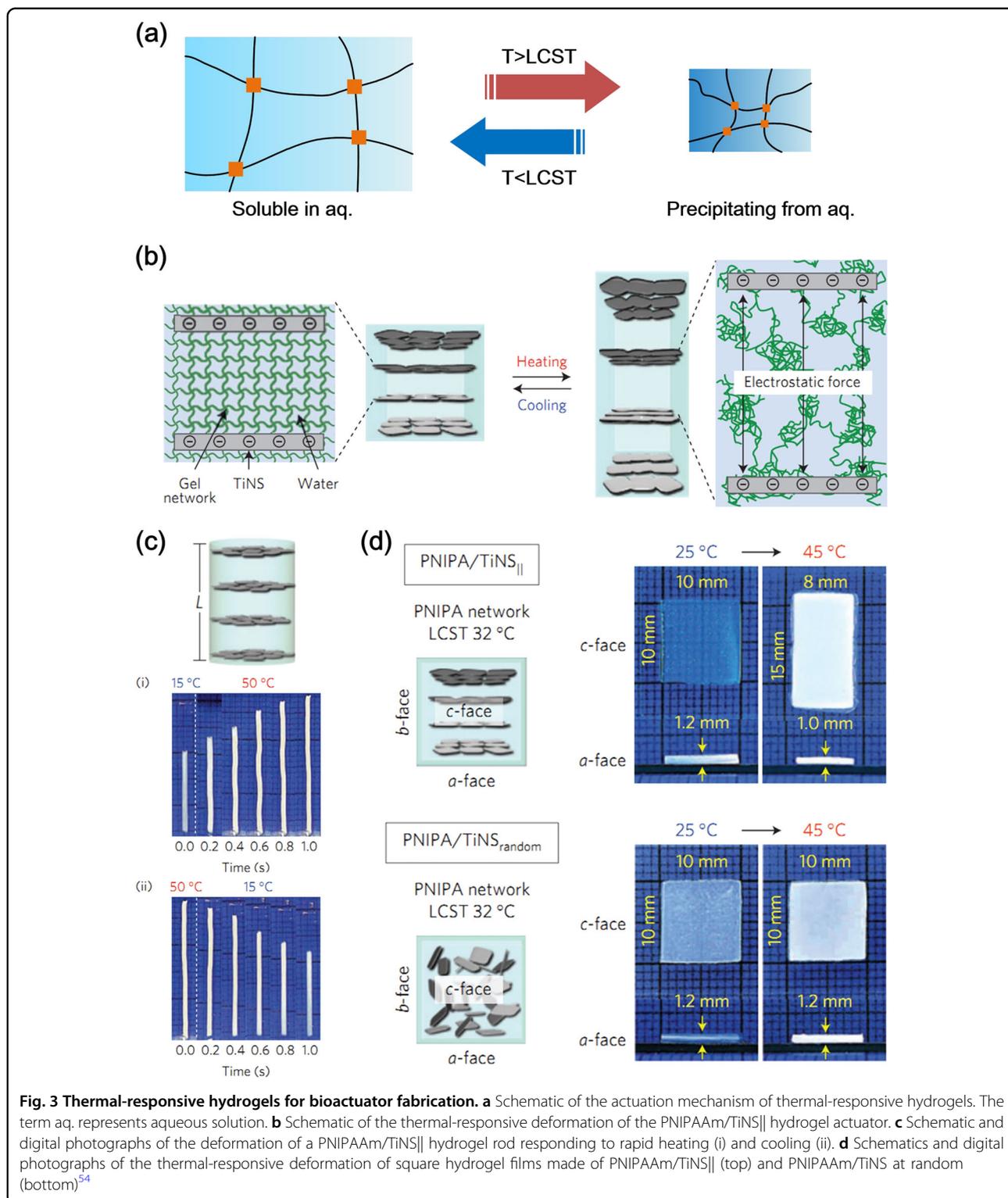
Thermal-responsive hydrogels

Thermal-responsive hydrogels are polymeric hydrogels that undergo reversible and dramatic shifts in



morphological features and physical phases in response to temperature variations in the surrounding environment. The underlying swelling/deswelling mechanism of thermal-responsive hydrogels can be attributed to their temperature-triggered solubility shift in aqueous solutions. Therefore, the critical solution temperature is usually regarded as an index to evaluate the volume-phase transition of a hydrogel. For instance, the very first investigated thermal-responsive hydrogel in history, poly (N-isopropylacrylamide) (PNIPAAm), possesses a lower critical solution temperature (LCST) of $32\ ^\circ\text{C}$ and a tunable swelling ratio ranging from 2 to 20⁵¹. Below this point, the hydrogel is soluble in water, whereas it shrinks and precipitates from the aqueous solution at an environmental temperature higher than its LCST due to the weakened interchain hydrogen bonding (Fig. 3a). When the system cools (to a temperature lower than the LCST), the PNIPAAm swells and returns to a soluble form, indicating its reversibility in a thermally induced volume-phase transition.

PNIPAAm has been intensively studied in bioactuation applications for its sensitivity to minimal local temperature oscillations^{52–55}. In a bilayer self-folding system consisting of a biodegradable, thermal-insensitive polycaprolactone (PCL) layer and a thermal-responsive PNIPAAm layer, the PNIPAAm layer experienced volume and phase transitions according to the temperature variation, while the attached PCL layer remained undeformed and thus restricted the deformation of PNIPAAm⁵². Correspondingly, the polymeric bilayer underwent folding and unfolding when the PNIPAAm layer swelled and shrank in response to the temperature, respectively. This self-folding actuator was further demonstrated to be capable of realizing cell capture/release in response to external temperature changes. To improve the response dynamics with a faster response rate, the molecular construct of PNIPAAm was tailored by grafting PNIPAAm single chains onto its polymeric network⁵³. The final architecture resembled a comb, where one terminal of the introduced PNIPAAm chain



was grafted to the polymer backbone and the other end was freely mobile. Due to the hydrophobic interaction between alkyl groups on the mobile end of terminally grafted chains, the aggregation of the polymer network

above the LCST that induces hydrogel deswelling could be intensified. Consequently, the comb-type gel presented improved deswelling kinetics and faster deswelling rates than those of the pristine homopolymer.

In an interesting study, the volume transition of PNIPAAm, which normally shrinks upon heating and swells upon cooling, was reversed by adopting cofacially arranged titanate nanosheets (TiNSs) in the hydrogel matrix (Fig. 3b)⁵⁴. In specific terms, the distance between cofacial TiNSs decreases and increases during heating and cooling, respectively, due to the corresponding hydrogel structural collapse and swelling. Therefore, the electrostatic repulsive forces inside the hydrogel are enhanced and weakened in response to the respective increase and decrease in temperature, further inducing elongation and shortening of the hydrogel actuator with a deformation rate of $70\% \text{ s}^{-1}$ (Fig. 3c). It is worth noting that this phenomenon is closely related to the orientation of the TiNSs. The hydrogel resists contraction perpendicular to the nanosheet arrangement direction while conforming to the deformation along the orientation. The anisotropic thermal-responsive behavior failed to occur when the TiNSs were randomly distributed in the PNIPAAm hydrogel (Fig. 3d). Additional work has been carried out to tune the LCST of PNIPAAm, especially to cater to *in vivo* biomedical actuating applications. The favorable temperature should be higher than 37°C (i.e., human body temperature) and lower than 42°C , at which point hyperthermia occurs. By incorporating AAm into the polymer chain, the LCST of the copolymer could be adjusted to a reasonable value of 39°C ^{56,57}.

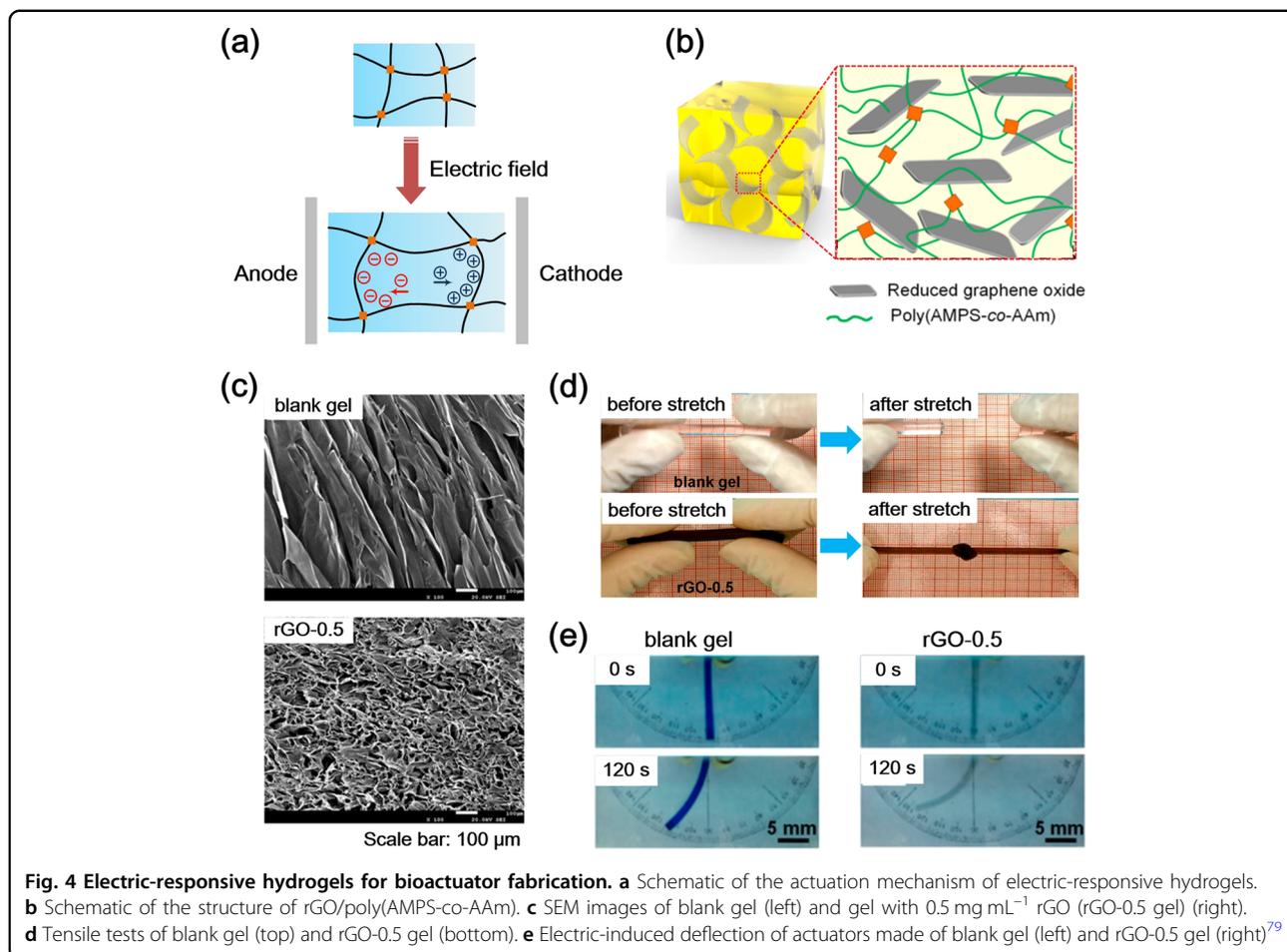
Despite their popularity in studies on hydrogels with thermal-responsive properties, PNIPAAm-based hydrogel actuators are intrinsically associated with a potential shortcoming for biomedical applications, since the unpolymerized residue for PNIPAAm polymerization, namely, acrylamide-based monomers, may cause neurotoxicity^{58,59}. To address this safety issue, alternatives such as poly(ethylene glycol) (PEG), poly(3-caprolactone) (PCL), and poly(propylene glycol) (PPG) have been used to provide a wider selection for thermal-responsive hydrogel materials^{60–62}. In a recent study, a supramolecular system was reported to overcome the deteriorated thermal-responsive performance of covalently bonded polymeric hydrogels upon continuous use⁶³. The thermally responsive phenomenon of this supramolecular gel actuator was influenced by the synergistic effect of hydrogen bonding and electrostatic repulsion, and the sol–gel transition feature was realized at 76°C with a response time of ~ 40 s.

Based on thermal-responsive hydrogels, thermal-induced actuation can be easily achieved via tuning the temperature. In addition, applications in drug transfer and release especially favor those hydrogels with critical solution points close to the human body temperature (i.e., 37°C). However, the relatively slow actuation rate and uncontrollability in the on/off state of actuation may hinder their extensive use.

Electric-responsive hydrogels

Electric-responsive hydrogels are able to change their geometrical shapes and sizes by swelling/deswelling when exposed to an electric field. The mechanism behind the electric-induced deformation phenomenon can be well explained as a synergy among Coulombic, electrophoretic, and electroosmotic interactions^{31,64,65}. In specific terms, the electric-responsive behavior of these hydrogels is often realized in an electrolyte aqueous environment, and the directional migration of mobile ions (i.e., cations to a cathode and anions to an anode) in the hydrogel–electrolyte solution system occurs under the applied electric stimuli, which induces contraction due to electrophoretic interactions. In addition, as a result of ion migration, the distribution of charged ions tends to be asymmetrical, and an osmotic pressure difference is then generated, owing to the concentration gradient of ions along the electric field, which induces electroosmotic movement of the water molecules³¹. In the end, the hydrogel swells and deswells when the osmotic pressure increases and decreases, respectively (Fig. 4a).

Due to attractive features such as a precisely controllable signal amplitude, frequency, and direction, the use of an electric stimulus to trigger hydrogel actuation has aroused remarkable interest⁶⁶. Commonly investigated electric-responsive hydrogels that deform upon electric stimulation include but are not restricted to chitosan^{67–69}, PAAc^{70–73}, 4-hydroxybutyl acrylate (4-HBA)^{73–75}, and poly(2-acrylamido-2-methylpropane-sulfonic acid) (PAMPS)^{76–79}. Chitosan is a natural macromolecular polymer made from chitin, a component abundantly distributed in the shells of crustaceans. Coupled with cutting-edge 3D printing technology, chitosan was employed to fabricate an electric-responsive hydrogel actuator in a layer-by-layer manner⁶⁹. In contrast to that in the form of a 2D film, the 3D-printed hydrogel actuator demonstrated a faster bending rate (reaching the maximum bending degree within 60 s) and a larger bending degree in response to electric stimuli (10 V) because of the increased surface-to-volume ratio, which further led to accelerated ion migration. The swelling ratio of this 3D-printed hydrogel was reported to be 70% when the water uptake reached equilibrium. To test the energy consumption and long-term durability of the hydrogel actuator, two major parameters that make this technology suitable for implantable biomedical applications (e.g., drug delivery), corresponding investigations were carried out on the electric-responsive bioactuator based on the 4-HBA hydrogel⁷³. The hydrogel actuator showed surprising and well-maintained functionality for continuous operation for as long as 6 months, with power supplied only from a 1.5-V AA battery. The excellent performance of 4-HBA hydrogels with low-energy consumption and long-term functional

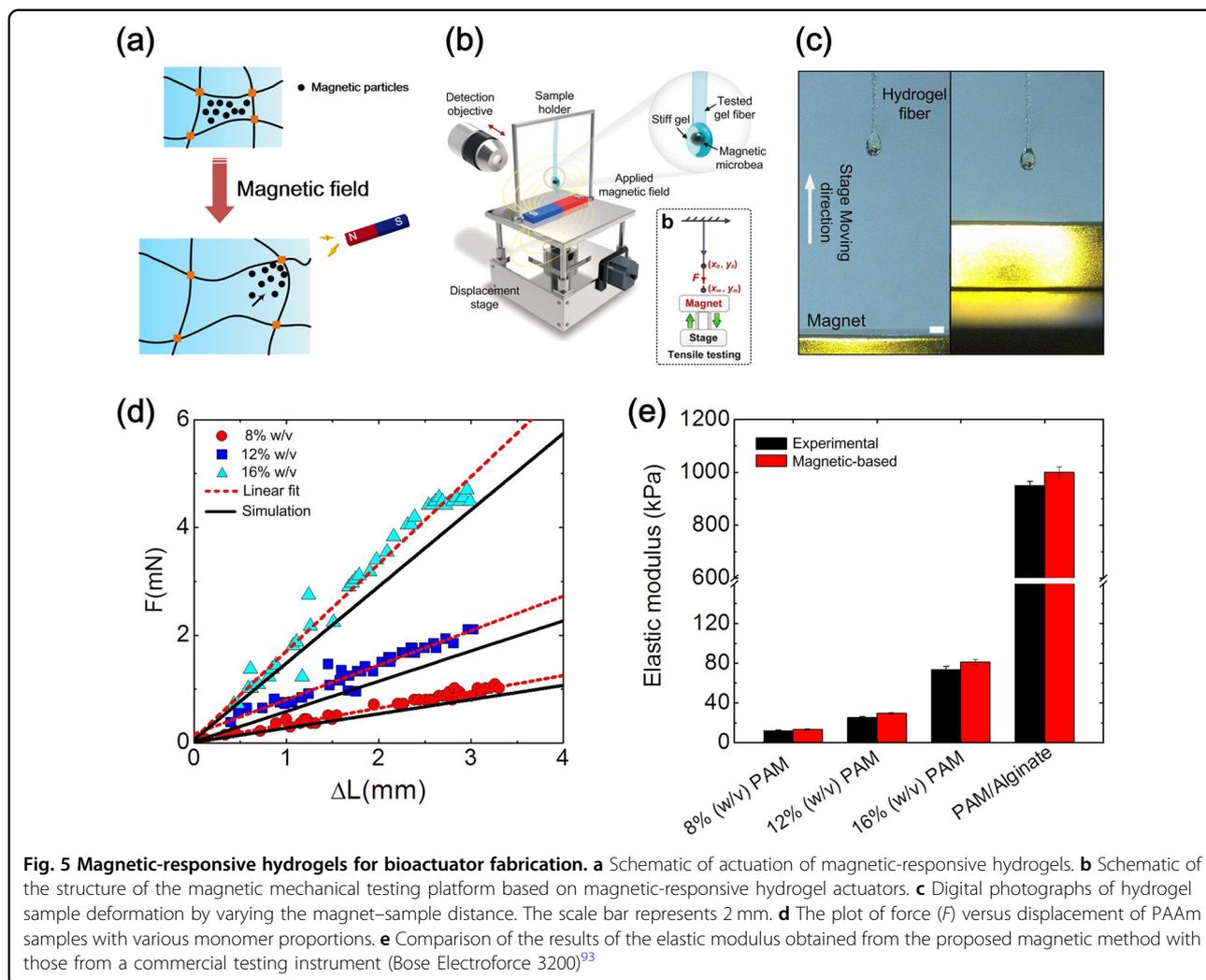


sustainability paves the way for their use as drug-delivery carriers and cell-sorting operators^{73,74}. Also aimed at lowering the actuation voltage for electric-responsive hydrogels, especially those with macrosizes to cater to unique application requirements, 4-vinylbenzenesulfonate (Na-4-VBS) was introduced for coupling with hydroxyethyl methacrylate (HEMA) (for better hydrophilicity) and polyacrylonitrile (AN) (for improved mechanical elasticity) to obtain a hybrid electric-responsive hydrogel system⁸⁰. The copolymer actuator revealed the capability to perform actuation with an electrical stimulation threshold down to 0.2 V and presented a fast response rate of 22°/s under a stimulation of 3 V.

PAAc has always been a popular candidate for electric-stimulated actuation, since the pendant carboxylic group on the polymer chain dissociates in an alkaline electrolyte solution, making the hydrogel negatively charged and ion-conductive. This property matches the requirement of realizing electric-responsive hydrogels. However, as mentioned above, the PAAc hydrogel is pH-responsive as well, showing significant differences in the exterior geometry and interior structure with varying pH. As a result,

its physiochemical properties, including its electric-responsive feature, are remarkably influenced by the pH of the surrounding environment, undermining its stable and reliable performance for practical applications⁸¹.

To address this issue, a PAMPS hydrogel, which shows complete ionization over a wide range of pH values (2–12), was employed to realize robust hydrogel-based electric-stimulated actuators regardless of pH changes. However, since the pristine PAMPS hydrogel is associated with inferior mechanical features ascribed to its irregularly distributed cross-linking points, its mechanical properties must be improved to achieve excellent actuation performance⁸². Luckily, a combination of acrylamide (AAm), which provides covalent linkage, and reduced graphene oxide (rGO) nanosheets, which form hydrogen bonds with polymeric chains, has been presented as a promising solution for the mechanical property enhancement of PAMPS (Fig. 4b)⁷⁹. The scanning electron microscopy (SEM) results showed that a dense and compact interior structure could be obtained after rGO dispersion compared with the porous architecture of the pristine hydrogel (Fig. 4c), validating the physical



interaction between rGO nanosheets and the hydrogel matrix. Tensile test outcomes clearly indicated an evidently increased tensile strength occurring in the gel with dispersed 0.5 mg mL^{-1} rGO (rGO-0.5 gel), which could tolerate intense mechanical stretching, while obvious fracture of the pristine gel could be detected under the same level of strain (Fig. 4d). By simply adjusting the rGO content, the deswelling ratio of the hydrogel can be tuned from 30% to 70%. Benefiting from the addition of highly electric-conductive rGO nanosheets, an additional conductive pathway can form inside the hydrogel network. Accordingly, the migration of mobile ions upon electric stimulation can be promoted, and intensified electric-responsive actuation can be realized (curvature value of 0.088 compared with 0.05 mm^{-1} for the blank hydrogel) (Fig. 4e).

By the utilization of adjustable electrical signals (e.g., voltage and current) as stimulation sources, electric-responsive hydrogels possess advantages in terms of a

highly controllable actuation state and tunable actuation degree compared with the aforementioned hydrogel actuators stimulated by pH and temperature. Benefiting from these features, these materials hold great potential for applications in muscular reconstruction and cellular electrical microenvironment engineering. However, due to the use of wire-connected bulky equipment, the practical convenience needs to be further improved.

Magnetic-responsive hydrogels

Unlike other actuation mechanisms where stimulus-responsive attributes are intrinsic properties of the hydrogel materials themselves, as reflected from interior physicochemical changes to exterior deformation, magnetic actuation is always carried out by modifying hydrogels with magnetic-responsive additives⁸³. With the integration of these magnetic materials, the inherently nonmagnetic-responsive hydrogels can be endowed with sensitivity to external magnetic stimuli, hence

demonstrating magnetically actuated deformation and movement (Fig. 5a). To date, a large number of magnetic materials, including Fe_3O_4 nanoparticles, Co nanoparticles, NdFeB microparticles, and Fe microspheres, have been introduced to magnetic hydrogel actuator applications as additive magnetic elements^{83–85}. Among all candidates, Fe_3O_4 nanoparticles have become the most popular and have received the most attention, mainly ascribed to their superparamagnetic features, biocompatibility, and noncytotoxicity, especially benefiting their use in biomedical and pharmaceutical territories^{86–88}. A typical example of a microscale hydrogel (microgel) with modification of Fe_3O_4 magnetic nanoparticles has clearly highlighted the fast and precise magnetic response of the microgel, as demonstrated by the uniformly arranged microgel pattern according to the applied magnetic field⁸⁸.

Since the magnetic response arises from the incorporation of magnetic particles rather than the inherent attributes of the hydrogel, a wide selection of hydrogel substrates are available for realizing magnetic-responsive hydrogels^{89–93}. It is additionally worth noting that the material selection principle is profoundly determined by the unique properties of the hydrogel associated with specific applications. Aimed at making muscle substitutes, PVA hydrogels with favorable elastic characteristics have been integrated with Fe_3O_4 microparticles dispersed in a concentration-gradient manner inside a hydrogel network⁸⁹. In this way, when activated by magnetic fields, the deflection degrees in different portions of the magnetic gel-based actuator vary owing to the heterogeneously distributed magnetic particles, resembling the motion of multijoint human fingers. Another study used an anionic PAMPS gel to realize magnetic hydrogel actuators⁹⁰. PAMPS, the swelling ratio of which is more than 8 in deionized water, is ionized and generates negative charges in aqueous media to adsorb cationic ions; thus, it can be used for the removal of heavy metal ions from wastewater for purification. Combined with magnetic responsiveness, the metal ion adsorption efficacy can be intensified because the additive magnetic nanoparticles also tend to induce ion adsorption at the surface⁹⁴. In addition, the magnetic-responsive capability enables the separation of a hydrogel loaded with toxic heavy metal ions from polluted water via a magnet controller, paving the way for practical water remediation and purification uses.

In a recent study⁹³, a mechanical testing platform based on a magnetic-responsive hydrogel actuator was established for the mechanical characterization of microscale biomaterials. The proposed scenario realized noncontact tensile loading of biological samples via magnetic actuation of an iron microbead-encapsulated poly(ethylene glycol) dimethacrylate (PEGDMA) layer attached to a terminal of the specimen (Fig. 5b). The specimen became

elongated as it was stretched by the magnetic PEGDMA layer; subsequently, the elongation could be measured, and the force could be calculated to finalize the mechanical characterization (Fig. 5c). The experimental results obtained from the developed method fit well with those from numerical simulations (Fig. 5d), and the accuracy of the presented magnetic mechanical testing platform was validated by similar results from a commercial mechanical testing instrument (Fig. 5e).

The integration of magnetic particles inside hydrogel matrices enables the hydrogels to respond and actuate according to the steering of the magnetic field in a tunable and wireless manner, facilitating applications in artificial muscles, drug delivery, and cell manipulation where actuation precision and convenience are favored. However, the compatibility and degradation of the magnetic additives remain a major challenge.

Other stimulus-responsive hydrogels

Apart from the aforementioned stimulus-responsive mechanisms, hydrogels with a phase-volume transformation behavior that responds to other stimulation signals, including light, humidity, redox state, and biomolecules, have also experienced development for different purposes^{26,27,95–104}. For instance, the realization of remote and invasive actuation with high spatial resolution via a light stimulus has great potential for applications in bio-related areas, especially when utilizing biofriendly near-infrared irradiation rather than potentially damaging ultraviolet light⁹⁶. The hydrogel actuator responds quickly to the exposure of near-infrared light, with a bending angle of 70° in 1 s. Meanwhile, hydrogels sensitive to humidity changes, which present a 30% increase in size when the humidity changes from 20% to 100%, may find use in real-time environmental monitoring⁹⁹. Regarding redox-responsive hydrogels that deform in response to the reduction or oxidation of specific attached moieties (e.g., ferrocene^{27,102}), they are advantageous for applications in working conditions with neutral pH and electric functionalization.

Emerging biomedical applications of bioactuators based on stimulus-responsive hydrogels

In recent years, bioactuators have stimulated increasing enthusiasm among researchers to develop novel materials for emerging biomedical applications. Among all potential candidates, a stimulus-responsive hydrogel is regarded as an ideal choice, as it outperforms other counterparts due to its high water content, sensitivity to ambient environment stimuli, excellent biocompatibility and biodegradability, and tunable structural and physiochemical properties. Benefiting from these appealing features, stimulus-responsive hydrogels have experienced widespread applications in biomedical areas. The diversity of

application cases of stimulus-responsive hydrogels, including biosensors for healthcare monitoring, drug-delivery carriers for disease therapeutics, and artificial muscles for sports-injury rehabilitation, as well as cell manipulators for engineering the cell microenvironment, has clearly depicted the extensiveness of these materials for novel scientific fields. In this section, state-of-the-art advances in the application of stimulus-responsive hydrogels for emerging biomedical uses are summarized and discussed in detail.

Biosensing

The burgeoning development of biosensors for the real-time, sensitive, and selective monitoring of biochemical indices of the natural environment and human internal environment is playing a crucial part in the human healthcare industry^{105–109}. Currently, popular biosensing platforms based on paper operate in a disposable manner^{108,109}. Despite the ubiquity, inexpensiveness, and mature fabrication of the substrate material, the practical difficulties in device collection and increased cost for reprocessing disposed devices can never be overlooked. To cope with this conundrum, stimulus-responsive hydrogels may provide a complementary option for fabricating biosensors. They can perform reusable sensing multiple times via capturing and releasing biochemical indicators along with reversible hydrogel swelling/deswelling, outperforming their paper-based disposable counterparts in durability and cost-effectiveness^{110–113}.

Hydrogels with a reversible phase-volume transition behavior in response to biophysical or biochemical stimuli have witnessed considerable application as biosensors for evaluating the levels of some healthcare-related indices, including protein^{114–116}, glucose^{117–119}, antibiotics¹²⁰, pH^{121,122}, and ions^{123,124}. For protein recognition, the molecular imprinting technique has been used to modify hydrogels, with the ability to recognize biomolecules with specific geometric structures^{114–116}. Through a precipitation polymerization procedure, the hydrogel can be imprinted with unique binding sites to adsorb lysozyme, a protein possessing potential efficacy for cancer diagnostics and therapeutics¹¹⁵. Compared with the pristine hydrogel, the molecularly imprinted hydrogel (MIH) exhibited markedly improved specificity and selectivity in lysozyme binding, benefiting from the specific interaction between the target analyte and polymer network. In addition, owing to the introduction of thermal-responsive PNIPAAm hydrogel, which experiences hydrophilic/hydrophobic transformation around its LCST and further impacts the protein–hydrogel bonding, the binding association constant of lysozyme for MIH decreases from 3.14×10^5 to $2.25 \times 10^4 \text{ M}^{-1}$ when the temperature decreases from 37 to 25 °C. Therefore, the hydrogel-based lysozyme recognition device carried out temperature-

dependent adsorption from/release to the target molecule, showing great promise as a controllable and reusable biosensor. Similar outcomes can also be realized for bovine serum albumin, offering a promising way to resolve the difficulty in employing MIHs for detecting proteins with large sizes and more complex structures¹¹⁶.

To perform ion screening, a DNAzyme sensitive to lead ions (Pb^{2+}) was introduced as a cross-linker to obtain an ion-responsive DNAzyme hydrogel¹²³. It should be noted that the hydrogel used was based on PAAm and conjugated with two DNA single strands (pA1 and pA2). Thus, when the enzymatic activity of the DNAzyme was initiated, and cleavage of the cross-linker strand occurred as a result of the presence of Pb^{2+} in the ambient environment, the embedded gold nanoparticles (AuNPs) were released from the collapsed hydrogel matrix for colorimetric detection (Fig. 6a). The proposed strategy presented highly sensitive detection of Pb^{2+} with a detection limit as low as 10 nM (Fig. 6b, c). In addition, the specificity of the hydrogel-based biosensor was validated with the selective detection of Pb^{2+} among many other metallic ions (including Cu^{2+} , Fe^{3+} , Mg^{2+} , Ca^{2+} , Mn^{2+} , Ni^{2+} , etc.), even when the concentration of Pb^{2+} (1 μM) was 1000 times lower than that of other cations (1 mM) (Fig. 6d, e). Moreover, the colorimetric sensor based on the ion-responsive DNAzyme hydrogel was successfully integrated with a portable microfluidic chip to visually and quantitatively read the Pb^{2+} level in an aqueous solution and blood samples, representing promising application potential in environmental monitoring and blood analysis.

Although the feasibility of applying stimulus-responsive hydrogels for biosensing has been validated, there are still some challenges to promoting these materials for practical applications. Since hydrogels are easily dehydrated in air, hydrogel-based biosensors are limited to applications in aqueous environments. In addition, the spontaneous swelling of the hydrogel network in an aqueous solution may cause errors in analyte-induced hydrogel swelling, which is closely related to the evaluation of the analyte levels.

Drug delivery

Since their emergence, hydrogels have long been used as carriers for targeted drug-delivery systems because of their abundant functional groups for drug immobilization, superior biocompatibility with in vivo environments, and adjustable degradation period for self-destruction^{125–128}. As a subgroup of hydrogel materials, novel stimulus-responsive hydrogels showing remarkable deformation after being triggered by multiple environmental stimuli might bring about additional benefits, such as precisely controllable drug release at the right time and at the right site. All these advantageous characteristics make

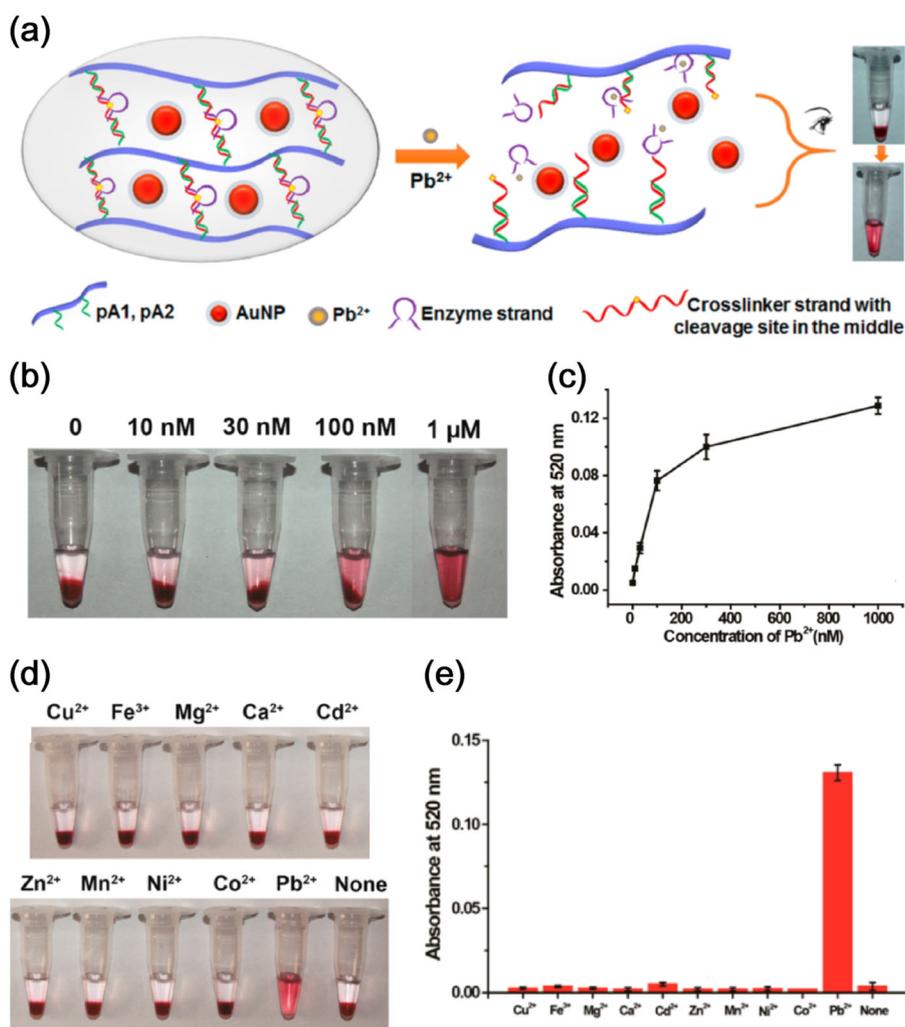


Fig. 6 Emerging applications of bioactuators based on stimulus-responsive hydrogels for biosensing. **a** Schematic diagram of the Pb^{2+} detection mechanism based on an ion-responsive DNAzyme hydrogel. **b** Digital photographs showing detection results of samples with different concentrations of Pb^{2+} . **c** AuNP absorbance at 520 nm as a function of Pb^{2+} concentration. **d** Digital photographs of the hydrogel responding to 1 mM solutions with different cations and 1 μ M Pb^{2+} . **e** Absorbance of AuNPs embedded inside a DNAzyme hydrogel at 520 nm responding to different cationic solutions¹²³

stimulus-responsive hydrogels an optimal option for the robust loading of therapeutic drugs and the subsequent controllable release of those drugs when and where they are needed through precise modulation of the surrounding environment (e.g., pH, temperature, and light) or the application of external physical fields (e.g., magnetic and electric fields)^{97,129–132}. The corresponding applications of drug-delivery systems based on stimulus-responsive hydrogels include neural disease treatment, cancer chemotherapy, and wound healing^{133–136}.

Having a volume-/phase-transition temperature (LCST of 32 °C) close to the human physiological temperature (37 °C), the drug release from the PNIPAAm-based system can be spontaneously activated as soon as the drug enters the human body. This drug-unloading mechanism

brings about unparalleled easy accessibility without any further applied stimuli; thus, PNIPAAm has been highlighted as an appealing candidate in bioactuator design and fabrication for thermally induced drug release^{56,133,136–138}. The PNIPAAm hydrogel has been shown to efficiently release drugs (e.g., doxorubicin and lysozyme⁵⁶) under heating, which can be explained by the collapse of chains and the shrinkage of the hydrogel network. The release process was rapid, showing a marked increase in drug concentration within 1 min after heating, and it took only 10 min to reach the maximum extent of unloading, which turned out to be comparable with the loaded value⁵⁶. However, since PNIPAAm is a synthetic polymer, its compatibility with tissues and living cells inside the human body is limited, and therefore, its

uses for implanted drug delivery have been questioned. To circumvent this issue, naturally derived chitosan with superior biocompatibility and low cytotoxicity was employed to provide the backbone for grafting PNIPAAm¹³³. With the contribution from chitosan, the hybrid gel exhibited largely improved biocompatible properties compared with those of pristine PNIPAAm, as demonstrated by the noncytotoxicity against HeLa and NIH3T3 cells. The designed chitosan-grafted PNIPAAm was further validated to be feasible for the loading, delivery, and temperature-triggered release of curcumin, showing satisfying killing effects toward malignant cells associated with severe diseases, including hepatocellular carcinoma, colorectal adenocarcinoma, and breast cancer.

Another intrinsic drawback that may restrict the application of PNIPAAm as a drug carrier lies in the fact that its spontaneous and quick release of a drug is uncontrollable, which may present challenges to drug release requiring accurate timing and precise targeting. Based on the pH dependence of the LCST of PNIPAAm, a strategy for pH-triggered thermal-responsive drug release from the PNIPAAm carrier was unveiled¹³⁶. The LCST of PNIPAAm varies with the surrounding pH (e.g., 56 °C for pH = 7.4 and 35 °C for pH = 5.0). According to the report, the drug release will not be initiated until the drug-loaded gel reaches the mildly acidic environment (pH = 5.0) resulting from disease-induced endocytosis inside the human body. More than 90% of the loaded drug can be released in 4 h under this condition (37 °C, pH = 5.0). Also operating under a pH stimulus, a chitosan–PMAA hybrid gel immobilized with quantum dots exhibited

simultaneous and integrated functionalities such as sensing, diagnosis, and therapy (Fig. 7a)¹²¹. The phase-volume transition behavior of the hybrid hydrogel is determined by the variation in pH in the ambient environment, with the hydrogel swelling markedly at pH values higher than 5.5 or lower than 5.0, but the swelling reaches a minimum when the pH is between 5.0 and 5.5. The quantum dots immobilized onto the polymer network were illuminated for B16F10 tumor cell imaging, enabling cellular diagnosis on the basis of the bioimaging results (Fig. 7b). The drug release behavior of the hybrid system varied with the surrounding pH due to changes in the PMAA–drug interaction intensity and mobility of charged drug molecules, as validated by a significantly faster release rate of the anticancer drug temozolomide at pH = 7.38 compared with that under acidic conditions (Fig. 7c). Furthermore, temozolomide released from the gel carrier exhibited excellent treatment efficacy, with the viability of B16F10 tumor cells descending to 30% after a 24-h incubation (Fig. 7d).

Apart from releasing loaded drugs in accordance with the temperature and pH of the internal bioenvironment, drug release can be triggered by applying external physical fields. In addition, applied electric or magnetic fields may outperform the thermally and pH-induced mechanisms in terms of controllability, as the on and off states of the release process can be manually switched with flexibly controlled timing and period^{129–131}. For instance, a magnetic-responsive hydrogel copolymerized from xanthan gum and alginate was synthesized for targeted drug delivery¹³⁴. The release of an anti-Parkinson drug, namely,

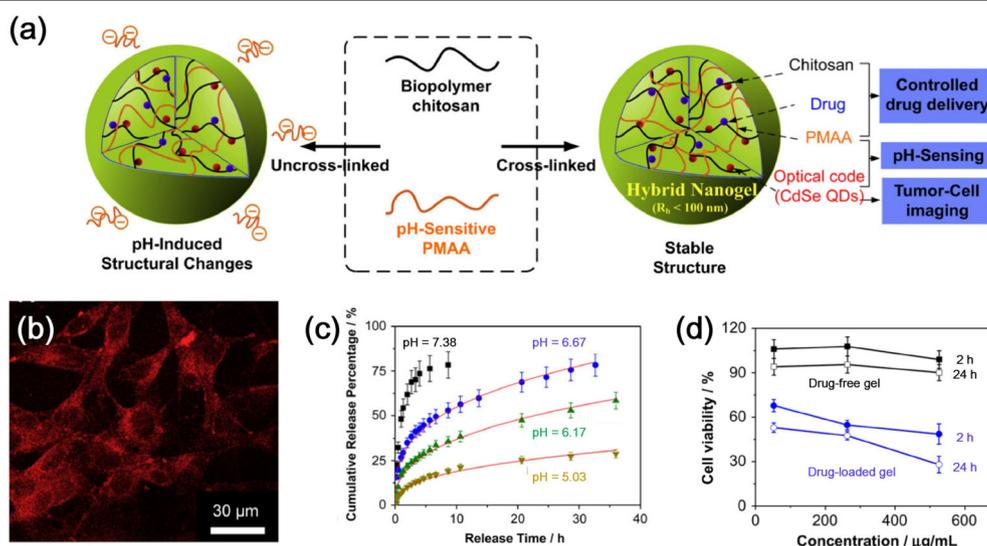


Fig. 7 Emerging applications of bioactuators based on stimulus-responsive hydrogels for drug delivery. **a** Schematics of pH-triggered thermal-responsive drug delivery based on a quantum dot-immobilized chitosan–PMAA hydrogel actuator. **b** Scanning confocal fluorescence image of B16F10 cells after a 2-h staining with the hybrid gel. **c** The amount of drug released from the hybrid gel with different pH conditions as a function of time. **d** Viability of B16F10 tumor cells after incubation with drug-free gels and drug-loaded gels at varying concentrations¹²¹

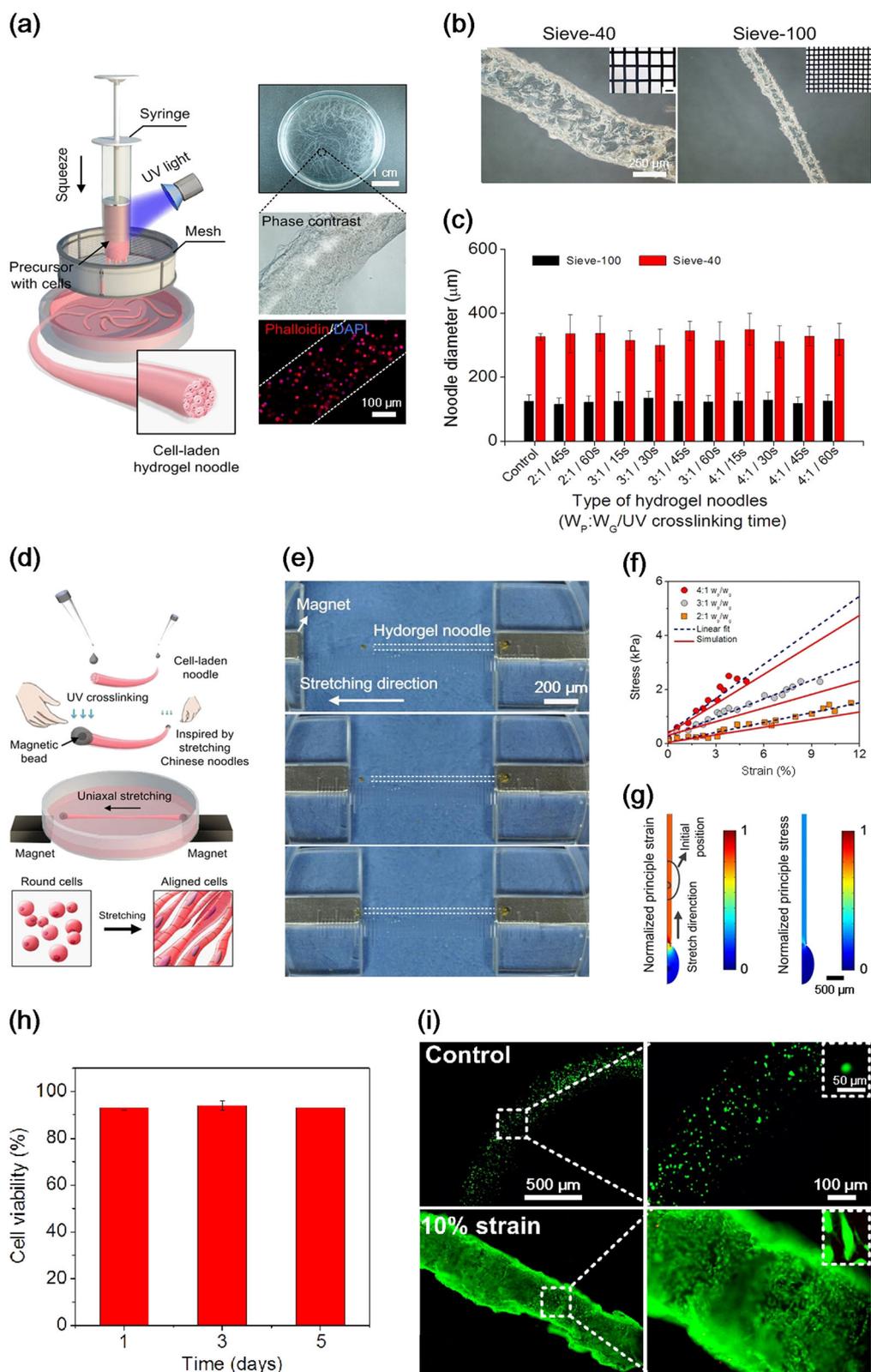


Fig. 8 (See legend on next page.)

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Fig. 8 Emerging applications of bioactuators based on stimulus-responsive hydrogels for artificial muscles. **a** Schematic of fabricating hydrogel fibers inspired by the fabrication process of a traditional Chinese dish, Hele noodles. **b** Bright-field microscopy images of the fabricated hydrogel fiber. **c** The effect of cross-linking time and prepolymer mass ratio on the size of the fabricated hydrogel fiber. **d** Schematic demonstration of realizing hydrogel-based artificial muscle by adding magnetically responsive terminals to the hydrogel fiber. **e** Digital photographs showing behaviors of artificial hydrogel muscle under magnetic actuation. **f** Experimental and simulation results characterizing the tensile properties of the hydrogel muscle. **g** Numerical simulation of strain (left) and stress (right) distribution on the stretched hydrogel. **h** Cell viability of C2C12 myoblasts cultured on unstretched fibrous hydrogel muscle. Error bars indicate the standard deviation, $n = 10$. **i** Live/dead fluorescence microscopic images of cells seeded on the hydrogel muscle with 0% and 10% strain³³

levodopa, was initiated in response to an external magnetic field with at most 70% unloading, and lasted for more than 30 h with maintained pharmaceutical efficacy, offering a sustained drug release tactic for Parkinson's disease treatment.

Due to their attractive attributes in terms of stimulus controllability, biocompatibility, and biodegradability, stimulus-responsive hydrogels have been extensively investigated for targeted drug delivery. However, most works merely focus on *ex vivo* studies in the current stage. The introduction of these hydrogel-based drugs into the *in vivo* environment, which is highly anticipated for solving practical questions in clinical and pharmaceutical cases, remains unexplored.

Artificial muscles

Biomimetic artificial muscles that resemble native muscular tissues with contractile and extensile behaviors have ignited extensive advances in bioinspired applications, including soft robotics such as grippers and locomotors, body-worn exoskeletons for sports rehabilitation, and implantable machines aimed at injured muscle substitution^{139–142}. In regard to the material selection for producing human-made muscles, the stimulus-responsive hydrogel stands out as an exceptional candidate, mainly owing to its biocompatibility, which guarantees integration with the human body in a human-friendly manner, as well as its stimulus-responsive feature, which facilitates manual controllability over the muscular performance of artificial substitutes^{33,143,144}. In this part of the review, a summary of artificial muscles made of stimulus-responsive hydrogels and their corresponding biomedical applications is presented.

Inspired by the fabrication of Hele noodles, a famous Chinese local dish, an ingenious methodology for preparing hydrogel-based muscular fibers with easy accessibility and high throughput was developed³³. GelMA and PEGDMA were employed as raw materials for fiber-shaped hydrogel muscle generation to produce ECM-like conditions for muscle cell proliferation and mechanical toughness, respectively. The fabrication process could be simply achieved by UV cross-linking the precursor mixture of GelMA and PEGDMA and subsequently pressing the polymerized hydrogel

through a sieve (Fig. 8a). The fabricated hydrogel noodle showed homogeneity in fiber diameter and could be well controlled by utilizing sieves with different pore sizes (Fig. 8b). Moreover, factors related to the cross-linking process, such as the photo-cross-linking time and hydrogel monomer ratio, had negligible effects on the fiber size (Fig. 8c). Next, with an additional procedure of UV coating a thin layer of iron microsphere-encapsulated PEGDMA on both ends of the fiber, a fibrous hydrogel muscle that expands and contracts in response to noncontact magnetic actuation could finally be realized (Fig. 8d, e). The as-fabricated hydrogel-based artificial muscle presented satisfactory elastic properties, with a maximum strain of ~10% and Young's modulus ranging from 12.19 to 42.05 kPa, mimicking the mechanical properties of natural muscles (Fig. 8f). Numerical simulation results further unveiled the uniform distribution of strain and stress caused by a magnetic stimulus along the hydrogel fiber (Fig. 8g). The biocompatibility and noncytotoxicity were validated by the high cell viability (>90%) of C2C12 myoblasts cultured in the gel for 5 days (Fig. 8h). More surprisingly, in comparison with cells in the unstretched case, muscular cells encapsulated in the stretched hydrogel muscle (10% strain) showed notable improvement in expansion and proliferation (Fig. 8i), making the proposed magnetic-actuated hydrogel myofiber a promising candidate for repairing native muscles with dysfunction due to pathological necrosis or sports injury.

In addition to the magnetic actuation strategy, stimuli such as electricity and temperature have been shown to actuate hydrogels for artificial muscle uses^{143,145–148}. An example is the composite of an electrically conductive polypyrrole and a biocompatible chitosan hydrogel, which functioned as artificial muscles responding to electric currents. The hydrogel actuator responded to electrical stimuli in <5 s even in electrolyte solutions as dilute as 0.01 M, resembling the real stimulation mechanism of natural muscle contraction triggered by a bioelectric potential¹⁴³.

Despite the fact that fabricating contractive hydrogel fibers is a promising route toward achieving artificial muscles, the current development of stimulus-responsive hydrogels for this application faces a major challenge.

Similar to the conundrum faced by the application of drug delivery, the feasibility of integrating these synthetic muscular fibers into the human body remains unknown. Therefore, it should be encouraged to boost studies on replacing injured and dysfunctional muscles with artificial hydrogel muscles for muscular tissue repair and substitution.

Cell manipulation

Cells in biological organisms do not live on their own, and their behaviors, including spreading, adhesion, proliferation, differentiation, migration, and apoptosis, are also strongly affected by the biophysical and biochemical conditions of the microenvironment in which they live^{149,150}. Therefore, it is of vital scientific significance and academic value to understand the mysterious principle of how and to what extent cellular behavior can be influenced by the ambient microenvironment. To achieve this goal, hydrogels are used as media to culture cells, as these polymeric materials offer ultimate compatibility with laden cells, and their 3D water-sufficient network largely resembles the ECM of cell living environments. More excitingly, microenvironmental cues can be further tuned with the aid of newly emerged stimulus-responsive hydrogels, through which externally applied physical and chemical stimuli can be transmitted to the hydrogel internal space, altering the culturing conditions of laden cells (e.g., mechanical microenvironment). The corresponding cell responses can be observed and recorded to investigate microenvironment-dependent cell behaviors.

The cell mechanical microenvironment consists of multiple mechanical cues, which cells perceive and respond to via transmembrane molecule adjustment and intracellular cytoskeleton reorganization^{151,152}. In recent years, deploying an external magnetic field to actuate cell-laden, magnetic-responsive hydrogels, and characterizing cell behaviors in response to a magnetic-induced strain of a hydrogel matrix, have emerged as appealing strategies for the discovery of the biomechanical responses of cells^{33,34,153}. In a cell-seeded hydrogel microfiber, C2C12 myoblasts demonstrated increased levels of proliferation and spreading under 10% strain of the hydrogel matrix with noncontact magnetic actuation³³. This favorable phenomenon could be associated with passive cellular stretching, which was subject to the magnetically triggered stretching of the hydrogel, and opened more space to facilitate cell occupation. In addition, improved cell alignment along the uniaxial stretch direction was found after 14 days of cell culture in a hydrogel fiber under a 10% strain, outperforming that in the unstretched hydrogel, where cells were oriented in a random way. Moreover, the myoblasts were further verified to successfully differentiate and form myotubes in the stretched case, as indicated by the content analysis results of myosin

heavy chain, a protein specifically expressed by skeletal muscle.

This magnetic-responsive microfiber was presented as a feasible system for characterizing cell responses to mechanical stretching with a strain of no more than 10%. However, for cells living in tissues sustaining larger levels of strain, such as cells in cardiac muscle, which undergo strain beyond 20%^{154,155}, the microfiber method may fail to mimic their real mechanical microenvironment. Luckily, a platform based on a magnetically actuated, cell-laden hydrogel that was demonstrated to experience a large strain (up to 60%) was established to investigate mechanically induced cell responses (Fig. 9a, b)¹⁵³. The experimental results revealed that the spreading of fibroblasts cultured in GelMA (Young's modulus of 6 kPa) was enhanced with increasing levels of strain induced by noncontact magnetic actuation, as validated by confocal microscopic images (Fig. 9c), cell volume analysis (Fig. 9d), and cell proliferation evaluation results (Fig. 9e). In addition, the existence of α -actinin and the highly aligned distribution of myosin heavy chain parallel to the stretch direction indicated the differentiation of C2C12 myoblasts and maturation of myofibers (Fig. 9f). An increased expression level of skeletal-muscle-specific proteins and nucleic acid with increasing culture time further sheds light on the practical applicability of this platform for probing the cell mechanoreponse (Fig. 9g).

Additional attention has been paid to the application of electrical currents for loading mechanical stretching onto cells encapsulated in electric-responsive hydrogels as a result of electrically modulated swelling/deswelling of the hydrogel matrix¹⁵⁶. With the addition of hydrophobic fibrin fibers and the alteration of cross-linker concentrations, the swelling ratio of the PAAc hydrogel can be tuned from 10 to 30. A prominent improvement in terms of cell proliferation and alignment of the smooth muscle cells was clearly observed when electrical pulses were activated and subsequently transduced into mechanical deformation of the PAAc hydrogel, inducing stretching of the embedded cells. In addition, the presented strategy may be used to modulate the cell electrical microenvironment, and the possible electrical–mechanical dual-modulation mechanism of cell living conditions may find widespread applications in cell microenvironment studies and vascular grafts for tissue engineering.

The latest research advances have revealed the capability of bioactuators based on magnetic-responsive and electric-responsive hydrogels to engineer mechanical and electrical cell microenvironments, respectively. However, due to the size of the biomimetic hydrogels, current systems are unable to realize single-cell analysis; thus, a clear principle illustrating the relationship between cell behaviors and cues from ambient microenvironments has yet to be established.

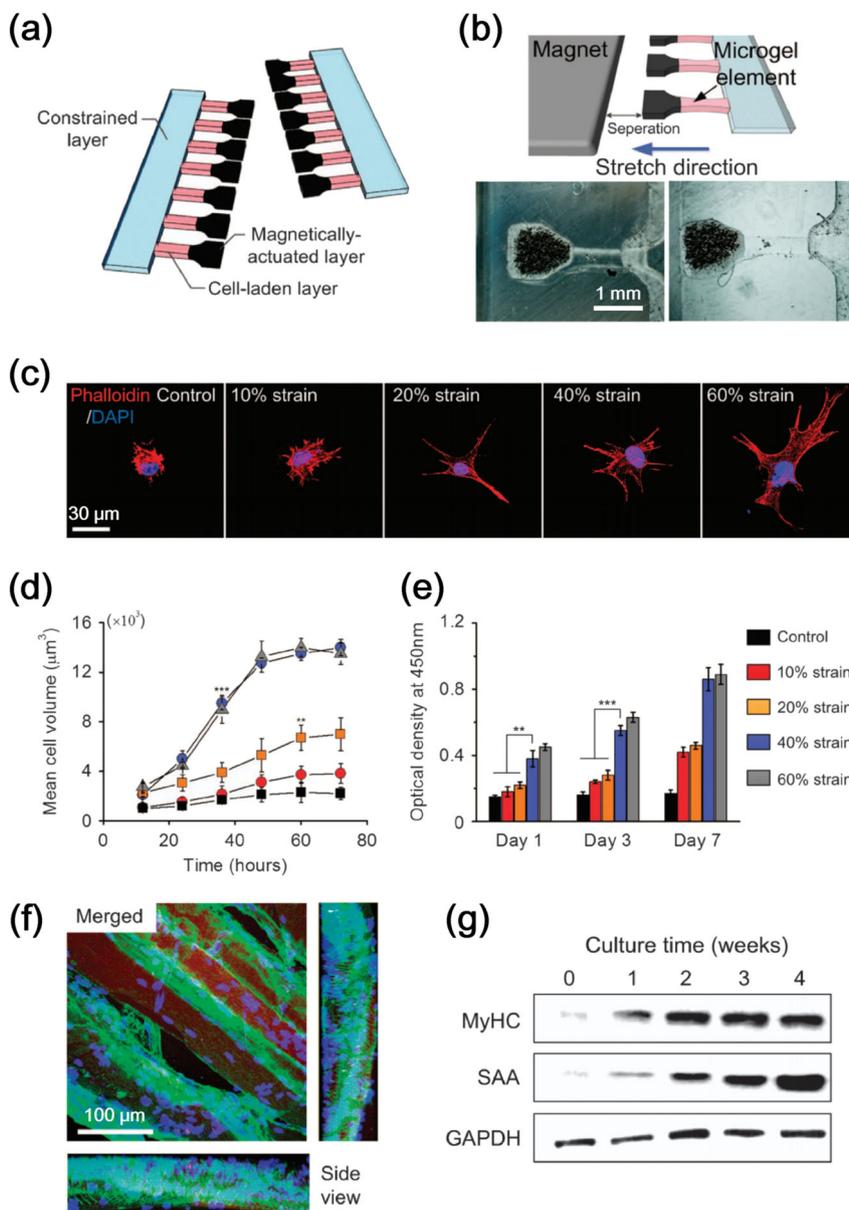


Fig. 9 Emerging applications of bioactuators based on stimulus-responsive hydrogels for cell manipulation. **a** Schematic of the cell mechanical microenvironment analysis platform based on magnetic-responsive hydrogels. **b** Schematic graph (top) and digital photographs (bottom) of noncontact magnetic-actuated stretching. **c** Fluorescence confocal microscopic images of the morphology of fibroblasts seeded in hydrogel under various levels of strain. **d, e** Mean cell volume **d** and cell density **e** analysis results from a hydrogel matrix with different levels of strain. Error bars represent the standard deviation, $n = 10$. **f** Merged confocal images to demonstrate C2C12 myoblast differentiation after 4 weeks of culture in a stretched hydrogel (40% strain). Green: α -actinin (SAA). Red: myosin heavy chain (MyHC). Blue: nucleus. **g** Expression of skeleton muscle-specific markers as a function of culturing time¹⁵³

Conclusions and future perspectives

To bridge the gap between soft actuating materials and novel biomedical applications, stimulus-responsive hydrogels that respond to ambient stimuli (e.g., pH, temperature, light, electricity, and magnetic field) with phase and morphology transformation have emerged as a

prominent group of materials for fabricating bio-oriented actuators (Table 1). Benefiting from their appealing characteristics, including but not limited to biocompatibility, cell ECM-mimicking features, structural porosity, tunable physiochemical properties, and responsiveness to multiple environmental stimuli, stimulus-responsive

Table 1 Material properties of stimulus-responsive hydrogels

Environmental stimuli	Hydrogels	Swelling ratio	Response time (s)	References
pH	PAAc	2.98–10	60–300	39,158,159
	PAAm	0.2–1	1.5–30	41–43
	PVA	0.25–0.3	100	48
	PMAA	5	–	46
Temperature	PNIPAAm	2.98–20	300–540	53,56,159–162
	PEG–PPG–PCL	0.23–1.5	1200	61
Electricity	PAAc	2.3–47	0.2–184.3	70–72,74
	Chitosan	0.65–0.74	7–60	67,69
	PAMPS	0.32–0.7	60–90	79
	Na-4-VBS	1.8–8.15	30–60	80,163
Magnetics	Alginate	1.06–1.32	–	134
	PEGDMA	–	<0.1	32,33,93
	PAMPS	50–85	–	90
Light	Elastin	–	<5	96

Table 2 Advantages, disadvantages, and potential applications of different stimulus-responsive hydrogels

Environmental stimuli	Advantages	Disadvantages	Potential applications	References
pH	Easy accessibility	Uncontrollable on/off state of actuation	Biosensors	121,122
	High specificity		Drug delivery	136
Temperature	Easy accessibility	Uncontrollable on/off state of actuation	Drug delivery	133,137,138
Electricity	Controllable on/off actuation state	Requirement for wired and bulky instrument	Artificial muscle	143
	Tunable actuation degree		Cell manipulation	156
Magnetics	Controllable on/off actuation state	Degradation issue of magnetic particles	Artificial muscle	33
	Wireless modulation		Drug delivery	134
	Tunable actuation degree		Cell manipulation	33,34,153
Light	High controllability with excellent spatial and temporal resolution	Inability of light sources to penetrate tissue for in vivo applications	Soft robots	96
	Remote modulation	Use of non-biofriendly light sources, like UV	Drug delivery	97,132

hydrogels have undergone unprecedented advances in application fields ranging from biosensing devices, targeted drug transfer and release, and artificial muscular reconstruction to cell microenvironment engineering (Table 2).

However, some challenges still need to be addressed before the widespread application of bioactuators based on stimulus-responsive hydrogels can ultimately be realized. One intrinsic obstacle of hydrogel-based actuators lies in the dependence on an aqueous environment, as the stimuli-triggered actuation phenomenon is based on the

transfer of water between the hydrogel matrix and the surrounding environment. Although some works have demonstrated that a stimulus-responsive hydrogel can be actuated in open air through moisture exchange with air¹⁵⁷, the actuation performance, such as the morphological expansion extent and response rate, turned out to be far inferior to the achieved performance in the water environment case. In addition, ascribed to the slow water diffusion-induced hydrogel volume transition, the actuation process of large-scale hydrogels is too slow to achieve real-time responses (e.g., several hours for cm-sized

hydrogel actuators). This problem may impede uses in artificial muscle reconstruction, where human-made muscles are required to replace injured muscular tissues of varying sizes. In addition, regardless of the recent emergence of hydrogel actuators able to sense multiple stimuli with a single device (e.g., pH/thermal-responsive hydrogel, pH/electric-responsive hydrogel, and light/thermal-responsive hydrogel), which remove the limitation of single stimulus-triggered hydrogel actuation, the reliability and specificity of these dual-mode-activated actuators need to be further studied, especially considering the potential cross-impact among different actuating mechanisms. A typical example is the pH, the stimulus of the pH-responsive hydrogel, which could affect the LCST of a thermal-responsive hydrogel and further impact its actuation performance. The last challenge is the trade-off between degradation and long-term durability of the hydrogel to cater to different application purposes. For example, applications such as drug delivery require the hydrogel to self-destruct after the drug is delivered to the target. However, usage as artificial muscle substitutes would require structural and functional stability of the hydrogel actuator even after a long period of time.

Despite these existing challenges, the appearance of stimulus-responsive hydrogels greatly stimulates the progress of biomaterial-based soft actuators and their applications in multiple bio-related areas. Future perspectives of this area should aim at proposing promising solutions to the existing challenges. First, quick-responding hydrogels are expected to be fabricated to largely enhance the response rate of the hydrogel to environmental stimuli. Especially for applications where instant response is a necessity (such as artificial muscles or neurons), the hydrogel response time (currently several minutes) is suggested to be at the second or millisecond level. Modifying the hydrogel molecular structure to have improved swelling kinetic performance, and implementing hydrogels with smaller sizes and thinner profiles, could be potential ways to address this aim. For the degradation issue, a promising tactic is to tune the degradation fate of hydrogels by designing and programming the molecular composition and the cross-linking extent of hydrogels. Thus, hydrogels with different degradation behaviors can be suitably employed to meet specific application demands. Further outlook regards the translation of fruitful research outcomes to solve practical medical problems. Based on state-of-the-art research advances, utilizing stimulus-responsive hydrogels for monitoring, diagnostics, and therapeutic purposes in clinics is highly anticipated. It can be expected that with the interdisciplinary collaboration among researchers from materials science, biomedical engineering, and advanced manufacturing technology, the development of

bioactuators based on stimulus-responsive hydrogels will be further driven.

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Conflict of interest

The authors declare that they have no conflict of interest.

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