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Magnetic bio-hybrid micro actuators

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Over the past two decades, there has been a growing body of work on wireless devices that can operate on the length scales of biological cells and even smaller. A class of these devices receiving increasing attention are referred to as bio-hybrid actuators: tools that integrate biological cells or subcellular parts with synthetic or inorganic components. These devices are commonly controlled through magnetic manipulation as magnetic fields and gradients can be generated with a high level of control. Recent work has demonstrated that magnetic bio-hybrid actuators can address common challenges in small scale fabrication, control, and localization. Additionally, it is becoming apparent that these magnetically driven bio-hybrid devices can display high efficiency and, in many cases, have the potential for self-repair and even self-replication. Combining these properties with magnetically driven forces and torques, which can be transmitted over significant distances, can be highly controlled, and are biologically safe, gives magnetic bio-hybrid actuators significant advantages over other classes of small scale actuators. In this review, we describe the theory and mechanisms required for magnetic actuation, classify bio-hybrid actuators by their diverse organic components, and discuss their current limitations. Insights into the future of coupling cells and cell-derived components with magnetic materials to fabricate multi-functional actuators are also provided.

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1. Introduction

In the past decade, there have been several remarkable developments in increasingly small wireless actuation systems for various biological and biomedical applications. Due to the length scale at which they operate, the physical forces which govern these devices are vastly different to those for macroscopic machines. Environmental parameters such as tempera-

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ture,[1] pressure,[2] and fluid properties[3] often also play more critical roles in the ability of these devices to function versus macroscale systems. In particular, surface forces dramatically impact these machines as these forces dominate over inertial forces.[4] To operate at small scales, where devices must account for the surface forces which resist actuator motion, several methods have been developed to overcome these resistive forces.[5] These actuation methods often take inspiration from nature, which has become adept at small-scale actuation. In particular, microorganisms, which through evolution have developed intricate and sophisticated molecular machines and stimuli-responsive macromolecules, have inspired the design of synthetic nano and micro-scale wirelessly controlled devices.[6,7] Two major categories of artificial devices that have utilized these mechanisms are chiral swimmers, which mimic the propulsion of flagellated prokaryotes, and flexible swimmers, which mimic the propulsion of many eukaryotes. However, while there has been significant progress in synthesizing small-scale synthetic machines,[8] it is still challenging to produce actuators of similar complexity and functionality as those displayed by natural molecular motors. Alternatively, many groups are developing living actuators using wild type[9] or engineered cells;[10,11] however, these cells are devoid of synthetic components, limiting their ability to be controlled externally with precision. Thus, to overcome the challenges of entirely artificial and completely biological systems, there is a growing class of hybrid devices that mimic the design of biological systems and incorporate organic components for actuation, sensing, and transport.

Bio-hybrid actuators integrate biological cells or subcellular parts with synthetic or inorganic components. Small-scale bio-hybrid devices harness the synergy of synthetic and natural materials for performing useful controlled tasks that can be difficult to achieve using purely biological or purely abiotic materials alone. As with all actuators, these require a suitable actuation mechanism for operation. To design these systems, the three main criteria of consideration are (1) ease of fabrication, as these devices often are designed to operate in swarms;[12] (2) controllability, which must overcome small-scale physics;[13] and (3) localization, enabling real-time tracking of mobile systems.[14] These design criteria have been used to develop a wide range of hybrid actuators with varied applications ranging from localized delivery of biomedical therapeutics[15–17] to remediation of toxic environmental chemicals[18] and microscale fabrication.[19]

Bio-hybrid actuators most often take the form of miniaturized vehicles, which require energy for propulsion by converting exogenous (*e.g.*, magnetic,[20] acoustic,[21] optical[22]) or endogenous (*e.g.*, chemical[23,24]) energy into mechanical work.[25–27] Here we focus on magnetic control as it is the most common method for manipulating actuators. Magnetic control offers efficient transfer of wireless energy, low hardware cost, the ability to penetrate non-magnetic or weakly magnetic materials, and can be precisely controlled.[28,29] Furthermore, weak rotating and oscillatory magnetic fields have been demonstrated to manipulate devices in a manner that mimics the propulsive motion of flagellated organisms.[30] As magnetic fields are biologically compatible,[31] a growing trend is the use of magnetic control in combination with other control modalities, which can be further facilitated due to the hybrid material properties. Recent examples are multimodal control of micro enzyme motors, which are guided over long rages using magnetic forces, and operated at small scales using enzymatic activity.[32] This example shows the potential of magnetic bio-hybrid actuators for advanced applications, despite being a subset of the larger field of small-scale magnetic actuators.

Recent advances in functional magnetic bio-hybrid devices have illustrated the ability of microactuators to be used for multiple tasks (*e.g.*, transport and sensing) while also being controlled using simultaneous signal inputs from external and internal energy sources. As these devices can possess various organic structures, it is convenient to categorize bio-hybrid devices by the type of biological material they utilize: those that incorporate whole living cells and others that integrate cell components. Here we briefly review the primary mecha-

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nism of magnetic actuation applied to small-scale actuators and then provide an up-to-date account of magnetic bio-hybrid actuators. Organized in terms of eukaryotic, prokaryotic, protein, and nucleic acids-based actuators, we highlight recently reported devices, emphasizing the unique hybrid material nature of these devices that enable their use for specific applications. Current limitations and potential directions for the development of future bio-hybrid actuators are also discussed.

2. Theory and mechanism of magnetic actuation

Magnetic actuation is widely used to wirelessly control and propel magnetic microactuators for precisely targeted delivery. An actuator's inherent ability to be controlled by a magnetic field stems from the choice of material used. Materials with various types of magnetism, such as ferromagnetism and paramagnetism, are widely used because they contain randomly oriented unpaired electrons that can be rearranged to give them a magnetic dipole moment. However, the material chosen when designing hybrid actuators depends on whether the magnetic particles have high magnetic moments, allowing objects to be controlled by an external field in biomedical and environmental applications. As a result, ferromagnetic materials, specifically superparamagnetic materials, are used because their susceptibility to applied magnetic fields generates often sufficient forces for actuation. Furthermore, because of the small size of these devices, aggregation caused by dipole-dipole interactions is reduced[33] and magnetic anisotropic is increased.[34] On the other hand, the magnetic susceptibility of paramagnetic material is positive and extremely small, resulting in a low force and torque response to a magnetic field. In an applied magnetic field a magnetic bio-hybrid micro actuator experiences a magnetic force ($\vec{F}$) and torque ($\vec{T}$) when exposed to an externally generated magnetic field.[35–38] The magnetic force and torque experienced by a magnetic actuator can be mathematically expressed as:

$$\vec{F} = \int \left(\rho \nabla (\vec{m}_0 \cdot \vec{B}) + \frac{\chi}{\mu_0} (\vec{B} \cdot \nabla) \vec{B} \right) \mathrm{d}V \quad (1)$$

$$\vec{T} = \int (\vec{m} \times \vec{B}) \mathrm{d}V \quad (2)$$

where $\vec{m}$ is the internal magnetization, $\vec{m}_0$ is the initial magnetization, ρ and V are the density and volume of the magnetic particle respectively, ∇ is the field gradient, χ is the susceptibility, and $\vec{B}$ is the magnetic flux density. In free space, $\vec{B}$ can be expressed as the product of $\mu_0 \vec{H}$, where μ_0 is the magnetic permeability of free space and $\vec{H}$ is the magnetic field strength.

The torque and force produced by an external magnetic field with no inertial effects and time dependencies has a linear relationship with the actuator's angular velocity $\vec{\Omega}$ and speed $\vec{U}$ and is defined by the symmetrical mobility matrix,[4] as shown in eqn (3).

$$\begin{bmatrix} \vec{U} \\ \vec{\Omega} \end{bmatrix} = \begin{bmatrix} \overline{\overline{\mathrm{M}}} & \overline{\overline{\mathrm{N}}} \\ \overline{\overline{\mathrm{N}}}^T & \overline{\overline{\mathrm{O}}} \end{bmatrix} \begin{bmatrix} \vec{F} \\ \vec{T} \end{bmatrix} \quad (3)$$

where $\overline{\overline{\mathrm{M}}}$, $\overline{\overline{\mathrm{N}}}$ and $\overline{\overline{\mathrm{O}}}$ represent a 3 × 3 symmetrical matrix and is time-dependent on the actuator's geometry. Bente *et al.* explained that if the shape of a magnetic actuator is asymmetrical, then the rotational motion of the propulsion system generates a force to push or translate the actuator forward.[39] The orientation of a magnetic moment can also be used to

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achieve a degree of asymmetry on a 3D actuator with one or two planes of symmetry.[40,41] This magnetic moment orientation shows that the force and torque associated with the mobility matrix induce controlled motion on the actuators. To this extent, electromagnetic coils provide controllable magnetic actuation to steer magnetic materials to the desired locations.[42]

The two classic coils are based on Maxwell and Helmholtz configurations[43,44] due to their experimental design and theoretical modeling simplicity. Helmholtz coils generate a uniform magnetic field with two identically sized electromagnetic coils positioned at an equal distance to their radius, whose strength depends on current input, the number of wires turned, and the coil radii. A rotating magnetic field is developed about the Cartesian coordinate axis by placing three orthogonal pairs and using a sinusoidal current input. Compared to Helmholtz coils, Maxwell coil configuration produces a gradient when the electromagnetic coil pair carries current in opposite directions, and the spacing is increased by a factor of $\sqrt{3}$.[45] To apply these magnetic configurations to *in vivo* applications, larger radii coils are required to operate and steer the microactuators in comparison to *in vitro* applications. As a result, more current is needed to produce the same magnetic field.

In this regard, permanent magnets are looked at as the alternative to generating the necessary magnetic fields.[46] Fountain *et al.* proposed using permanent rotating magnets *via* a robotic controlled arm to propel helical swimmers.[47] It was determined that a diametrically magnetized magnet works better than an axially magnetized cylindrical magnet as it utilizes the volume of the magnet creating a stronger magnetic field. The helical swimmers' actuation was limited by the attractive forces produced by the magnet and its one degree of freedom propulsion.

The axial configuration was also shown to actuate a swarm of magnetic bio-hybrid swimmers *in vivo.*[57] While permanent magnets may seem to provide a solution for scaling magnetic devices, they introduce many issues due to limited steering and the inability to be switched off.[58] Therefore, novel mechanisms have been designed to control single or multiple microactuators using electromagnetic coils. For example, Chowdhury *et al.* developed a specialized substrate containing micro coils to generate magnetic gradients to control numerous robots simultaneously.[59] Other electromagnetic configurations include BigMag, a closed-loop magnetic navigation system, and Octomag,[60–62] which introduce higher degrees of freedom (>3). These electromagnetic systems can also be combined with other actuation methods, such as acoustic, leading to increased functionality.[63]

3. Discussion

Bio-hybrid miniature actuators often utilize a magnetic component, such as magnetic particles, to drive directed motion in response to magnetic signals. This integration relies on the proposed application which affects the actuator's size.[64] Decreasing an actuator's size results in complex assembly techniques as well as limits the amount of integrated magnetic materials. Alapan *et al.* recently discussed bio-hybrid actuator scaling limitations, such as alteration of physical and chemical properties of the components affecting the fabrication strategies. Further, Alapan *et al.* also discussed current fabrication and control strategies for bio-hybrid actuators.[16]

In this article, we have classified magnetic actuators based on their proposed applications, fabrication methods, and integrated biological component (eukaryotic, prokaryotic, nucleic acid, and protein functionalization (see Fig. 1a)). Statistics were collected from the 'Web of Science' and 'Engineering Village' databases to evaluate the number of peer-reviewed publication citations for over three decades (1990–2021) to evaluate the scope and growth of various magnetic hybrid actuators. We used various keyword searches that describe each actuator type (*e.g.*, magnetic DNA origami, magnetically actuated bacteria, *etc.*). As shown in Fig. 1b, the accumulated peer-reviewed magnetic bio-hybrid publications have been biased toward eukaryotic actuators attributed to their large size, non-hazardous properties, and accessibility, followed by prokaryotic actuators. There is also a growing field of protein and nucleic acid-based bio-hybrid actuators, which are operated by magnetic stimulation.

Magnetically controlled manipulation of micron-sized bio-hybrid (eukaryotic and prokaryotic) actuators is less challenging than their smaller nano and molecular scale counterparts.[58] The use of eukaryotic and prokaryotic actuators are favored because the magnetic actuation force is often proportional to the cell volume (see eqn (1)); decreasing the size of the actuator reduces the magnetic force and torque generated on the actuator. Many of these reported magnetically controlled miniature actuators are designed to be used in the human body one day. However, the majority of investegations today are still preformed *in vitro*, aimed at answering fundamental questions necessary for successful future *in vivo* applications. Some of the critical challenges for a successful journey are summarized in Table 1.

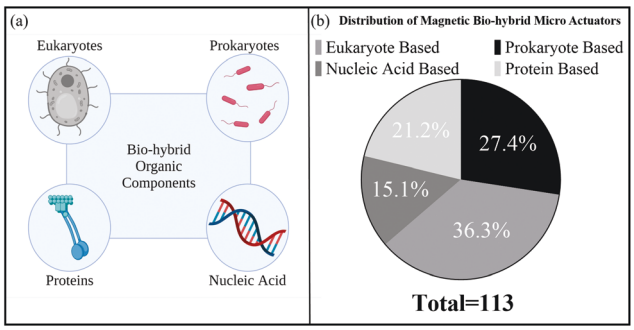

Fig. 1 Classification of magnetic microactuators. (a) Biological material used in the fabrication of bio-hybrid magnetic actuators. (b) Distribution of bio-hybrid magnetic actuators categorized based on their biological appendage, found using databases such as 'Engineering Village' and 'Web of Science'.

Table 1 Questions being addressed in microactuator fabrication, control, and localization

Fabrication	Control	Localization
How can micro actuators be designed to increase their propulsive efficiency and speed[48]?	How can microrobot swarms be deployed with high precision control[49]?	How can micro actuators be localized in real-time *in vivo*[14]?
How to allow the interaction between the actuator and the local environment so that the actuator processes information and starts to learn[50]?	How can the actuators be controlled such that they can be collected and reused[51]?	How can payloads be released at precise locations[52]?
How can microrobots be made such that they are biocompatible and do not elicit immune responses[53,54]?	How can microrobots be controlled to intelligently respond to a range of stimuli (*i.e.*, actuate, percept, respond, and assess[55]?	How to enhance/switch propulsion modes in different terrains[56]?

The diverse scope and transformative potential of bio-hybrid magnetic robots have caused experts from different fields to solve the problems (fabrication, control, and localization) listed in Table 1.

3.1. Eukaryotic based actuators

Eukaryotic cells consist of a well-defined nucleus and other organelles, such as mitochondria, ribosomes, and proteins. Living cells from protozoa and fungi[80] to plants,[49,51,66,81] and animals[73] have been used to fabricate bio-hybrid eukaryotic actuators. Micromotors based on eukaryotes have distinctive advantages due to their relative size, ranging from 10–100 μm, and inherent biochemically driven motion. This innate self-actuation mechanism can significantly attenuate effective external control, as cell-driven processes are challenging to manipulate over short time scales. However, controlling the motion/actuation of these eukaryotic cells and the ability to steer them in fluidic microenvironments will be essential in the future use of these systems as tools for exploring eukaryotic cell biology and for illuminating the intricacies of self-assembled living systems.

The aforementioned applications are performed using an external magnetic field and require magnetic material for actuation. Most microorganisms in nature consist of a certain level of magnetic material. For example, in many cells, the protein ferritin is responsible for reversible formation and dissolution of magnetic iron oxide and its storage.[82] Although magnetic iron oxides are often present in living organisms, they usually exist in trace amounts and thus have inadequate volumes for magnetic field actuation. To accommodate effective magnetic control, eukaryotic cells must be functionalized to be sufficiently magnetic. Thereby sufficient magnetic force and torque can then be applied to these organisms to steer them wirelessly to a location of interest *in vitro* and *in vivo*. For this purpose, researchers have looked towards genetically modifying and controlling the formation of ferritin in these cells to augment them with desired functionality. To this extent, Kim *et al.* genetically modified mammalian cells through the ectopic creation of the protein human ferritin heavy chains (hFTH1), driving increased uptake of iron ions, resulting in the cell displaying superparamagnetic behavior.[11] When a magnetic field was applied to these cells containing superparamagnetic particles, the cells experienced transitional motion. The cells achieved velocities up to 30 μm s^{-1} and could be separated efficiently from complex mixtures. Kim *et al.*'s research paved the way to genetically modify eukaryotic cells to aid in practical cell separation studies for advanced diagnostics and cell-based therapies.[11]

Recently in 2019, through localized cellular heating of magnetic material, Ito *et al.* magnetically remote-controlled transgene expression in mammalian cells (Human cervical carcinoma HeLa and Human hepatoblastoma HepG2 cells).[83] Magnetic cells were generated by engineering cells with a ferritin gene and tagging cells with magnetite nanoparticles. With exposure to an alternating magnetic field, transgene expression was induced in the cell, demonstrating a novel approach to controlling the appearance of therapeutic genes in cell-based regenerative medicine. While genetically engineered cells can improve the actuator's response to magnetic fields, there is an alternative manufacturing method that can produce similar results. This method involves attaching synthetic magnetic particles to eukaryotes through layer coating or internalization without modifying the organism's genetics. The microrobots made as a result are referred to as magnetic hybrid eukaryotic actuators.

3.1.1. Hybrid eukaryotic actuators. Bio-hybrid systems are composed of cells and inorganic appendages that enhance the device's functionality. Table 2 summarizes the various mammalian organisms used to fabricate eukaryotic bio-hybrid microactuators.

3.1.1.1. Plant & fungi-based actuators. Derivatives of cell-walled organisms such as plants and fungi have been used to fabricate actuators because of their renewability, low harvest cost, thermal stability, and diverse structural morphology.[84,85] For example, Liu *et al.* manufactured a porous magnetic micromotor based on Kapok fibers, hollow tubular structures.[51] The motor's manganese dioxide coating allowed for hydrogen peroxide fuelled actuation, while iron/aluminum metal oxides permitted guidance by an external magnetic field to remove organic pollutants from water. Similarly, Li *et al.* fabricated lotus pollen template-based magnetically actuated robots by coupling magnetic particles with a hydrogel layer, achieving absorption and release of erythromycin.[81] Here, trimanganese tetraoxide served as a catalyst, allowing propulsion *via* oxygen bubble generation, while cobalt ferrite allowed steering *via* an external magnetic field.

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Table 2 Types of magnetic bio-hybrid actuators

Author	Magnetic material	Organic material	Average speed ($\mu m\ s^{-1}$)	Average speed (body length per s)	Actuator
Guo *et al.*[65]	Fe_3O_4 nanoparticles, and polymeric materials	Red blood cell membrane	N/A	N/A	Rebuilt red blood cells
Yasa *et al.*[54]	Double-helical microswimmer	Macrophage	46.7	2.3	Both synthetic and organic parts contribute to swimming
Liu *et al.*[51]	$Al(NO_3)_3{\cdot}9H_2O$ $Fe(NO_3)_3{\cdot}9H_2O$	Kapok fibers	150 (ZIF-8MEOH) 105 (ZIF-8DMF)	1.5 (ZIF-8MEOH) 0.99 (ZIF-8DMF)	O_2 bubbles
Sun *et al.*[49]	Magnetic particles iron oxide	Pine pollen	175.19 (tumbling), 108.25 (rolling)	2.9 (tumbling), 1.8 (rolling)	Magnetic particles iron oxide
Sun *et al.*[66]	Nickel coating	Sunflower pollen grain	125	4.2	Nickel coating
Xie *et al.*[67]	Iron oxide nanoparticles	Spirulina platensis	N/A	N/A	Spirulina platensis
Yan *et al.*[68]	Iron oxide nanoparticles	Spirulina platensis	N/A	N/A	Spirulina platensis
Yasa *et al.*[69]	Magnetic spherical	C. reinhardtii microalga	51.89 ± 1.67 (2D), 135.92 ± 4.82 (3D)	0.5 (2D), 1.4 (3D)	C. reinhardtii microalga
Santomauro *et al.*[70]	Terbium	C. reinhardtii microalga	217 ± 7.1	2.2	C. reinhardtii microalga
Magdanz *et al.*[71]	Iron oxide particles	Bull spermatozoa	30	1	Iron oxide particles
Xu *et al.*[52]	Tetrapod	Sperm	41 ± 10	2.1	Sperm
Magdanz *et al.*[72]	Iron oxide	Bovine sperm	1	0.01	Iron oxide
Magdanz *et al.*[73]	Maghemite nanoparticles	Bovine sperm	6.8 ± 4.1	0.2	Maghemite nanoparticles
Xu *et al.*[74]	Magnetic horned caps	Sperm	76 ± 17 (in blood)	1.5	Sperm
Stanton *et al.*[75]	Electropolymerized Microtube	*E. coli*	5 ± 1	0.5	*E. coli*
Zhang *et al.*[76]	Silica coated iron oxide nanoparticle in poly vinyl alcohol network	Staphylococcus aureus *E. coli*	N/A	N/A	Bacteria
Stanton *et al.*[77]	Janus particles	*E. coli*	Pt/Ps: 0.4 ± 0.1, Pt/SiO_2: 0.7 ± 0.2	Pt/Ps: 0.2, Pt/SiO_2: 1.2	*E. coli*
Li *et al.*[56]	Iron oxide nanoparticles	*Salmonella typhimurium*	5.82	0.77	*Salmonella typhimurium*
Li *et al.*[78]	Fe_3O_4 particles	*Magnetospirillum magneticum*	N/A	N/A	*Magnetospirillum magneticum*
Alapan *et al.*[79]	Iron oxide nanoparticles	Red blood cell, *E. coli*	10.2 ± 3.5	2.0	*E. coli*

Another plant-based actuator was recently reported by Sun *et al.*, who manufactured a pollen-based micromotor.[49] In the pollen-based micromotor, magnetic particles and doxorubicin (an anti-cancer drug) were encapsulated into two hollow air sacs of the pine pollen. Through magnetization, three swimming modes were attained – rolling, tumbling, and spinning. The complete controllability of the actuator was demonstrated through path planning. This work demonstrated individual and swarm plant-based actuators, which can precisely traverse complex fluids, thereby demonstrating the potential as cargo carriers in targeted release applications. Sun *et al.* also fabricated a sunflower grain, nickel layer coated, magnetic actuator (see Fig. 2a), with two modes of swimming, rolling (at the surface) and rotation (in bulk fluid).[66] The actuator was shown to autonomously pierce the cell membrane of cancer cells to deliver therapeutics. Both Liu *et al.*[51] and Li *et al.*[81] used artificial plant-based eukaryotic systems and magnetic control for the detection of toxic bacteria and the purification of contaminated water. Sun *et al.*[49,66] fabricated actuators that demonstrated various swimming modes aiding in the delivery of drugs. Here, artificial intelligence and path planning were also incorporated, achieving autonomous navigation, swarm control, and obstacle avoidance in complex environments.

Besides plant components, fungi have the capabilities to supply important organic features for eukaryotic actuators, as demonstrated by Zhang *et al.*, who designed a magnetic actuator for detection, real-time tracking, and removal of *Clostridium difficile* bacteria from clinical stool specimens.[80] In this work, *Ganoderma lucidum* spores were encapsulated by a layer of magnetic iron nanoparticles, functionalized with 3-mercaptopropionic acid (MPA), and finally actuated by a rotating magnetic field. In addition to magnetic field control, localization was improved with carbon nanodots, which gave fluorescence properties to the spore actuator. Zhang *et al.*[80] article explored magnetic control utilizing nanoparticle fluorescence which meets the real-time localization and stimuli-responsive challenges outlined in Table 1.

3.1.1.2. Erythrocyte & leukocyte derived actuators. For biomedical applications, actuators made of materials foreign to the body can elicit destructive immune responses affecting

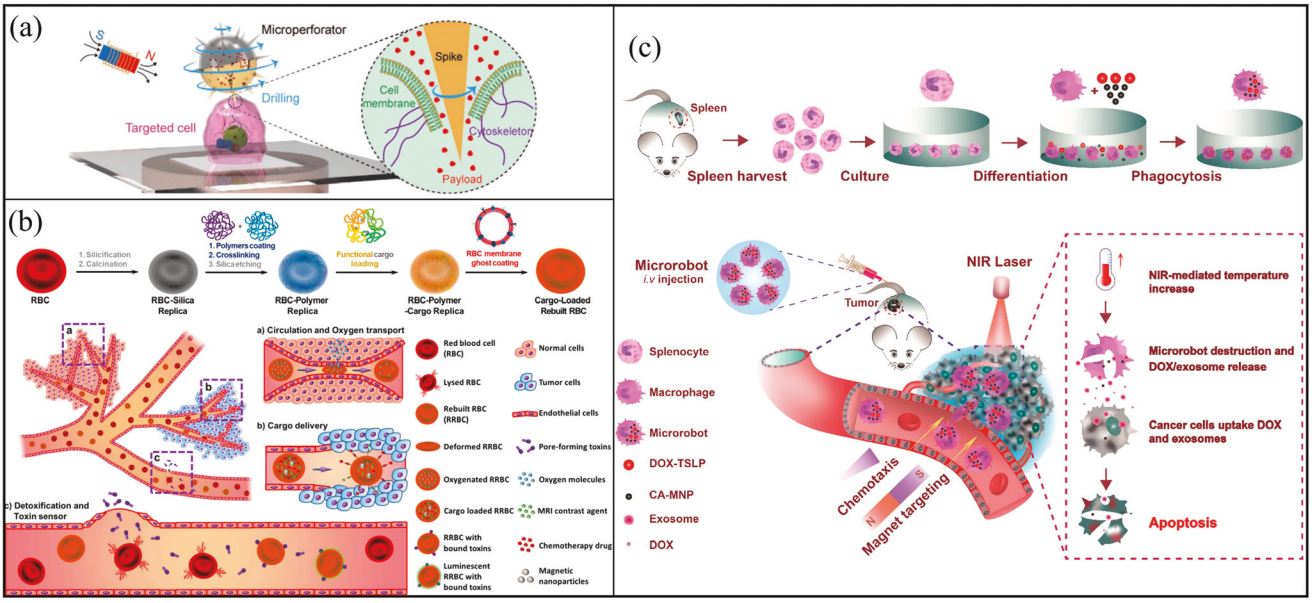

Fig. 2 Eukaryote hybrid actuators. (a) Sunflower grain hybrid actuator magnetically controlled to pierce the cell membrane to deliver drugs. Adapted with Permission.[66] Copyright 2020, Wiley. (b) Schematic diagram of the fabrication steps of the rebuilt red blood cells as wells as images showing the RRBC functionalities, which include cargo delivery, detoxification, and toxin senor and circulation, and oxygen transport. Adapted with Permission.[65] Copyright 2020, ACS (c) Illustration of macrophage hybrid micro actuator fabrication. Adapted with Permission.[53] Copyright 2020, ACS.

therapeutic delivery.[86] To overcome this issue blood cells have been explored for developing bio-hybrid actuators. These cells possess intrinsic biocompatibility, surface immunosuppressive properties, deformability, cargo carrying ability, and chemotactic responsiveness.[63,87] For instance, Wu *et al.* demonstrated a multi cargo-carrying artificial red blood cell (RBC), loaded with quantum dots, doxorubicin, and magnetic nanoparticles.[88] The ultrasound-powered, magnetic guided RBC micromotor has been shown to retain its propulsion properties and can be potentially used in therapeutic and diagnostic applications. Later, Guo *et al.* fabricated an artificially reconstructed red blood cell (RRBC) that mimics mammalian RBCs' structural, mechanical, and functional characteristics.[65] The four-step process used during manufacturing is shown in Fig. 2b. The manufacturing process involves a layer-by-layer infusion of polymer decomposition of iron oxide nanoparticles. The RRBC's were magnetically steered to deliver cargo and target oxygen-deficient regions in the human body, acting as a detoxification and toxin sensor. Other biological cells found in the body have also been used in the manufacturing of a hybrid magnetically actuated micro actuators. For example, Yasa *et al.* investigated the interaction between a magnetically controlled immunobot actuator with macrophage cells.[54] The immunobot was a 3D printed helical micromotor made of nickel, gold, and polyethylene glycol (PEG) coating. Phagocytosis of the actuators was then performed by the macrophage cells, which exhibited different forms of motility such as rolling, crawling, and rowing. More recently, Nguyen *et al.* constructed a multifunctional micromotor using macrophages isolated from mice.[53] These micromotors retain the chemotactic ability of the macrophage and contain responsive agents allowing steering through the use of magnetic fields and drug release in response to near infrared (NIR) laser irradiation (Fig. 2c). Work from Wu *et al.*,[88] Guo *et al.*,[65] Yasa *et al.*,[54] and Nguyen *et al.*[53] has shown that artificially implanted magnetic actuators can be used to transport, control, and influence eukaryotic cell behavior and immune response for biomedical applications.

3.1.1.3. Microorganism-based eukaryotic actuators. Eukaryotic actuators can also be fabricated through the integration of whole microorganisms with magnetic components. This is fabrication strategy can be advantageous as the living actuator incorporates the organism's innate propulsion mechanisms and physical properties. To illustrate this, Yasa *et al.* and Santomauro *et al.* have created a microactuator powered by the unicellular freshwater microalgae *Chlamydomonas reinhandtii.*[69,70] Yasa *et al.* used polyelectrolyte-functionalized magnetic spherical cargos attached to the surface of microalgae that allowed the hybrid actuator to be magnetically steered.[69] On the other hand, Santomauro *et al.* used microalgae incorporated with terbium as a bio-cyborg actuator.[70] Terbium enables the organism to be controlled and localized through permanent magnetic fields. Additionally, terbium did not affect the velocity of the microalgae cyborg actuator. Another actuator integrating the entire microorganism was shown by Yan *et al.*, who fabricated Spirulina microalgae coated with magnetite, allowing *in vivo* fluorescence imaging and remote diagnostic sensing.[57] To demonstrate its effectiveness, a swarm was also shown to be tracked in a rodent's stomach, guided by an external magnetic field. Yan *et al.* further explored the ability to functionalize the Spirulina cells to transport and release molecular cargoes by exploiting

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the cell's dehydration and rehydration capabilities.[68] The Spirulina actuator could be used to deliver molecular agents to the gastrointestinal tract through its low magnetic field. Later, Xie *et al.* imaged and tracked a swarm of Spirulina, actuated with electromagnetic coils, designed with an off-on fluorescence diagnosis enhanced by a polydopamine (PDA) coating.[67] These investigations show that cyborg actuators can retain their intrinsic functionalities while guided by an external magnetic field.

Sperm cells serve as another type of microorganism that can be utilized to create a bio-hybrid eukaryotic actuator. Interest in sperm robots has been accentuated because of their potential uses in treating reproductive tract infections and their potential use in the enhancement of non-motile sperms. For example, Xu *et al.* designed a tetrapod coupled with bovine sperm cells that can be magnetically guided and released when the four fins are pressed on (see Fig. 3a).[52] These hybrid sperm actuators laden with doxorubicin were steered using a magnetic field towards HeLa cancer cells. Doxorubicin was released upon impact, penetrating the cancerous cells. After 8 hours, a significant reduction in the cancer cells was noticed. Later, Xu *et al.* demonstrated a sperm micro actuator fabricated with a streamlined horned cap that can actively swim against flowing blood and deliver heparin, actuated by a permanent magnet (Fig. 3b).[74]

The eukaryotic flagella actuators investigated previously relied on the motility of the sperm. Considering motility as one of the parameters during actuator designs, non-motile sperm is also being explored. For example, Magdanz *et al.* fabricated a hybrid actuator using iron oxide nanoparticles that bought motility to a non-motile sperm.[72] Magdanz *et al.* have also recently developed an IRON-sperm by exploiting the difference in charges of bovine sperm cells and rice grain-shaped maghemite nanoparticles.[73] This coupling increased the echogenicity of the actuator, allowing swarms to be localized using ultrasound imaging. The embedded magnetic particles allowed controllability, helical propulsion, and complex maneuvers by an external magnetic field. In addition, drug loading was achieved by incubating doxorubicin-hydrochloride, which demonstrated its potential for biomedical applications. Table 2 also includes a list of different flagella micro/nano actuators. These investigations with eukaryotic flagella actuators have paved the way for further research into the use of magnetic actuation for advance targeted therapeutics.

3.2. Prokaryotic based actuators

Prokaryotes are unicellular microorganisms that form two of the three domains of life – bacteria and archaea. While both types of prokaryote are ubiquitous in nature, bacteria have been by far the most investigated in part to their dominant abundance in nature and their role in human health. Some bacteria have also demonstrated up to six modes of motion,[89] of which swimming motility using bacterial flagella is the minimum requirement for designing prokaryotic actuators. These parameters add to the favourability of bacteria in biomedical applications and environmental monitoring. Therefore, researchers are investigating various methods for incorporating magnetic properties into the bacteria to be controlled by an external magnetic field, ultimately leading to prokaryotic flagella actuators.

A unique species of bacteria exist that creates enough ferritin to control it by an external magnetic field known as magnetotactic bacteria (MTB).[90] These naturally occurring organisms contain magnetosomes, intracellular iron-rich granules, which get synthesized naturally in the body (see Fig. 4a), enabling the

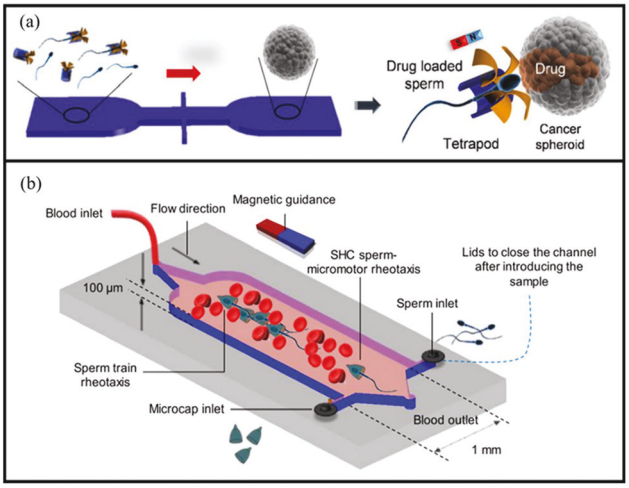

Fig. 3 Eukaryotic flagella actuators (a) Transport of drug loaded hybrid sperm actuators to HeLa cancer cell. Adapted with Permission.[52] Copyright 2017, ACS. (b) Schematic of the streamlined-horned caps hybrid sperm micromotors in blood. Adapted with Permission.[74] Copyright 2020, ACS.

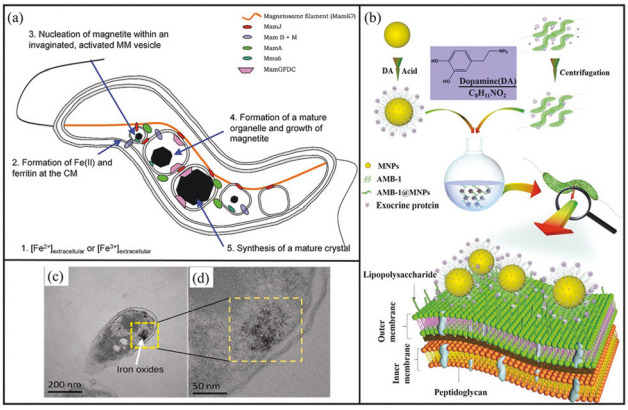

Fig. 4 Magnetotactic bacteria actuators. (a) Model of the iron reaction pathway for the bio-mineralization of the magnetosomes and its chain assembly. Adapted with Permission.[90] Copyright 2008 ACS. (b) Process of embedding iron oxide particles onto the surface of magnetotactic bacteria to enhance the actuator's magnetic response. Adapted with Permission.[78] Copyright 2019, Wiley. (c) TEM images of modified E. Coli (cultured with iron) expressing mineralized mCherry-ferritin. (d) A zoomed-in image of the ferritin formed in the cytosol of modified *E. coli*. Adapted with Permission.[91] Copyright 2020, ACS.

bacteria to align with an external magnetic field. Bacteria that are non-magnetic can be genetically modified with magnetic properties, which can be used in addition to MTB to design magnetic actuators.[91] Alternatively, non-magnetic bacteria, when functionalized with synthetic magnetic particles, attain controllability through magnetic fields. Here these bacterial magnetic devices are termed hybrid prokaryotic actuators.

3.2.1. Hybrid prokaryotic based actuators

3.2.1.1. MTB-based actuators. Magnetotactic bacteria have served as a platform for many researchers to explore prokaryotic actuation. For example, Li *et al.* navigated magnetotactic bacteria *Magnetospirillum magneticum* (AMB-1) in complex fluid environments to deliver drugs while tracked in real-time.[78] In addition to the internally present magnetosomes, Fe_3O_4 magnetic nanoparticles were attached to the surface of the AMB-1 swimmers through electrostatic interactions, enhancing the controllability of the hybrid actuator using an external magnetic field Fig. 4b. Stanton *et al.* also used a magnetotactic bacteria *Magnetospirillum gryphiswaldense* (MSR-1) to design controllable microactuators.[92] The bacteria, MSR-1, were then integrated with drug-loaded mesoporous silica microtubes and used to penetrate a biofilm made of *E. coli*. These microactuators were used in delivering and releasing drugs triggered by the biochemical properties of the biofilm.

3.2.1.2. Genetically modified prokaryotic actuators. Most bacterial species are non-magnetic and must be augmented with magnetic material to allow for magnetic functionality. One approach is genetic engineering, as shown by Aubry *et al.*, who modified *E. coli* to express mCherry ferritin and named the modified bacteria MagEcoli (Fig. 4c and d).[91] The iron stored in the cytosol of the bacteria resulted in paramagnetic behavior when exposed to an external magnetic field. By modifying the surface properties of the MagEcoli, the researchers were able to apply their MagEcoli to trap, and transport targeted bacteria using a magnetic force.

3.2.1.3. Non-magnetic prokaryotic actuators. Bacteria with internalized or genetically induced magnetosomes formations are well suited for the creation of hybrid magnetically steered actuators. However, the alternative method is to attach magnetic material to the surface of the bacteria. By connecting multiple bacteria to superparamagnetic beads, Carlsen *et al.* showed that weak magnetic fields could be used to fabricate and guide the hybrid actuators, achieving average speeds of 1.2 body length per second using *Serratia marcescens.*[30] Further research into controllability determined that a single-celled hybrid actuator results in a more predictable motion compared to the stochastic movement of utilizing multiple bacteria. Using a single-celled actuator, Li *et al.* proposed a hybrid bacteria-bot actuated in large blood vessels by an electromagnet and in small vessels by bacterial-driven motion.[56] By attaching magnetic microparticles to the bacteria, this group has shown that using an external magnetic field can enhance the actuation capability allowing the actuator to operate in different hydrodynamic environments. Another similar bio-hybrid actuator was shown by Stanton *et al.*, who created a bacterial Janus particle by taking advantage of the cell adhesion capabilities of the bacteria's basal body to a variety of metals.[77] The Janus particle consisted of polystyrene or silicon dioxide particles capped separately with platinum, iron, gold, or titanium. Of all the metal coatings, the bacteria exhibited a high adherence to the platinum-coated hemisphere. The hydrophobic nature of the platinum-coated Janus particle and contact angle plays a key role during bacterial adhesion. A hydrophobic material provides significant surface energy for bacterial attachment;[93] however, despite polystyrene's high surface energy determined by a contact angle greater than 100°, bacteria did not attach to its surface.[77] Furthermore, the authors attached the bacteria to iron-coated Janus particles achieving magnetic guidance. Stanton *et al.* also demonstrated the first hybrid prokaryotic actuator that utilizes a microtube functionalized with magnetic properties.[75] The microtube included an inner layer of bacteria-attracting polydopamine and a bacterial kill trigger to stop bacteria from swimming on demand. The work demonstrated this new generation of biocompatible prokaryotic micromotors' potential tools for minimally invasive medical applications.

Magnetically guided prokaryotic actuators have also been harnessed to deliver antibiotics. For example, Zhang *et al.* encapsulated the antibiotic vancomycin into a polymer matrix, which was later internalized by two strains of bacteria (refer to Table 2).[76] Here the antibiotic was loaded into poly-vinyl alcohol (PVA), which was then used to coat iron oxide nanoparticles. The particles were then internalized by the bacteria enabling magnetic control. The small size of the bacteria limited the number of antibiotics delivered by magnetic actuation. For this purpose, investigators integrated the ability of prokaryotic cells' high maneuverability with eukaryotic cells' large loading capacity. A hybrid system coupling prokaryotic and eukaryotic cells were explored by Alapan *et al.*, who designed an erythrocyte-based bacterial actuator, and achieved higher load-carrying capacity while being biocompatible and biodegradable.[79] First, the erythrocytes were loaded with small molecule therapeutics and superparamagnetic iron oxide nanoparticles. Motile *E. coli* were then attached to the surface of red blood cells using biotin-avidin-biotin binding complexes, providing strong non-covalent bonding. The hybrid swimmer was then actuated by the bacteria and guided by an external magnetic field. Finally, optical stimulation was used to add additional functionality leading to cell death. These investigations demonstrated a myriad of hybrid bacteria actuator designs ranging from combining prokaryotes with eukaryotic cells to Janus magnetic particles, allowing the actuator to be potentially applied in various applications, including environmental monitoring and biomedicine.

It is also important to note that the living cells of hybrid prokaryotic actuators can often retain their chemotactic behavior, which can be exploited for cancer therapy.[94] Over long distances, magnetic fields can be used to guide these hybrid actuators to specific regions. Then, across short distances, the bacteriabots can rapidly utilize self-generated bacterial motion to navigate to local targets using chemotaxis. This control strategy can be further enhanced through the use of genetic

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modification, where the bacteria's virulence or desired characteristics can be adjusted. The tunability of bacteria, along with the ease at which they can be integrated with magnetic particles and other natural or synthetic components, provides evidence as to why prokaryotic actuators are highly desirable for small-scale applications.

3.3. Nucleic acid and protein-based actuators

In nature, nucleic acids and proteins often act as supramolecular machines essential for all life, driving biochemical reactions, transporting molecular payloads, and serving as information carriers for cellular tasks. By combining these large molecules with magnetic structures, advanced multifunctional materials have been reported with many potential applications.

3.3.1. Protein-based actuators. Numerous proteins and protein complexes act as biomolecular machines synthesized by cells. These ubiquitous natural actuators have the properties of self-replication and have high operating efficiency. Protein's active properties have led to their use in various multi-degree-of-freedom nanodevices, which have received extensive attention. Molecular machines have been extensively investigated, with myosins,[95] kinesins,[96,97] and dyneins[98] being the most well understood. These molecular motors convert the chemical energy present in a fluidic environment (*e.g.* ions and adenosine triphosphate (ATP)) into nanoscale linear, oscillatory, or rotary mechanical motion, often through minute changes in protein structure.

Recently, research in protein bio-hybrid actuators has been expanding due to novel propulsion and power-like mechanisms being used in fabrication. In 2019, Pena-Francesch *et al.* fabricated one such novel magnetic protein-based nanomotors by integrating a protein matrix from squid ring teeth onto superparamagnetic iron oxide nanoparticles (Fig. 5a).[99] The magnetically controlled protein motor showed higher performance and efficiency than others that employ Marangoni forces for propulsion. The reconfigurable nature of protein actuators was revealed by Ali *et al.*, who fabricated a protein actuator allowing morphological changes of its geometry in response to environmental stimuli.[100] The flagellin protein from *Salmonella typhimurium* bacteria was depolymerized and repolymerized to create functionalized flagella filaments. It was then attached to a superparamagnetic nanoparticle, finally actuated by a rotational magnetic field. Kurinomaru *et al.* designed another reconfigurable protein actuator consisting of serum albumin and magnetic nanoparticles to capture and release cells.[101] This robot was manipulated using the weak fields of a permanent magnet to deliver several intact cells to the desired target on a matrix and in an enclosed space. Kobayakawa *et al.* also used human serum albumin to create a microtube actuator propelled by oxygen bubbles due to the reaction between platinum nanoparticles and hydrogen peroxide (H_2O_2).[102] The tubular robot demonstrates the ability to add motility to a protein-laden polycarbonate membrane, enhancing the removal of cyanine dye and bacteria (Fig. 5b). The first construction of a magnetic targeting pro-coagulant protein for embolic therapy of solid tumors was shown by Zou *et al.*[103] The fabrication involved the surface modification of Fe_3O_4 nanoparticles *via* silanization to facilitate the binding of *O*-carboxymethyl chitosan and the fusion protein tTF-EG3287 (Fig. 5c). Extensive thrombosis was induced in the tumor vessels by attracting the hybrid actuators to the solid tumors using a permanent magnet. A permanent magnet[104] also achieved the actuation of a virus-based bio-hybrid robot. Then, viral particles were additionally functionalized to release Killer red enabling photodynamic therapy under light irradiation.

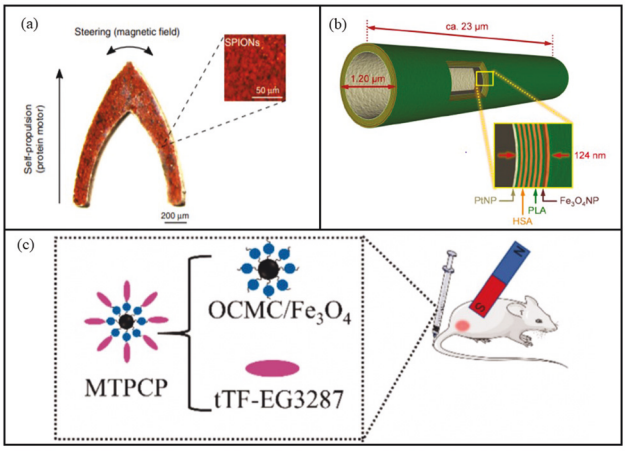

Fig. 5 Magnetic protein actuators (a) Actuator fabricated by integrating superparamagnetic iron oxide nanoparticles into a protein matrix. Adapted with Permission.[99] Copyright 2019, Springer Nature. (b) Dimensions of human serum albumin (HSA) microtube actuator fabricated using wet templating synthesis. The actuator was propelled through a platinum reaction with hydrogen peroxide. Adapted with Permission.[102] Copyright 2017, John Wiley and Sons. (c) Magnetic targeting pro-coagulant protein intravenously administered at the tail and directed by a permanent magnet to the tumor site. Adapted with Permission.[103] Copyright 2019, Taylor & Francis Group.

With protein's ability to self fold into complex hierarchical nanostructures, exist in harsh environments, and be coupled with magnetic actuation for steering, there is a growing interest in the fabrication of these hybrid protein actuators. Therefore, magnetic protein-driven robots have inherent biocompatibility and self-propulsion capabilities and have broad application prospects in biomedical treatment *via* targeted delivery.

3.3.2. Enzyme powered actuators. Another class of protein actuators is enzyme-powered micro/nanomotors, which have become increasingly more prominent in fabricating microactuators due to their biocompatibility and versatility. Through the catalytic action of an enzyme, conventional fuels, including H_2O_2,[105,106] urease,[107–110] and glucose,[111] can be decomposed, providing energy for the actuation of these motors. Combining these natural catalysts with magnetic materials embedded in small-scale structures allows for remote guidance when exposed to external fields. For example, Ma *et al.* proposed a magnetically bio-catalytic Janus motor conjugated with catalase and coated with a metallic nickel (Ni) layer.[32] The catalase triggered the decomposition of H_2O_2 to produce driving force

by bubble propulsion, while the Ni layer facilitated the controllable motion of motors (Fig. 6a). To further enhance the propulsion of enzyme-powered motor, Luo *et al.* reported a Janus Au/magnetic microparticles (MMPs) motor.[110] Multilayers of biotinylated ureases were asymmetrically immobilized on the micromotor through streptavidin and boosted the decomposition of urea, thus improving the swimming ability of micromotors. Due to the magnetic property of MMPs, the micromotors can perform fast magnetic separation and controllable motion direction under the external magnetic field (Fig. 6b). In addition, enzyme-powered micro and nano scale motors have enormous application value in the biomedical scenario. In 2019, Patiño *et al.* introduced a combination of FRET labelled three-strand DNA nano-switch and urease driven mesoporous silica-based micromotors.[107] Here, this device traversed the surrounding microenvironment through pH changes on urea decomposition (Fig. 6c). In the same year, the same group also reported an enzyme-powered nanomotor with pH-triggered drug release caused by the dethreading of the supramolecular nano-valves (see Fig. 6d).[108] In short, enzyme-powered motors can be considered a promising tool in various biomedical applications due to their biological origin, catalytic propulsion, and ability to be integrated with magnetic components.

3.3.3. Nucleic acid-based actuators. Protein and nucleic acid actuators are of growing interest (refer to data trend from Fig. 1b) to researchers because of their built-in biocompatibility, reconfigurable prospective, and modification potential. However, these natural actuators require a different mode of control to remove positional uncertainty in their spatiotemporal operation effectively. The integration of these macromolecules with magnetic materials makes it possible to design motion control systems with greater functionality.

DNA structures formed through folding or 'origami' based processes have become one of the most promising nano actuators. The advantage of DNA origami is that this technique can be designed to include complex structures and mechanisms, such as a cavity that can be open or closed to transport payloads. DNA origami structures are also chemically modifiable and can be functionalized with specific molecules to meet different biomedical needs. For example, Li *et al.* studied the mouse model of breast cancer by combining the DNA nano actuator carrying thrombin with tumor-related endothelial cells.[112] The shape of the nanoactuator was modified to an open folded state with the thrombin exposed explicitly to the tumor site; this helped inhibit tumor growth and induce tumor necrosis. Real-time control of the movement of DNA nano actuators to the target site will further improve the precision of treatment in the biomedical field. At present, the methods of driving DNA nano actuators include the insertion of chains, photoexcitation, electric fields, and magnetic fields.

For actuation using magnetic fields, the size of the magnetic particle is required to be larger than one micron.[113] This condition is necessary as the forces and torque needed to actuate DNA origamis are on the order of magnitude of piconewtons,[114] and magnetic nanoparticles provide forces on the femtonewton scale.[115] In 2005, the first magnetic DNA microrobot was reported using micron-sized superparamagnetic particles.[116] The chemically bound particles on DNA, move similarly to sperm in an oscillating magnetic field. Maier *et al.* connected the tile tube to the DNA-modified magnetic beads through biotin-streptavidin coupling to generate DNA tile tube magnetic bead hybrids driven by a uniform rotating magnetic field.[117] Shape controllable DNA flagella expanded the function of biocompatible nanorobots (see Fig. 7a). Similar work

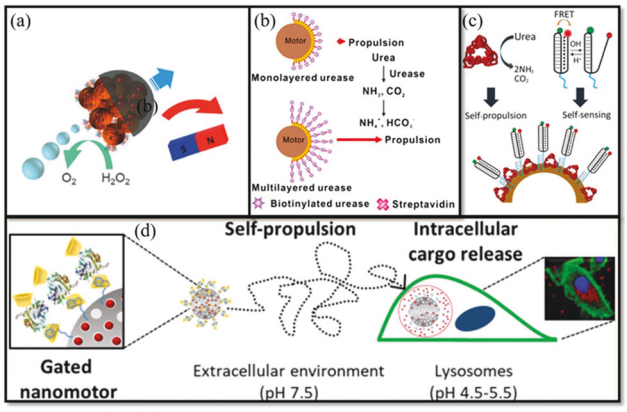

Fig. 6 Enzyme-powered actuators; (a) A magnetically bio-catalytic Janus motor coated with catalase. Adapted with Permission.[32] Copyright 2015, RSC. (b) Schematic illustration of a Janus Au/magnetic microparticles (MMPs) micromotor with multilayers of biotinylated ureases. Adapted with Permission.[110] Copyright 2020, ACS. (c) Enzyme-powered micromotors functionalized with a FRET-labelled triplex DNA nano-switch for pH sensing. Adapted with Permission.[107] Copyright 2018, ACS. (d) Schematic diagram of enzyme-powered mesoporous silica nanomotors' intracellular payload delivery. Adapted with Permission.[108]Copyright 2019, ACS.

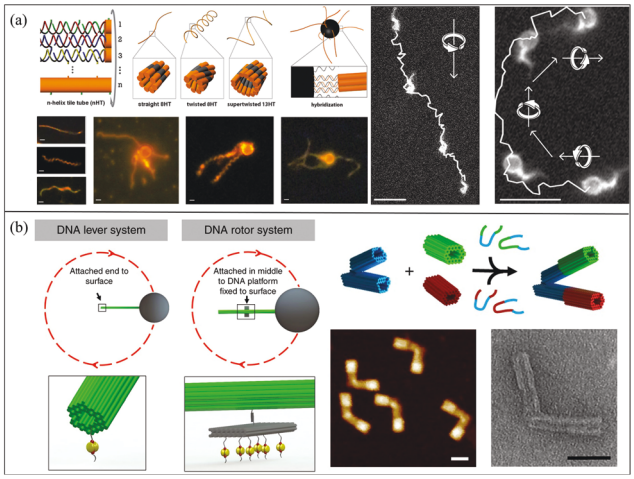

Fig. 7 Magnetic propulsion of DNA nano actuators (a) Structure and directed motion of DNA-flagellated magnetic bead hybrids. Adapted with Permission.[117] Copyright 2016, ACS. (b) Employing external magnetic fields to control DNA origami movement. Adapted with Permission.[119] Copyright 2018, Springer Nature.

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was also performed by Harmatz *et al.*, who was able to precisely construct DNA-microsphere hybrid actuators using a hybrid top-down and bottom-up assembly.[118] The aforementioned design allowed interaction between the DNA-microsphere actuator and its local environment to be controlled using both a rotating and oscillating magnetic field. Therefore, DNA appendages induce actuation capabilities to microspheres by introducing flexibility which breaks the cyclic swimming strokes.

In the recently reported work, Lauback *et al.* demonstrated the control of DNA origami through external magnetic fields (see Fig. 7b).[119] Furthermore, the driving structure can be synthesized by assembling using three types of DNA units: levers, rotors, and hinges. Through biotin-streptavidin, the axis or edge is anchored on the base platform to become the free-moving part of the system, and the free end is connected with magnetic particles. Finally, Tang *et al.* developed a DNA soft robot based on DNA hydrogel material.[120] The soft robot has both super soft and super elastic mechanical properties and can deliver cells to the confined space under the driving of magnetic navigation. More importantly, DNA hydrogel has a three-dimensional porous structure and excellent biocompatibility. It can be used as a three-dimensional material for cell culture and deliver cells to confined space under magnetic navigation without affecting cell activity. The DNA soft robot is expected to be used in diagnosis and treatment, implantable medical equipment, minimally invasive surgery, and other relevant biomedical-related fields.

The hybridization between magnetic particles and DNA was used to construct a biosensor that had improved signal amplification, processivity and can be used for sensitive and label-free cancer detection.[121] The magnetic nanoparticle provided a 3D surface for the DNA conjugated gold nanoparticles to roll, releasing large amounts of gold nanoclusters in the presence of target DNA. This work demonstrates the methods used to create machines that can respond to a stimulus, as mentioned in Table 1.

4. Current challenges and outlook

Here we discussed the latest development in a new class of small-scale actuator that combine cells and sub cellular components with magnetic materials. Categorizing these bio-hybrid actuators based on their biological appendage, we highlight their ability to address current challenges in fabrication, control, and localization. Actuators that utilize eukaryotic components achieve diverse structural and mechanical characteristics and can possess relatively large storage capacity. Additionally, actuators incorporating prokaryotes can benefit from cancer-targeting chemotactic behavior and high propulsion speeds. Integrating proteins and nucleic acid into magnetic actuators allows morphological toughness, catalytic propulsion, and programmable biomarkers. The biological appendage chosen for a magnetic bio-hybrid actuator determines the ability to be utilized for a specific task. Recent achievements of these tiny devices demonstrate the potential of magnetic bio-hybrid actuators for future environmental and biomedical applications.

Despite recent advances, most experiments are still performed *in vitro*, not capturing the heterogeneous microenvironment of tissues, cells, and other complex structures. In these environments, if a device is too small, controlled magnetic actuation can be complex, and if it is too large, it can be cleared by the body's defense systems. Also, rigid swimmers can become entangled, preventing actuators from reaching intended targets. Solutions are provided in designing magnetic bio-hybrid actuators that can acquire different gaits or undergo polymorphic transformations through multi-stimuli response mechanisms. Actuators responsive to multiple stimuli can be directed though external and internal energy sources, including chemical and optical.[122–124] The former is usually inherent to the biological component of the actuator. The latter is currently the most used actuating technique after magnetic manipulation and offers high temporal resolution for precise control of multiple actuators.[122–125] Non-ionizing irradiation also allows for controlling complex tasks such as release and binding cargo[126] and increasing metabolic activity.[127] To this extent, artificial intelligence is currently being explored for identifying design criteria and optimizing the 'physical intelligence[128]' of hybrid systems in dynamic heterogonous environments.

Moving towards *in vivo* investigations necessitates capturing the complex microenvironment and precisely localizing the position of micromotors within the human body in real-time. For localization, fluorescence can be used with bio-hybrid actuators to enable live imaging. Fluorescent dyes or quantum dots can be added to organisms or particles that do not significantly autofluorescence.[57,67,79] This has made fluorescence imaging an appealing approach for biomedical applications;[80] however, its penetration depth is limited. Ultrasound,[129–131] positron emission tomography (PET), computed tomography (CT), multispectral optoacoustic tomography,[132] and magnetic resonance imaging (MRI)[57] are other imaging methods that have been used to overcome the penetration depth limitation. Ultrasound is helpful for guidance and deep tissue penetration; however, the microrobot should be larger than the sonographic detection limit, and bubbles are usually required to improve contrast. Similarly, PET and CT have been used to improve contrast, but ionizing radiation and radioactive energy can be detrimental to practical long-term *in vivo* localization. MRI imaging has demonstrated its versatility for imaging and actuating magnetic microrobots with sub-millimeter scale spatial and temporal resolution. Magnetic particle imaging, a new imaging technique that uses superparamagnetic iron oxide tracers to capture informative 3D images, has recently shown promise for *in vivo* applications.[133] Its ability to localize and control swarms also reduces the complexity of the previously mentioned image modalities.[134] Finally, one common aspect among the imaging methods mentioned is the high concentration of actuators needed to improve contrast for localization. As a result, control strategies

directed at manipulating swarms of magnetic bio-hybrid actuators will also need to be considered for the targeted applications.[49,57,135]

The use of micro and nanoscale wireless actuators for routine clinical procedures is still in the distant future, however, we foresee that future developments in bio-hybrid microrobotic systems will bridge the gap to realizing the long-sought 'fantastic voyage.'

Author contributions

Review article conceptualization, P.B., D.Q., U.K.C., and J.A.; methodology; validation, J.A., U.K.C., and P.B.; data curation, P.B. and D.Q; writing—original draft preparation, P.B and J.A.; writing—review and editing, J.A, Y.K., D.Q and P.B, U.K.C.; visualization, P.B. and J.A.; supervision, J.A., U.K.C.; project administration, J.A. Writing, review, and editing Z.C., Z.W., X. M., X.S., T.J., Y.Z. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1 A. Ghosh, D. Paria, G. Rangarajan and A. Ghosh, *J. Phys. Chem. Lett.*, 2014, **5**, 62–68.
2 J. J. Abbott, K. E. Peyer, M. C. Lagomarsino, L. Zhang, L. Dong, I. K. Kaliakatsos and B. J. Nelson, *Int. J. Rob. Res.*, 2009, **28**, 1434–1447.
3 D. Li, C. Liu, Y. Yang, L. Wang and Y. Shen, *Light: Sci. Appl.*, 2020, **9**, 84–94.
4 E. M. Purcell, *Am. J. Phys.*, 1977, **45**, 3–11.
5 S. Jeon, S. Kim, S. Ha, S. Lee, E. Kim, S. Y. Kim, S. H. Park, J. H. Jeon, S. W. Kim, C. Moon, B. J. Nelson, J.-y. Kim, S.-W. Yu and H. Choi, *Sci. Rob.*, 2019, **4**, eaav4317.
6 A. V. Singh, M. H. Ansari, M. Mahajan, S. Srivastava, S. Kashyap, P. Dwivedi, V. Pandit and U. Katha, *Micromachines*, 2020, **11**, 448–465.
7 L. Zhang, K. E. Peyer and B. J. Nelson, *Lab Chip*, 2010, **10**, 2203–2215.
8 C. Hu, S. Pané and B. J. Nelson, *Annu. Rev. Control Rob. Auton. Syst.*, 2018, **1**, 53–75.
9 S. Kriegman, D. Blackiston, M. Levin and J. Bongard, *Proc. Natl. Acad. Sci. U. S. A.*, 2020, **117**, 1853–1859.
10 N. Buss, O. Yasa, Y. Alapan, M. B. Akolpoglu and M. Sitti, *APL Bioeng.*, 2020, **4**, 026103.
11 T. Kim, D. Moore and M. Fussenegger, *J. Biotechnol.*, 2012, **162**, 237–245.
12 B. Yigit, Y. Alapan and M. Sitti, *Adv. Sci.*, 2019, **6**, 1801837.
13 S. Palagi and P. Fischer, *Nat. Rev. Mater.*, 2018, **3**, 113–124.
14 A. Aziz, S. Pane, V. Iacovacci, N. Koukourakis, J. Czarske, A. Menciassi, M. Medina-Sánchez and O. G. Schmidt, *ACS Nano*, 2020, **14**, 10865–10893.
15 M. M. Stanton and S. Sánchez, *Trends Biotechnol.*, 2017, **35**, 910–913.
16 Y. Alapan, O. Yasa, B. Yigit, I. C. Yasa, P. Erkoc and M. Sitti, *Annu. Rev. Control Rob. Auton. Syst.*, 2019, **2**, 205–230.
17 G. Saper and H. Hess, *Chem. Rev.*, 2020, **120**, 288–309.
18 F. Soto, M. A. Lopez-Ramirez, I. Jeerapan, B. Esteban-Fernández de Ávila, R. K. Mishra, X. Lu, I. Chai, C. Chen, D. Kupor, A. Nourhani and J. Wang, *Adv. Funct. Mater.*, 2019, **29**, 1900658.
19 J. Yang, C. Zhang, X. Wang, W. Wang, N. Xi and L. Liu, *Sci. China: Technol. Sci.*, 2019, **62**, 1–20.
20 Q. Cao, Q. Fan, Q. Chen, C. Liu, X. Han and L. Li, *Mater. Horiz.*, 2020, **7**, 638–666.
21 B. Esteban-Fernández de Ávila, P. Angsantikul, D. E. Ramírez-Herrera, F. Soto, H. Teymourian, D. Dehaini, Y. Chen, L. Zhang and J. Wang, *Sci. Rob.*, 2018, **3**, eaat0485.
22 K. Yuan, J. Bujalance-Fernández, B. Jurado-Sánchez and A. Escarpa, *Microchim. Acta*, 2020, **187**, 581.
23 C. Chen, X. Chang, P. Angsantikul, J. Li, B. Esteban-Fernández de Ávila, E. Karshalev, W. Liu, F. Mou, S. He, R. Castillo, Y. Liang, J. Guan, L. Zhang and J. Wang, *Adv. Biosyst.*, 2018, **2**, 1700160.
24 B. V. V. S. P. Kumar, A. J. Patil and S. Mann, *Nat. Chem.*, 2018, **10**, 1154–1163.
25 M. Sitti and D. S. Wiersma, *Adv. Mater.*, 2020, **32**, 1906766.
26 H. Hess, *Annu. Rev. Biomed. Eng.*, 2011, **13**, 429–450.
27 F. Soto and R. Chrostowski, *Front. Bioeng. Biotechnol.*, 2018, **6**, 170.
28 N. Ebrahimi, C. Bi, D. J. Cappelleri, G. Ciuti, A. T. Conn, D. Faivre, N. Habibi, A. Hošovský, V. Iacovacci, I. S. M. Khalil, V. Magdanz, S. Misra, C. Pawashe, R. Rashidifar, P. E. D. Soto-Rodriguez, Z. Fekete and A. Jafari, *Adv. Funct. Mater.*, 2021, **31**, 2005137.
29 A. M. Mustafa and N. Khaled, *IOP Conf. Ser.: Mater. Sci. Eng.*, 2020, **717**, 012006.
30 R. W. Carlsen, M. R. Edwards, J. Zhuang, C. Pacoret and M. Sitti, *Lab Chip*, 2014, **14**, 3850–3859.
31 D. Jang, J. Jeong, H. Song and S. K. Chung, *J. Micromech. Microeng.*, 2019, **29**, 053002.
32 X. Ma and S. Sanchez, *Chem. Commun.*, 2015, **51**, 5467–5470.

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33 P. Tartaj, M. a. d. P. Morales, S. Veintemillas-Verdaguer, T. González-Carreño and C. J. Serna, *J. Phys. D: Appl. Phys.*, 2003, **36**, R182–R197.

34 S. Oyarzún, A. Tamion, F. Tournus, V. Dupuis and M. Hillenkamp, *Sci. Rep.*, 2015, **5**, 14749.

35 S. S. Shevkoplyas, A. C. Siegel, R. M. Westervelt, M. G. Prentiss and G. M. Whitesides, *Lab Chip*, 2007, **7**, 1294–1302.

36 D. Jiles, *Introduction to Magnetism and Magnetic Materials*, CRC Press, 3rd edn, 2015, pp. 626, DOI: 10.1201/b18948.

37 J. Lipfert, M. M. van Oene, M. Lee, F. Pedaci and N. H. Dekker, *Chem. Rev.*, 2015, **115**, 1449–1474.

38 X. J. A. Janssen, A. J. Schellekens, K. van Ommering, L. J. van Ijzendoorn and M. W. J. Prins, *Biosens. Bioelectron.*, 2009, **24**, 1937–1941.

39 K. Bente, A. Codutti, F. Bachmann and D. Faivre, *Small*, 2018, **14**, 1704374.

40 U. K. Cheang, F. Meshkati, D. Kim, M. J. Kim and H. C. Fu, *Phys. Rev. E: Stat., Nonlinear, Soft Matter Phys.*, 2014, **90**, 033007.

41 K. I. Morozov, Y. Mirzae, O. Kenneth and A. M. Leshansky, *Phys. Rev. Fluids*, 2017, **2**, 044202.

42 J. J. Abbott, O. Ergeneman, M. P. Kummer, A. M. Hirt and B. J. Nelson, *IEEE Trans. Robot.*, 2007, **23**, 1247–1252.

43 K. B. Yesin, K. Vollmers and B. J. Nelson, *Int. J. Rob. Res.*, 2006, **25**, 527–536.

44 P. Chakrabarty, S. Roy and R. Paily, *Analysis of Electromagnetic Actuation System for Different Coil Topologies*, TENCON 2019 – 2019 IEEE Region 10 Conference (TENCON), 2019, pp. 679–683.

45 Y.-L. Liu, D. Chen, P. Shang and D.-C. Yin, *J. Controlled Release*, 2019, **302**, 90–104.

46 J. Lipfert, X. Hao and N. H. Dekker, *Biophys. J.*, 2009, **96**, 5040–5049.

47 T. W. R. Fountain, P. V. Kailat and J. J. Abbott, *IEEE Int. Conf. Rob. Autom.*, 2010, 576–581.

48 J. Miao, X. Li, B. Liang, J. Wang and X. Xu, *Sensors*, 2021, **21**, 494.

49 M. Sun, X. Fan, X. Meng, J. Song, W. Chen, L. Sun and H. Xie, *Nanoscale*, 2019, **11**, 18382–18392.

50 P. Fischer, *Adv. Mater.*, 2020, **32**, 1905953.

51 J. Liu, J. Li, G. Wang, W. Yang, J. Yang and Y. Liu, *J. Colloid Interface Sci.*, 2019, **555**, 234–244.

52 H. Xu, M. Medina-Sánchez, V. Magdanz, L. Schwarz, F. Hebenstreit and O. G. Schmidt, *ACS Nano*, 2018, **12**, 327–337.

53 V. D. Nguyen, H.-K. Min, H. Y. Kim, J. Han, Y. H. Choi, C.-S. Kim, J.-O. Park and E. Choi, *ACS Nano*, 2021, **15**, 8492–8506.

54 I. C. Yasa, H. Ceylan, U. Bozuyuk, A.-M. Wild and M. Sitti, *Sci. Rob.*, 2020, **5**, eaaz3867.

55 F. Soto, J. Wang, R. Ahmed and U. Demirci, *Adv. Sci.*, 2020, **7**, 2002203.

56 D. Li, H. Choi, S. Cho, S. Jeong, Z. Jin, C. Lee, S. Y. Ko, J.-O. Park and S. Park, *Biotechnol. Bioeng.*, 2015, **112**, 1623–1631.

57 X. Yan, Q. Zhou, M. Vincent, Y. Deng, J. Yu, J. Xu, T. Xu, T. Tang, L. Bian, Y.-X. J. Wang, K. Kostarelos and L. Zhang, *Sci. Rob.*, 2017, **2**, eaaq1155.

58 B. J. Nelson, I. K. Kaliakatsos and J. J. Abbott, *Annu. Rev. Biomed. Eng.*, 2010, **12**, 55–85.

59 S. Chowdhury, B. V. Johnson, W. Jing and D. J. Cappelleri, *J. Micro-Bio Rob.*, 2017, **12**, 21–31.

60 J. Sikorski, I. Dawson, A. Denasi, E. E. G. Hekman and S. Misra, *IEEE Int. Conf. Rob. Autom.*, 2017, 3594–3599.

61 J. Nam, W. Lee, E. Jung and G. Jang, *IEEE Trans. Ind. Electron.*, 2018, **65**, 5673–5681.

62 A. Pourkand and J. J. Abbott, *IEEE Rob. Autom. Lett.*, 2018, **3**, 2957–2962.

63 Z. Wu, T. Li, J. Li, W. Gao, T. Xu, C. Christianson, W. Gao, M. Galarnyk, Q. He, L. Zhang and J. Wang, *ACS Nano*, 2014, **8**, 12041–12048.

64 R. D. Field, P. N. Anandakumaran and S. K. Sia, *Appl. Phys. Rev.*, 2019, **6**, 041305.

65 J. Guo, J. O. Agola, R. Serda, S. Franco, Q. Lei, L. Wang, J. Minster, J. G. Croissant, K. S. Butler, W. Zhu and C. J. Brinker, *ACS Nano*, 2020, **14**, 7847–7859.

66 M. Sun, Q. Liu, X. Fan, Y. Wang, W. Chen, C. Tian, L. Sun and H. Xie, *Small*, 2020, **16**, 1906701.

67 L. Xie, X. Pang, X. Yan, Q. Dai, H. Lin, J. Ye, Y. Cheng, Q. Zhao, X. Ma, X. Zhang, G. Liu and X. Chen, *ACS Nano*, 2020, **14**, 2880–2893.

68 X. Yan, J. Xu, Q. Zhou, D. Jin, C. I. Vong, Q. Feng, D. H. L. Ng, L. Bian and L. Zhang, *Appl. Mater. Today*, 2019, **15**, 242–251.

69 O. Yasa, P. Erkoc, Y. Alapan and M. Sitti, *Adv. Mater.*, 2018, **30**, 1804130.

70 G. Santomauro, A. V. Singh, B.-W. Park, M. Mohammadrahimi, P. Erkoc, E. Goering, G. Schütz, M. Sitti and J. Bill, *Adv. Biosyst.*, 2018, **2**, 1800039.

71 V. Magdanz, M. Guix, F. Hebenstreit and O. G. Schmidt, *Adv. Mater.*, 2016, **28**, 4084–4089.

72 V. Magdanz, J. Gebauer, D. Mahdy, J. Simmchen and I. S. M. Khalil, Sperm-templated magnetic microrobots, *2019 International Conference on Manipulation, Automation and Robotics at Small Scales (MARSS)*, 2019, pp. 1–6.

73 V. Magdanz, I. S. M. Khalil, J. Simmchen, G. P. Furtado, S. Mohanty, J. Gebauer, H. Xu, A. Klingner, A. Aziz, M. Medina-Sánchez, O. G. Schmidt and S. Misra, *Sci. Adv.*, 2020, **6**, eaba5855.

74 H. Xu, M. Medina-Sánchez, M. F. Maitz, C. Werner and O. G. Schmidt, *ACS Nano*, 2020, **14**, 2982–2993.

75 M. M. Stanton, B.-W. Park, A. Miguel-López, X. Ma, M. Sitti and S. Sánchez, *Small*, 2017, **13**, 1603679.

76 W. Zhang, R. Taheri-Ledari, Z. Hajizadeh, E. Zolfaghari, M. R. Ahghari, A. Maleki, M. R. Hamblin and Y. Tian, *Nanoscale*, 2020, **12**, 3855–3870.

77 M. M. Stanton, J. Simmchen, X. Ma, A. Miguel-López and S. Sánchez, *Adv. Mater. Interfaces*, 2016, **3**, 1500505.

78 Q. Li, H. Chen, X. Feng, C. Yu, F. Feng, Y. Chai, P. Lu, T. Song, X. Wang and L. Yao, *Small*, 2019, **15**, 1900427.

79 Y. Alapan, O. Yasa, O. Schauer, J. Giltinan, A. F. Tabak, V. Sourjik and M. Sitti, *Sci. Rob.*, 2018, **3**, eaar4423.
80 Y. Zhang, L. Zhang, L. Yang, C. I. Vong, K. F. Chan, W. K. K. Wu, T. N. Y. Kwong, N. W. S. Lo, M. Ip, S. H. Wong, J. J. Y. Sung, P. W. Y. Chiu and L. Zhang, *Sci. Adv.*, 2019, **5**, eaau9650.
81 J. Li, F. Ji, D. H. L. Ng, J. Liu, X. Bing and P. Wang, *Chem. Eng. J.*, 2019, **369**, 611–620.
82 E. C. Theil, *J. Nutr.*, 2003, **133**, 1549S–1553S.
83 A. Ito, R. Teranishi, K. Kamei, M. Yamaguchi, A. Ono, S. Masumoto, Y. Sonoda, M. Horie, Y. Kawabe and M. Kamihira, *J. Biosci. Bioeng.*, 2019, **128**, 355–364.
84 S. Iravani and R. S. Varma, *Nano-Micro Lett.*, 2021, **13**, 128.
85 B. Han, Z. Weng, Y. Wu, X. Zhao, J. Li, Q. Zhang, K. Qu, B. Liang, F. Zhou, G. Liu and Z. Wang, *J. Bionic Eng.*, 2021, **18**, 915–926.
86 Y. Gao, F. Wei, Y. Chao and L. Yao, *Biomed. Microdevices*, 2021, **23**, 52.
87 Y. Zhang, Q. Wang, T. Ma, D. Zhu, T. Liu and F. Lv, *J. Controlled Release*, 2020, **328**, 127–140.
88 Z. Wu, B. Esteban-Fernández de Ávila, A. Martín, C. Christianson, W. Gao, S. K. Thamphiwatana, A. Escarpa, Q. He, L. Zhang and J. Wang, *Nanoscale*, 2015, **7**, 13680–13686.
89 J. D. Shrout, *Trends Microbiol.*, 2015, **23**, 244–246.
90 D. Faivre and D. Schüler, *Chem. Rev.*, 2008, **108**, 4875–4898.
91 M. Aubry, W.-A. Wang, Y. Guyodo, E. Delacou, J.-M. Guigner, O. Espeli, A. Lebreton, F. Guyot and Z. Gueroui, *ACS Synth. Biol.*, 2020, **9**, 3030–3041.
92 M. M. Stanton, B.-W. Park, D. Vilela, K. Bente, D. Faivre, M. Sitti and S. Sánchez, *ACS Nano*, 2017, **11**, 9968–9978.
93 H. H. Tuson and D. B. Weibel, *Soft Matter*, 2013, **9**, 4368–4380.
94 M. Holay, Z. Guo, J. Pihl, J. Heo, J. H. Park, R. H. Fang and L. Zhang, *ACS Appl. Bio Mater.*, 2021, **4**, 3830–3848.
95 K. Kitamura, M. Tokunaga, A. H. Iwane and T. Yanagida, *Nature*, 1999, **397**, 129–134.
96 S. M. Block, *Cell*, 1998, **93**, 5–8.
97 M. J. Schnitzer and S. M. Block, *Nature*, 1997, **388**, 386–390.
98 C. Shingyoji, H. Higuchi, M. Yoshimura, E. Katayama and T. Yanagida, *Nature*, 1998, **393**, 711–714.
99 A. Pena-Francesch, J. Giltinan and M. Sitti, *Nat. Commun.*, 2019, **10**, 3188.
100 J. Ali, U. K. Cheang, J. D. Martindale, M. Jabbarzadeh, H. C. Fu and M. J. Kim, *Sci. Rep.*, 2017, **7**, 14098.
101 T. Kurinomaru, A. Inagaki, M. Hoshi, C. Nakamura and H. Yamazoe, *Mater. Horiz.*, 2020, **7**, 877–884.
102 S. Kobayakawa, Y. Nakai, M. Akiyama and T. Komatsu, *Chem. – Eur. J.*, 2017, **23**, 5044–5050.
103 M. Zou, P. Xu, L. Wang, L. Wang, T. Li, C. Liu, L. Shi, J. Xie, W. Li, S. Wang, G. Wu, F. Luo, T. Wu and J. Yan, *Artif. Cells, Nanomed., Biotechnol.*, 2020, **48**, 116–128.
104 S. J. Tseng, K.-Y. Huang, I. M. Kempson, S.-H. Kao, M.-C. Liu, S.-C. Yang, Z.-X. Liao and P.-C. Yang, *ACS Nano*, 2016, **10**, 10339–10346.
105 A. A. Solovev, S. Sanchez, M. Pumera, Y. F. Mei and O. G. Schmidt, *Adv. Funct. Mater.*, 2010, **20**, 2430–2435.
106 K. K. Dey, X. Zhao, B. M. Tansi, W. J. Méndez-Ortiz, U. M. Córdova-Figueroa, R. Golestanian and A. Sen, *Nano Lett.*, 2015, **15**, 8311–8315.
107 T. Patiño, N. Feiner-Gracia, X. Arqué, A. Miguel-López, A. Jannasch, T. Stumpp, E. Schäffer, L. Albertazzi and S. Sánchez, *J. Am. Chem. Soc.*, 2018, **140**, 7896–7903.
108 T. Patino, A. Porchetta, A. Jannasch, A. Lladó, T. Stumpp, E. Schäffer, F. Ricci and S. Sánchez, *Nano Lett.*, 2019, **19**, 3440–3447.
109 A. Llopis-Lorente, A. García-Fernández, N. Murillo-Cremaes, A. C. Hortelão, T. Patiño, R. Villalonga, F. Sancenón, R. Martínez-Máñez and S. Sánchez, *ACS Nano*, 2019, **13**, 12171–12183.
110 M. Luo, S. Li, J. Wan, C. Yang, B. Chen and J. Guan, *Langmuir*, 2020, **36**, 7005–7013.
111 J. Wu, S. Ma, M. Li, X. Hu, N. Jiao, S. Tung and L. Liu, *ACS Appl. Mater. Interfaces*, 2021, **13**, 31514–31526.
112 S. Li, Q. Jiang, S. Liu, Y. Zhang, Y. Tian, C. Song, J. Wang, Y. Zou, G. J. Anderson, J.-Y. Han, Y. Chang, Y. Liu, C. Zhang, L. Chen, G. Zhou, G. Nie, H. Yan, B. Ding and Y. Zhao, *Nat. Biotechnol.*, 2018, **36**, 258–264.
113 J. K. Daljit Singh, M. T. Luu, A. Abbas and S. F. J. Wickham, *Biophys. Rev.*, 2018, **10**, 1283–1293.
114 A. E. Marras, L. Zhou, H.-J. Su and C. E. Castro, *Proc. Natl. Acad. Sci. U. S. A.*, 2015, **112**, 713.
115 H. Xu, S. Jones, B.-C. Choi and R. Gordon, *Nano Lett.*, 2016, **16**, 2639–2643.
116 R. Dreyfus, J. Baudry, M. L. Roper, M. Fermigier, H. A. Stone and J. Bibette, *Nature*, 2005, **437**, 862–865.
117 A. M. Maier, C. Weig, P. Oswald, E. Frey, P. Fischer and T. Liedl, *Nano Lett.*, 2016, **16**, 906–910.
118 I. M. Harmatz, M. J. Travers and R. E. Taylor, *J. Microelectromech. Syst.*, 2020, **29**, 990–995.
119 S. Lauback, K. R. Mattioli, A. E. Marras, M. Armstrong, T. P. Rudibaugh, R. Sooryakumar and C. E. Castro, *Nat. Commun.*, 2018, **9**, 1446.
120 J. Tang, C. Yao, Z. Gu, S. Jung, D. Luo and D. Yang, *Angew. Chem., Int. Ed.*, 2020, **59**, 2490–2495.
121 N. Wu, K. Wang, Y.-T. Wang, M.-L. Chen, X.-W. Chen, T. Yang and J.-H. Wang, *Anal. Chem.*, 2020, **92**, 11111–11118.
122 S.-J. Park, M. Gazzola, K. S. Park, S. Park, V. Di Santo, E. L. Blevins, J. U. Lind, P. H. Campbell, S. Dauth, A. K. Capulli, F. S. Pasqualini, S. Ahn, A. Cho, H. Yuan, B. M. Maoz, R. Vijaykumar, J.-W. Choi, K. Deisseroth, G. V. Lauder, L. Mahadevan and K. K. Parker, *Science*, 2016, **353**, 158–162.
123 R. Raman, C. Cvetkovic, S. G. M. Uzel, R. J. Platt, P. Sengupta, R. D. Kamm and R. Bashir, *Proc. Natl. Acad. Sci. U. S. A.*, 2016, **113**, 3497.
124 E. Yamatsuta, S. P. Beh, K. Uesugi, H. Tsujimura and K. Morishima, *Engineering*, 2019, **5**, 580–585.
125 Z. Lin, T. Jiang and J. Shang, *Bio-Des. Manuf.*, 2022, 107–132.

 This journal is © The Royal Society of Chemistry 2022

126 O. I. Sentürk, O. Schauer, F. Chen, V. Sourjik and S. V. Wegner, *Adv. Healthcare Mater.*, 2020, **9**, 1900956.
127 D.-W. Zheng, Y. Chen, Z.-H. Li, L. Xu, C.-X. Li, B. Li, J.-X. Fan, S.-X. Cheng and X.-Z. Zhang, *Nat. Commun.*, 2018, **9**, 1680.
128 M. Sitti, *Extreme Mech. Lett.*, 2021, **46**, 101340.
129 S. Tognarelli, V. Castelli, G. Ciuti, C. Di Natali, E. Sinibaldi, P. Dario and A. Menciassi, *J. Rob. Surg.*, 2012, **6**, 5–12.
130 Q. Wang, L. Yang, J. Yu, P. W. Y. Chiu, Y. P. Zheng and L. Zhang, *IEEE Trans. Biomed. Eng.*, 2020, **67**, 3403–3412.
131 A. Carovac, F. Smajlovic and D. Junuzovic, *Acta Inform. Med.*, 2011, **19**, 168–171.
132 A. Aziz, M. Medina-Sánchez, J. Claussen and O. G. Schmidt, *Nano Lett.*, 2019, **19**, 6612–6620.
133 C. Billings, M. Langley, G. Warrington, F. Mashali and J. A. Johnson, *Int. J. Mol. Sci.*, 2021, **22**, 7651.
134 K. Bente, A. C. Bakenecker, A. von Gladiss, F. Bachmann, A. Cēbers, T. M. Buzug and D. Faivre, *ACS Appl. Nano Mater.*, 2021, **4**, 6752–6759.
135 Q. Wang and L. Zhang, *ACS Nano*, 2021, **15**, 149–174.

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