Citation: Zhao, S., Sun, D., Zhang, J., Lu, H., Wang, Y., Xiong, R. & Grattan, K. T. V. (2022). Actuation and biomedical development of micro-/nanorobots – A review. Materials Today Nano, 18, 100223. doi: 10.1016/j.mtnano.2022.100223

Actuation and biomedical development of micro-/nanorobots – A review

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ABSTRACT

Over the past 20 years, there has been considerable progress in the development of research in the micro-/nanorobotics area. Through major, rapid, investment in the field and the use of, for example, correlation techniques, many successes have been seen in both theoretical and experimental work, which have had applications in emerging areas such as clinical medicine. As a result, this article aims to introduce and review some of the most influential and advanced research work carried out over the past few years. To do so, the approach taken has been to categorize micro-/nanorobots by their propulsion modes, analyzing their advantages and drawbacks in detail, looking at medical applications such as such as the delivery of medical supplies, medical imaging and so on. Additionally, the article looks at future directions in micro-/nanorobot development, including important areas such as biocompatibility (as well as biodegradability), autonomy and accurate operation in the complex and dynamic environment of the human body.

1. Introduction

Micro-/nanorobots are usually defined as mechanical robots on the micrometer or nanometer scale, that can be controlled through proper programming to accomplish specific tasks [1]. Taking advantage of their small-scale structure, they can reach areas that cannot be accessed by normal methods and thus minimize damage, which creates a new direction in the medical field – this was envisaged in the famous sci-fi movie ‘Fantastic Voyage’ where a miniature submarine was injected into a dying scientist's brain to remove a blood clot. Quoting the famous physicist, Richard Feynman's words at an American Physical Society meeting, ‘it would be interesting in surgery if you could swallow the surgeon’- this idea could offer new possibilities in future surgery [1–4] Under these circumstances, flexibility, adaptability, robustness and accuracy of a specific mission could then be guaranteed, when performed on a miniature scale. More importantly, using clusters of these robots, more complex tasks can be performed through the cooperative behavior of these multiple robots [5,6].

Currently, micro-/nanorobots (MNRs) can be applied across a wide range of areas [7]. In addition to their significant role in energy security and environmental engineering [8,9], there is major potential for the application of MNRs in the biomedical field, as we mentioned above. Taking advantage of their small size, the capacity to send micro-/nanorobots directly into target locations presents a dynamic platform for delivering various types of cargos, such as pharmaceutical drugs [10,11], biological specimens [12,13], inorganic chemical agents [14,15] and living cells [16,17], thereby improving the accuracy of delivery. In addition, performing as surgical tools, they can operate on a microscale or nanoscale [18], where blades or catheters cannot, in order undertake biopsies or sample collection [19–21], penetrate human tissue [22,23], deliver cargos intracellularly [24–26] and realize biofilm degradation [27,28]; The improvement in the accuracy of the binding of the target substance to the receptor also opens up many advanced aspects of engineering, such as biosensors [29–32] or physical sensors [33–35] designed to detect internal media and in the isolation and purification of biotargets [36–39]; Through adopting the combination of cluster and individual control, the medical imaging achieved by MNRs can be divided into optical [40,41], ultrasonic

[42], magnetic [43] and radionuclide imaging [44,45]. Developments and innovation in this area, addressing challenges such as biocompatibility and good design, are moving the field forward step by step [46].

In this article, a review of recent research in the field of microrobots and nanorobots (MNRs) is presented (Fig. 1). Categorizing MNRs by their driving modes, this work divides the subject into magnetic, optical, chemical, biological and hybrid actuation. Propulsion methods using electrical fields and ultrasound are not included in this review — although they have made a huge contribution to the development of the MNR field, the fact that they are not readily compatible with biological or biomedical applications limits their prospects. In each section, the mechanism of the MNRs considered, their advantages and disadvantages, as well as important, cutting-edge research are reviewed. The article concludes with a summary, an analysis and discussion of existing problems, and points to future developments in the current field.

2. Magnetic propulsion

As one of the most mature and promising technology, magnetic propulsion is well famous for its remote drive and strong penetration, making it very suitable for biological and health-care area [47–50]. By putting magnetic particles into various magnetic field and manipulating it, their movements can be controlled with magnetic force and torque changing correspondingly. Of all designs concerning magnetic field, there modes of magnetic fields can contain most of the conditions: rotating ones, oscillating ones and gradient ones [51], which, though have pros and cons in different situations, show similarity and consistency in propulsion principle. The designs and function mechanisms of MNRs, however, exhibit great diversity among large amounts of researches and result in different conclusions. As a consequence, in the following essay, light shall be shed upon them as well as some related perspectives of our own.

2.1. Helical MNRs

The concept of an artificial helical structure was inspired by the unique swimming strategies of helical bacterial flagella in a fluid environment, which rotates their bases under a rotating magnetic field to motivate MNRs, which function like a corkscrew [58]. The helical structure, which is human-engineered or self-assembled dynamically [59,60], can handle navigation in most types of biofluids and alter between various motion modes [61] and the material of the artificial flagella can be derived from an organism, such as DNA [62]. Nelson's group at ETH Zurich first produced a helical magnetic swimmer, in 2007 [63], and in 2009, his group made a great breakthrough and related work [64] has been considered as a pioneering discovery in this field as well as Fischer's achievement [65]. The structure, termed ABF (which stands for Artificial Bacterial Flagellum), whose principle is roundly concluded afterwards [66], consists of a magnetic head and a helical semiconductor tail, constructed with an InGaAs (or GaAs) bilayer thin film, which enables it to rotate along the microrobot's axis and move forwards or backwards in the vertical direction of the rotation plane. In 2009, after thorough comparison between methods of microrobot swimming, his group drew a conclusion that helical MNRs had best performance in vivo and discussed related reasons [67]. Then, they have continued to influence the field and in 2012 reported another kind of helical microrobot, powered and steered wirelessly using low-strength rotating magnetic fields and this has demonstrated important advantages, especially in pipe flow conditions or 3D swimming in an open environment Afterwards, the same group has made great contribution to this field continuously. They reported another kind of helical microrobots powered and steered wirelessly using low-strength rotating magnetic fields in 2012 and demonstrated great advantages especially in pipe flow conditions or 3D swimming in open environment [68]. Meanwhile, at the time of publication, Fischer's group has manufactured the smallest helical robot (on a nanoscale level) using Glancing-Angle Deposition

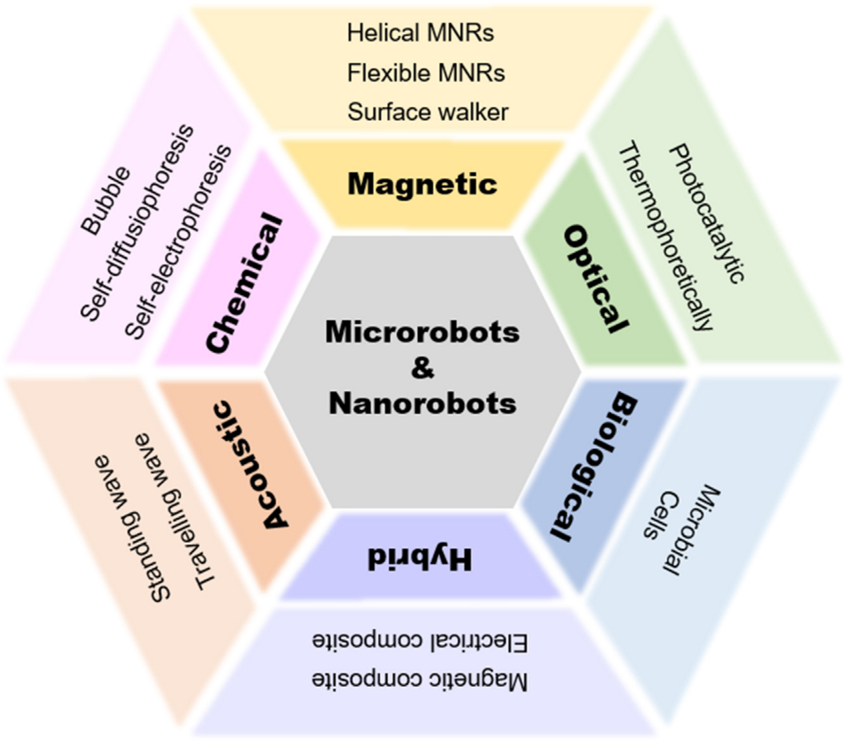

Fig. 1. Overview of this review. Categorizing MNRs by their driving modes, this work divides the subject into magnetic, optical, chemical, biological and hybrid actuation.

(GLAD), which is an efficient approach to fabrication and an alternative to methods such as Direct Laser Writing (DLW) [59,65,69–71] In addition, the modification of plants has formed the basis of new approaches and an excellent way to fabricate ABFs from helical plant vessels, later used in magnetic MNRs, has been reported by Wang's group. They extracted helical xylem vasculature from plants and coated it with Ni and Ti (see Fig. 2A) [56]. This fabrication method, described as biotemplated synthesis, was extensively used by Zhang's group. Utilizing spirulina as a template, it was wrapped in crystalized Fe3O4 using a sol-gel method and high-temperature annealing, as a result demonstrating a high specific surface area and excellent biocompatibility [43]. Many other studies of helical MNRs, processed in a similar way, (called membrane electrochemical codeposition) have been reported [72,73], and nowadays such helical MNRs have been widely applied in 'real life' situations [74–80]. For example, a double-helical microrobot which was driven by a magnetic field and stimulated by an external light source has been presented by Bozuyuk and co-workers [57], where this can be used to release drugs automatically, 'on demand', and then degrade into a non-toxic substance. In this way, it has shown good biocompatibility and relevance (see Fig. 2B). Being one of the most promising methods, helical MNRs, combining metal–organic framework and relevant materials together, continue developing at a rapid speed. Recently, Prof.

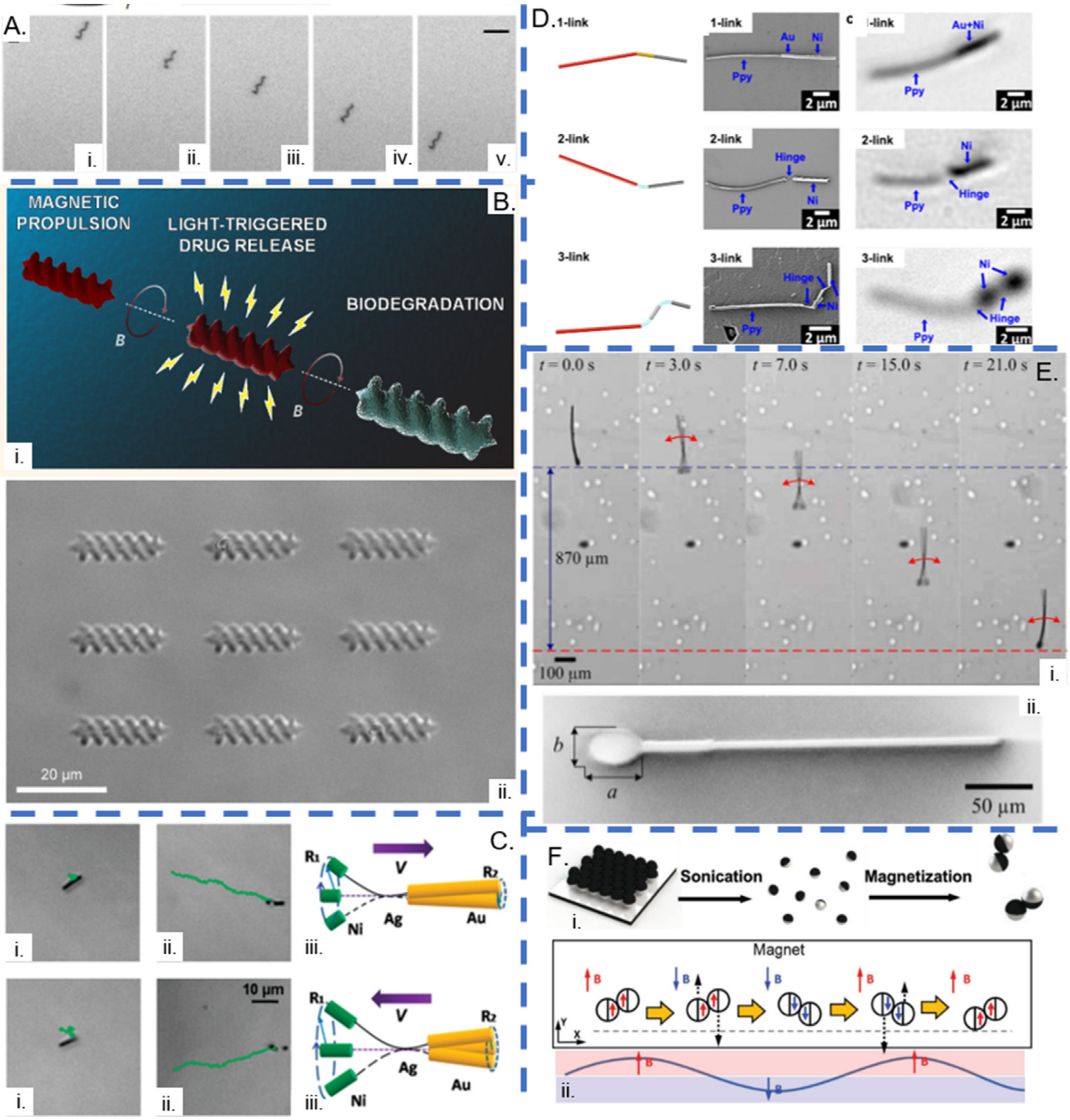

Fig. 2. (A) Schematic of Ni–SiO2 magnetic Janus microdimer. (i) Fabrication of the Ni–SiO2 magnetic Janus microdimers, which will combine and form a dimer in the magnetic field. (ii) Walking mechanism of the Ni–SiO2 magnetic Janus microdimer under an oscillating magnetic field, as a surface walker. (Reproduced with permission from Ref. [52]. Copyright 2018 (Wiley)) (B) Comparison of (i) rigid Au/Ag/Ni and (ii) flexible Au/Agflex/Ni when moving forwards or backwards, with the flexible mechanism used. (Reproduced with permission from Ref. [53]. Copyright 2010 (American Chemical Society)) (C) Structure of 1-, 2-, and 3-link flexible microrobots. (Reproduced with permission from Ref. [54]. Copyright 2015 (American Chemical Society)). (D) Time lapse images (over 500 ms intervals) showing the track of a plant-derived helical microswimmer, remolded from the spiral vessel of R. indica, propelled under a 10 G, 70 Hz rotating magnetic field. (Scale bar, 50 μm). (Reproduced with permission from Ref. [55]. Copyright 2014 (American Institute of Physics)). (E) (i) The movement of MagnetoSperm under a weak oscillating (25 Hz) magnetic field (5 mT) and (ii) its structure (a = 42.6 lm, b = 27.6 lm). (Reproduced with permission from Ref. [56]. Copyright 2013 (American Chemical Society)). (F) Chitosan-based helical microswimmer showing (i) basic mechanism of the chitosan-based helical microswimmer system and (ii) shape of swimmers seen under optical microscopy. (Reproduced with permission from Ref. [57]. Copyright 2018 (American Chemical Society)).

Pane's group has proposed many excellent researches that ensures biodegradability and motility using functional materials or proper organism, putting forward many insightful ideas [81–84].

2.2. Head-tail configuration MNRs

Imitating specific soft - bodied microorganisms, flexible magnetic micro-swimmers and nanoswimmers can perform symmetry deformation to activate movement through altering their shapes, responding to various environments [85,86]. They can even pass through a small-sized gap or survive violent impact, although this cannot be achieved with current technology. One of the first products developed using this mechanism was a microscopic magnetic swimmer, demonstrated by Deryfus and co-workers. Its movement depended on flexible an artificial flagellum fabricated with DNA-linked chains of paramagnetic colloidal beads, and the velocity as well as the direction could be controlled by adjusting the magnetic field characteristics, such as strength and frequency [87] A flexible three-segment nanorobot was reported by Gao et al. fabricating this in a simplified way, compared to what was done before. After gold, silver, and nickel segments were sequentially deposited in an anodic aluminum oxide (AAO) membrane template, the silver part was dissolved partly in hydrogen peroxide solution and this formed a flexible joint. In a rotating magnetic field, the final products of the electrochemical reaction mentioned above could be motivated by cone-shaped rotation of Ni (see Fig. 2C) [53]. Another flexible magnetic nanorobot fabricated in a similar way was reported by Nelson's group where propelled by an oscillating magnetic field, the flexible multiple-segment nanorobot started with Ni and ended with an elastic eukaryote-like polypyrrole (PPy) (see Fig. 2D) [54]. Later, the same group created a prototyping process for building a self-folding microrobot driven by a magnetic field, to satisfy a complicated body program, with a transformable shape and steerable motility [88]. The multiple-linked structure has appeared in many other studies, such as hinged nanoswimmers containing Au at both ends, Ni in the middle and Ag as the interconnection. Wang et al. [89] reported a similar model for adjusting the motion of a swinging flexible nanomotor (SFN), propelled by an oscillating magnetic field, which also was presented by Zhang and co-workers [90]. In the meantime, bionic MNRs of other structures have also been reported recently. Mimicking the motility of sperm, Misra et al. presented a microrobot named ‘MagnetoSperm’, which was composed of an ellipsoidal magnetic Co—Ni head and a flexible SU-8 tail (see Fig. 2E) [55]. Scallop-formed microrobots developed by Fischer's group also showed a simplified propulsion method, which can propel in both shear thickening and shear thinning (non-Newtonian) fluids [91]. Afterwards, millirobot integrating actuation and power generation in a thin multilayer film was reported. The millirobot consisted of a lower magnetic composite limb collocated with multiple feet to impart locomotion, and a flexible piezoceramic composite film to recover energy autonomously [92]. Further, a magnetic MNR fabricated and based on a new material, chitosan hydrogel, had been reported by Hoop and co-workers, whose surface could expand when being placed in an acidic environment and used to release drugs [93]. Nowadays, many groups have continued developing MNRs using such designs, and some advanced technology such as learning-based methods has also been applied and demonstrated satisfying results [94–96].

2.3. Magnetic surface walkers

A particular type of magnetic-driven MNRs is the surface walker, which relies on a surface to break the spatial symmetry, to provide the propulsion power [97–100] Considerable recent research has been done in this field, as it is a promising mechanism for propulsion. One of the pioneering studies in this field was the DNA-linked anisotropic doublets reported by Tierno et al. where the robots were formed with two different diameters of paramagnetic colloidal microparticles and their velocity could be manipulated by the related properties of the magnetic field [99] Wang reported a magnetic surface walker acting in an oscillating magnetic field, whose maximum available speed can be up to 18.6 μm/s when the frequency of the magnetic field reaches 25 Hz, moving by using a microdimer formed with two magnetic Janus microspheres, through magnetic dipolar interactions (as shown in Fig. 2F) [52] Also, a peanut-shaped hematite colloid micromotor was reported in the same year by He, Xie and co-workers, working as a magnetic surface walker [38]. It was capable of not only moving in fluids with a rolling or wobbling mode but also operating to allow contactless single cell manipulation and pattering. Using this mechanism, different shapes of hematite colloidal particle swarms such as liquid, chain, vortex or ribbon could be programmed by the changing magnetic fields and transform these into other forms freely and reversibly, to allow performing multiple missions cooperatively, in a confined environment [101] Another example of swarm guidance in a dynamic environment has been illustrated recently where using ultrasound Doppler imaging, a microswarm, on the nanoscale level, was able to fulfill active endovascular delivery with a knowledge of the real-time, precise localization of the swarm [102].

3. Optical propulsion

3.1. Photocatalytic propulsion

A light-induced catalytic process, which is mainly semiconductor based, is used to active MNRs in a solution, creating so-called photocatalytic propulsion [107]. After light was used to photoexcite the semiconductor, electrons in the valence band get excited, cross the forbidden band and enter the conduction band, causing the generation of electrons and holes pairs. Moving under an electric field or by diffusion, they tend to react with the material adsorbed on the surface of the catalyst particle and create a strong REDOX potential, to enable a reaction to occur. In a way that is similar to chemical propulsion, the principle of photocatalytic propulsion can be divided into three similar cases, in light of the difference in the catalytic mechanism, when compared to the propulsion mechanism [108]. The first of these cases is using self-diffusiophoresis in a way demonstrated by Ibele and co-workers [103] where being exposed to UV light AgCl particles decompose asymmetrically and create a localized electrolyte gradient, leading to autonomous movement (see Fig. 3A). As one of the most common compound semiconductors, TiO2 has been widely utilized in this field. In the work reported by Hong [106], microrobot propulsion can be induced by UV light, which can be easily controlled and which transforms optical to mechanical energy, with no requirement to have a substrate or artificial catalytic conditioning (see Fig. 3D). In addition, Au—TiO2 Janus micromotors that were photocatalytically driven using UV light were demonstrated by Dong et al. This can be triggered even with extremely low intensity light and the micromotors can reach specific speeds which are as fast as have been seen with chemical propulsion, but offering greater convenience and high repeatability in performance. When coated with gold film, the TiO2 particles will be immediately activated on exposure to UV light and propelled efficiently in pure water, based on using the self-diffusiophoresis principle (see Fig. 3B) [104]. Also, the combination of Pt and TiO2 has also been proven to offer a major breakthrough in the field of organic pollutant degradation [109]. In addition to UV irradiation, visible light can be used for convenience as, for example, photocatalytic metal oxide-

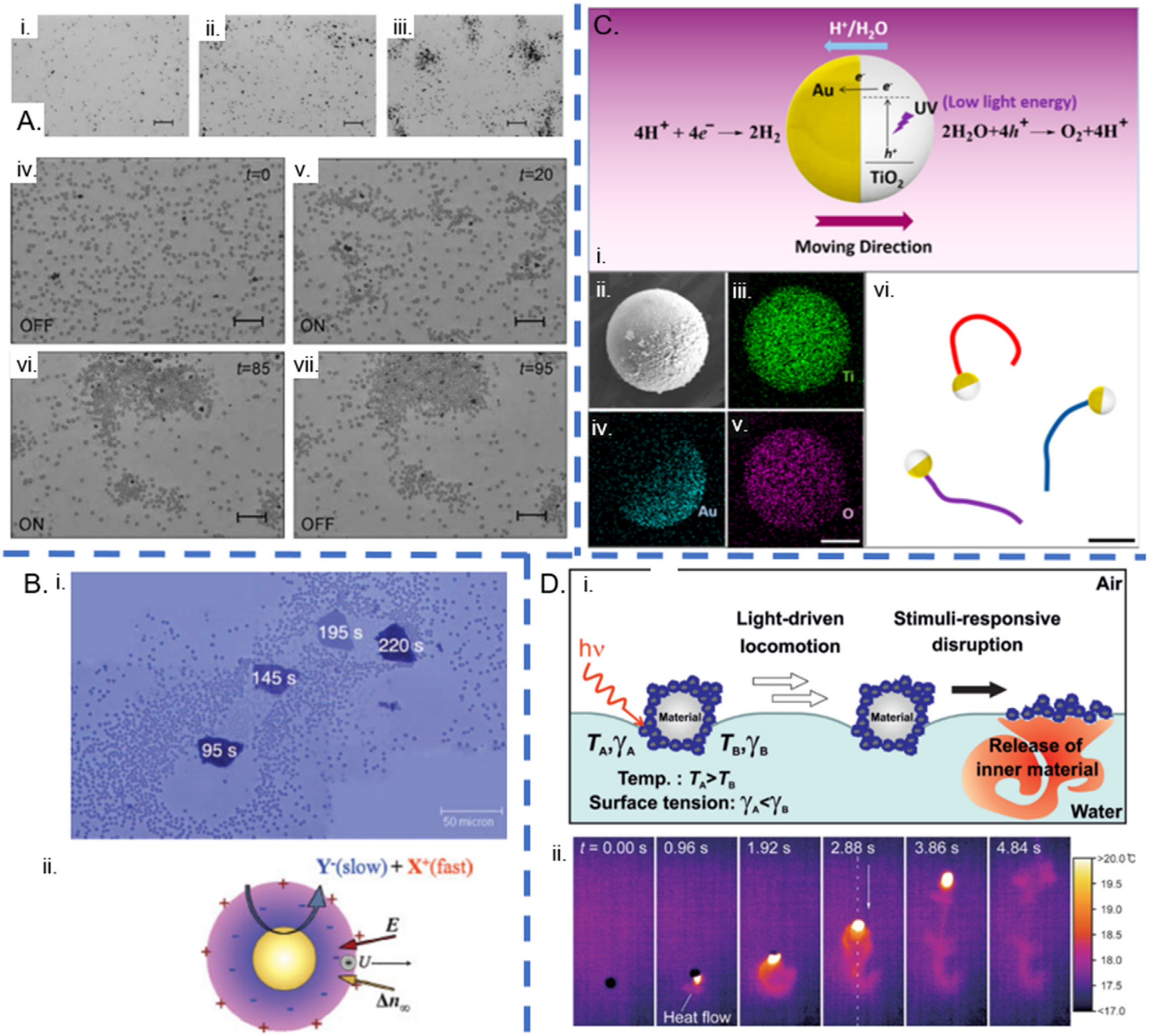

Fig. 3. (A) AgCl particles and their reaction to UV light. (i-iii) AgCl particles (in de-ionized water) – (i) before UV irradiation, (ii) after exposure to UV light for 30s and (iii) for 90s. (iv-vii) Illustrating 'Predator–prey' behavior. The addition of AgCl and 2.34 mm diameter silica spheres to de-ionized water – when exposed to UV light, the silica spheres will autonomously find AgCl particles and encase them. After removing the UV light, the 'prey' behavior terminates. (Reproduced with permission from Ref. [103] (Copyright 2018 (Wiley)). (B) Catalytic TiO2–Au Janus micromotors. (i) Schematic of catalytic TiO2–Au Janus micromotors activated by UV radiation in water. (ii-v) Images of microrobots seen using a Scanning Electron Microscope (SEM) and dispersive X-rays for Ti, Au, and O respectively. (scale bar – 0.5 μm). (vi) Microrobot trajectory (over a 1s time) in pure water illustrated by the tracking lines shown. (Reproduced with permission from Ref. [104] (Copyright 2016 (American Chemical Society)). (C) Light-driven liquid marble. (i) Schematic illustrating the delivery of material by Liquid Marbles (LMs). (ii) Snapshots of the movement of a CB-stabilized thermophoretically driven LM (1.8 mm in diameter) taken using thermography. (Reproduced with permission from Ref. [105]. Copyright 2016 (Wiley)). D) TiO2-based microrobots, driven by UV radiation. (i) A TiO2 boat cutting through SiO2 particles, under UV irradiation (image edited by Photoshop). (ii) TiO2-based microrobot propulsion mechanism: self-diffusiophoresis in an electrolyte solution. (Reproduced with permission from Ref. [106]. Copyright 2010 (Wiley)).

incorporated Janus copper oxide-Au microparticles can be propelled in H2O2 solution under visible light, through self-electrophoresis [110]. Also, the ability of Near Infra-Red Radiation (NIR) to penetrate biological tissues without any damage presents great potential in the medical field [111].

3.2. Thermophoretic propulsion

In thermophoretically light-driven propulsion, a thermal gradient is generated by asymmetric forced light sources [112], which enables the MNRs to move along the direction of this temperature gradient. In another word, such phenomenon is an extension of thermophoresis in colloidal suspensions, which set forth that particles in the medium are motivated by forces in the opposite direction of the temperature gradient [113,114]. In this case, the maneuverability and stability can be carefully controlled, while the key shortcoming is that special substrates are required to transform light into heat and thus this form of actuation can only be applied in 2D [115]. Silica hemispheres coated with a thin Au layer are the first generation of thermophoretically, light-driven robots. While NIR radiation has essentially no effect on silica hemispheres, the Au layer used tends to absorb the NIR illumination, creating an asymmetric temperature distribution which can be used to activate the microrobots [116]. The fact that Janus nanorobots can be propelled in the same way was demonstrated by the same team a decade later [117], proving the feasibility of this mechanism. Following the same principle, the Au-coated side of the Janus nanomotor was propelled at an ultra-high speed using the self-thermophoresis mechanism, this presenting a great potential frothier use in the medical field. Another related example is the polymer multilayer microtube rockets driven by NIR radiation, developed by Wu et al. The gold nanoshells inside the rockets led to a plasma absorption resonance under the NIR irradiation and this created an asymmetry of local heat distributions, which caused the movement of the (PSS/PAH)20 AuNP microrocket [118]. By comparison to traditional mechanical millirobots, liquid marbles (as millirobots), can also be actuated in a thermophoretically light-driven way. When their surface is covered with black powder, liquid marbles can be guided to targets under NIR irradiation along the thermal gradient created by the black powder's ability to convert light into heat (see Fig. 3C) [112].

4. Acoustic propulsion

As an essential external power when propelling MNRs, acoustic propulsion has been considered as an indispensable method and widely applied in this field recently. Due to its immense advantages concerning biocompatibility and large propulsive force, acoustic propulsion, especially ultrasound one, is highly welcome in biomedical area, which not only brings efficiency but also ensures safety of the whole process. Mechanism of it, which still remains challenges and shows a great diversity among various researches, can be generally concluded as forming a standing or traveling wave to activate particles in short.

In 2012, Wang' group firstly proposed that a type of asymmetric segmented metal rods could perform different acts such as axial movement in the field of a standing wave, which was driven by forces produced by an uneven distribution of acoustic pressures [119]. Then, due to the fact that no standing wave in vivo can be predicted, a new class of artificial nanoswimmers that can be propelled in both standing and traveling acoustic waves was reported by Nelson's group. Comprising a flagellum-like flexible tail and a rigid bimetallic head, it can generate force by oscillating its tail in a small amplitude [120]. It's worth being mentioned that experimental results demonstrate great quality in the acoustic field according to Wang's group. In 2013, they reported a three-segment Au—Ni—Au nanowire, whose construction has been widely in magnetic field, driven by acoustic waves generated by a piezoelectric transducer [121]. Based on their previous work, they brought up a an ultrasound-powered nanowire motors with nanoporous gold segment, possessing great advantages such as tunable size, high capacity and high surface area. With the character of near-infrared-light triggered release, it was tested in medicine delivery of DOX and displayed relatively satisfying results [122]. Later, the same group performed another approach for target-specific payloads (insulin, in this article) transport with gold nanowire motors in ultrasound field. By using pH-responsive supramolecular nanovalves as release mechanism, the nanomotors depicted more efficiency in speed with the guarantee of accuracy, giving great hope to diabetes treatment [123]. Another typical design is bullet-like propulsion under ultrasound field. In 2012, a micromachine with biocompatible fuel inside was represented by Wang's group. By utilizing ultrasound, fuel inside will be vaporized to provide massive momentum, giving micromachine ability to penetrate through tissues with high velocity [124]. Afterwards, based on similar principle, the same group presented acoustically triggered microcannons. Forced ultrasound pulse triggered the perfluorocarbon emulsions vaporization, which enriched material that can be utilized in such design and improved its universality [125]. One of the most interesting and valuable application of ultrasound-based propulsion is the idea of ‘acoustic tweezers’ in order to manipulate micro-scaled even nanoscaled objects such particles, cells or organism. In 2012, it has been achieved in a standing surface acoustic wave field by Huang's group, which are able to successfully fulfill relevant tasks under real-time control. Moreover, biocompatibility of such design has also been proved with cell-viability tests, presenting a promising future in both science and engineering areas [126].

However, downsides have been shown in acoustic propulsion that ultrasound may have problems building predictable field with bones or air existing in vivo and more studies need to be done before being put into practical usage.

5. Chemical propulsion

5.1. Bubble propulsion

Bubble propulsion is defined as the specific way that MNRs can be driven by the force caused by high-speed gases formed in chemical reactions, especially in fuel decomposition with the use of catalytic effects [127—129]. Generally speaking, this mechanism is mostly considered as using force generated by growth and detachment of recoiling bubbles to propel MNRs, according to momentum theorem, though some of examples also involve action of gravity [128—130]. This approach was commonly used in the early days of the technology and now is fully developed [130—132], as sufficient energy can be generated. Research has shown that enlarging the particles has made it possible for diffusiophoretic propulsion to be switched to bubble propulsion, as this has also been proven feasible [133—135]. The earliest example of bubble propulsion was the decomposition of hydrogen peroxide by Pt to produce oxygen, used to propel a robot (see Fig. 4A) [136]. In further work, by modifying the catalyst using either carbon nanotubes, an Ag—Au alloy or hydrazine, the average speed of the particle motion was improved significantly [137,138]. Furthermore, the propulsion speed can be modulated dynamically by adjusting several external factors, to give reproducible on/off control which can be achieved with ultrasound [139] or by using folding and unfolding control from temperature changes (see Fig. 4B) [140]. It is worth noting that the direction of the bubble-propelled robot in the 3D environment is random and must be controlled by applying a radio frequency field, which means that this technique cannot be used alone to achieve the necessary control [141]. More importantly, a toxic fuel source or catalyst (like hydrazine in the examples above) may cause serious damage to health and Pt is an expensive metal to us as a catalyst. To avoid the problems of residue from toxic substances, magnesium and zinc are used as they can react with water, producing hydrogen gas as bubbles to activate MNRs and hydroxides. However, this demonstrates the biggest shortcoming: the irreversible increase in the pH [130,142] and so it can only be counted as a second choice for biomedical applications. Another serious problem is that the driving force in bubble propulsion decreases as the fuel concentration decreases, limiting any long-term medical applications [143] and so better propulsion methods are currently being developed at the moment.

5.2. Self-diffusiophoresis propulsion

Self-diffusiophoresis propulsion is defined as a type of propulsion driven by forces generated by a concentration gradient, caused by a chemical reaction on an asymmetrical surface [144—146]. More particularly, this mechanism has been depicted in two ways: the conventional one [147] that believes that surface chemical reaction leads to the non-equilibrium concentration, which generates a

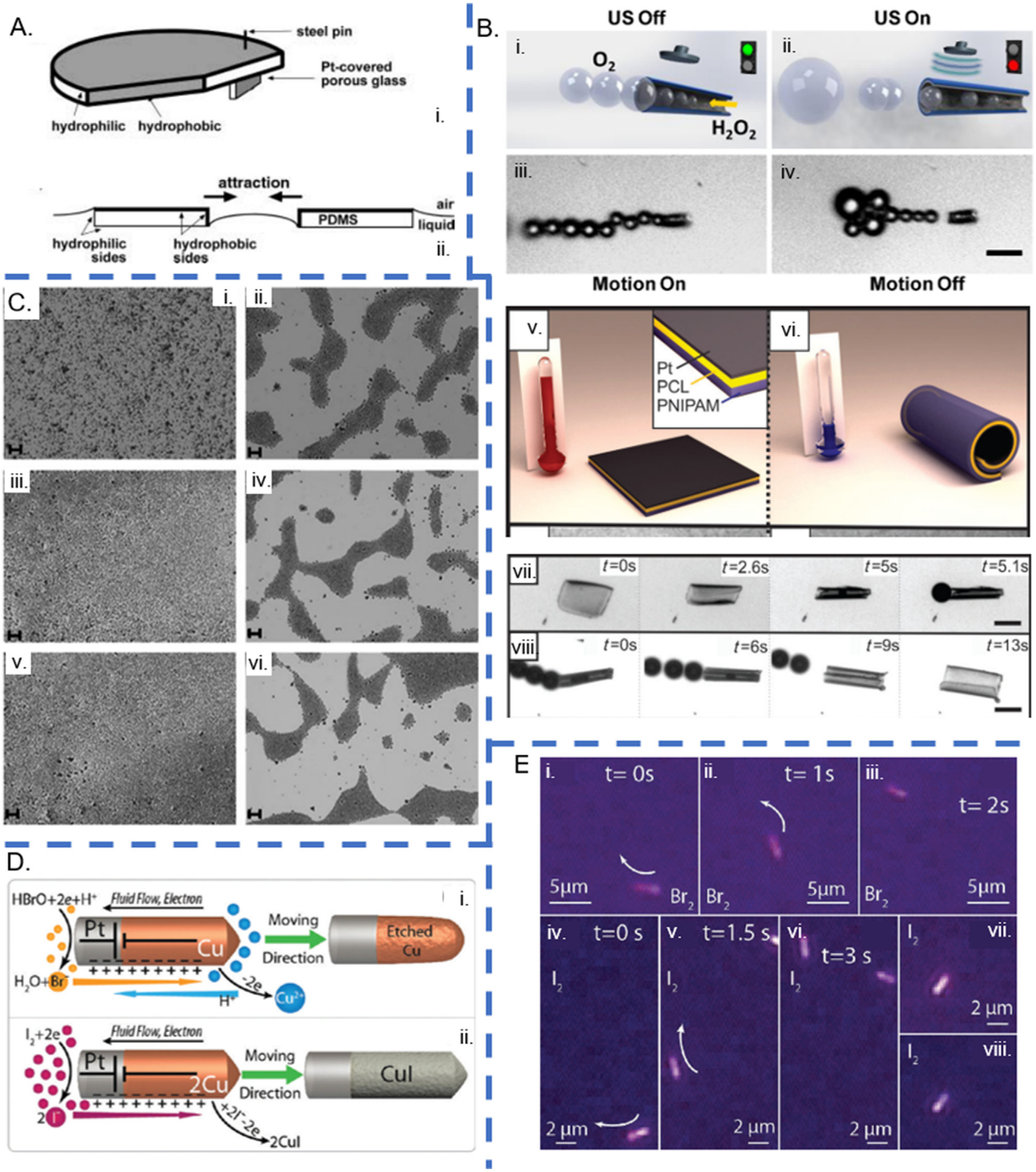

Fig. 4. (A) (i). Structure of the system activated by bubble propulsion. (ii). An image demonstrating self-assembly through capillary interactions. (Reproduced with permission from Ref. [136]. Copyright 2002 (Wiley)) (B) (i-ii). Schematic of ultrasonic modulated motion of PEDOT/Ni/Pt engine in a micron scale. (iii-iv). Their microscope images. (Reproduced with permission from Ref. [139]. Copyright 2014 (American Chemical Society)) (v-vi). Representation of polymer-Pt films. (vii-vii). Forming bubbles to propel through folding (top) and unfolding after bubble formation (bottom). (Reproduced with permission from Ref. [140]. Copyright 2014 (Wiley)) C) Reversible swarm formation triggered by repetitive hydrazine additions. (Reproduced with permission from Ref. [150]. Copyright 2011 (Wiley)) D) Schematic and mechanism of Cu–Pt self-powered nanomotor in aqueous Br_2 and I_2. (Reproduced with permission from Ref. [151]. Copyright 2011 (American Chemical Society)) E) Movement of nanomotor in Br_2/I_2 in optical microscope and its images before and after reaction with I_2. (Reproduced with permission from Ref. [151]. Copyright 2011 (American Chemical Society)).

osmotic pressure imbalance as proportion and the innovational one [148] that describes the engaged particles as colloidal ones undergoing Brownian motion. Despite the slight differences in theory, it's acknowledged that the formation of a local gradient makes this much more efficient when it comes to cluster control of MNRs. Research has also suggested that a self-diffusiophoresis mechanism could also been applied to a traditional bubble-propelled material, hydrogen peroxide. For instance, by adding PS-Pt Janus particles into H2O2 solutions, the decomposition of the H2O2 generates gradients on the Pt surface to provide the driving force, illustrating an important usage of the self-diffusiophoresis mechanism [149]. To improve the biological compatibility to the greatest extent, a more bio-enzyme catalyst such as urease or glucose oxidase (GOx) was considered [144,152,153]. Recently, the remote-controlled Janus particles powered by two biological fuels were reported, whose reaction products are oxygen and water (instead of toxic combustion products, achieved by employing the tandem reaction between GOx and catalase (Cat) [154]. Also, Prof. Ma's group at HIT has made huge progress about Janus particles, whose motion are triggered by different chemical reactions catalyzed by non-compatible enzymes [155] and compatible ones [144,156], while Prof. Ma's group at HIT mostly focus on propelling Janus particles in other ways such as photochemical and magnetic ones [52,157,158]. In multiple MNR collaborative operation, the self-diffusiophoresis mechanism enables the formation of robot swarms which can fulfill collective and cooperative behavior. According to the work of Wang et al. [150], the electrolyte gradient which is triggered by the chemical reaction in hydrogen peroxide solution with hydrazine can be used to organize Au microparticles (Au MPs) into swarms, whose size, shape and formation rate can be altered through controlling the MP density or modifying the catalytic gold surface (Fig. 4C). Considered to be highly promising, the self-diffusiophoresis mechanism has attracted considerable investment and this field is developing significantly as a result.

5.3. Self-electrophoresis propulsion

Self-electrophoresis propulsion is defined as propulsion which is driven by a force produced by the interaction between a charged swimmer surface and a self-generated electric field, unlike ordinary electrophoresis which responds to an external electric field [145]. After self-proportion by ions exchange with solution firstly brought up by Peter Mitchell in 1956 [159], now it has been proved right and further discovered concerning velocity calculation method, which is proportional to the particle's charge and self-generated electric field [160]. This mechanism is widely accepted in the community and has been proven experimentally to be the one of the most useful [161]. The bimetallic particle nanorobots propelled by hydrogen peroxide were first developed by Paxton and co-workers in 2004, the non-Brownian movement of Pt/Au nanorods catalyzing the deposition of hydrogen peroxide, which becomes the most common assembly method in the self-electrophoresis field [162]. For instance, in the case of the use of Pt–Au, the bipolar electrochemical reaction between the Pt–Au and hydrogen peroxide leads to a high concentration of protons around the Pt and a low concentration at both ends of the Au. The asymmetric distribution of the protons forms a local electric field and drives the MNRs [163,164]. As time has gone on, many other fuel materials and swimmers have been presented, such as using Br_2 and I_2 solution as fuel (see Fig. 4E,F) [151], to active nanobatteries or using glucose to activate carbon filter motors [165].

6. Biological propulsion

Bacteria that have been modified can be applied to fabricating MNRs, due to their extraordinary abilities. They are on the micro-/nanoscale, and can swim through their environment not only using local energy storage but also additionally being steered by external inputs. One of the most commonly used kinds of bacteria is *E. coli*, whose uses have been developed rapidly recently [166–168]. One efficient way of transporting drugs is to attach them to the surface of *E. coli* on a patterned silicon substrate using a two-antibody-based method and then to release cargo-carrying E. coli into a specific location, whenever that is needed (see Fig. 5A) [169]. Also, the method of attaching *E. coli* to the surface of a drug-loaded polyelectrolyte multilayer (PEM), with inlaid magnetic nanoparticles has been shown to be feasible by Byung-Wook Park and his co-workers and to be efficient for the transportation of doxorubicin anticancer drug molecules (see Fig. 5B) [170]. By optimizing the fabrication method, the speed of the swimmer has been increased to 22.5 μm/s. The flagellar of the bacteria are considered to be a perfect natural drive motor, converting ion-motive force into mechanical force. Besides adding artificial flagellar (like DNA as mentioned above) to other cells, bacteria driven by flagella originally such as alginolyticus are also being widely studied [171]. Magnetotactic Bacteria (MTB) use two flagella bundles to assist the rotary molecular motors to create a propulsion system, where the highest transportation speed can be up to 300 μm/s [172] In addition to the reliable results coming from the MC-1 bacterium propelled through using a magnetic field [173–175], the flagella motor can also be planted inside the intact cell of the *Serratia marcescens* bacteria to allow for controllable propulsion of microrobots [176]. During the experiment carried out, the modified microrobots were attached to 10 μm polystyrene (PS) beads. By following these PS beads, it was proved successfully that the displacement of the bacteria was caused by the flagella motor, rather than by Brownian motion [177]. In addition, it can be observed that through sensing the pH of the flagellated bacteria, multi-bacteria propelled microrobotic action can be robustly controlled. The experiment presents both the use of *S. marcescens* employed as a specific actuator of this microsystem and a detailed analysis of the trajectories of the microrobots, in order to demonstrate the feasibility of the approach and the rules underpinning it [178].

Moreover, Scientists are drawn to the use of animal (and especially human), as a consequences of the greatest advantages of this approach: natural biocompatibility and biodegradability. Therefore, by using human tissue-based MNRs, the side-effects created by MNRs can be almost eliminated, except for a slight immunoreaction occurring. Further, their adaptability to the environment provides MNRs with a large living space, which prolongs their survival time. To date, many discoveries have been made in this field and many kinds of eukaryotic cells have been put into practice to create the MNRs, such as skeletal and cardiac muscle cells, and these have demonstrated excellent results [179–182].

Of all the tissues inside the human body, red blood cells (RBCs) are one of the most widely used in transportation in medicine. As the most abundant type of blood cell [183], RBCs serve as an ideal container [184] with power being provided for in vivo drug delivery, this arising from their qualities such as a long circulation time, high cargo loading capacity, excellent biocompatibility, and low immunogenicity [183,185]. For example, it was demonstrated in 2015 that red blood cells could be turned into microrobots after merging with magnetic nanoparticles, quantum dots and doxorubicin, which could travel at high speeds and with high spatial precision through micro-sized networks, when used for transporting medicine and imaging efficiently, without causing severe biofouling [186]. Meanwhile, the combination of an RBC and another type of human blood cell, thrombocyte, has also been considered to be highly promising in the medical field due to the properties that this offers, taking advantage of the functional proteins that are contained in it. Although multiple advantages can be seen in the RBC field, at the same time challenges still exist such as eliminating cytomembrane damage and allowing independent local release [187,188]. Hemamebas used as microrobots are also considered to be highly promising in the medical field, due to their abilities to target specific pathogens and eliminate them autonomously [189]. Utilizing the mediated permeabilization of the ATPs, hemamebas can allow a high loading and concentration of the

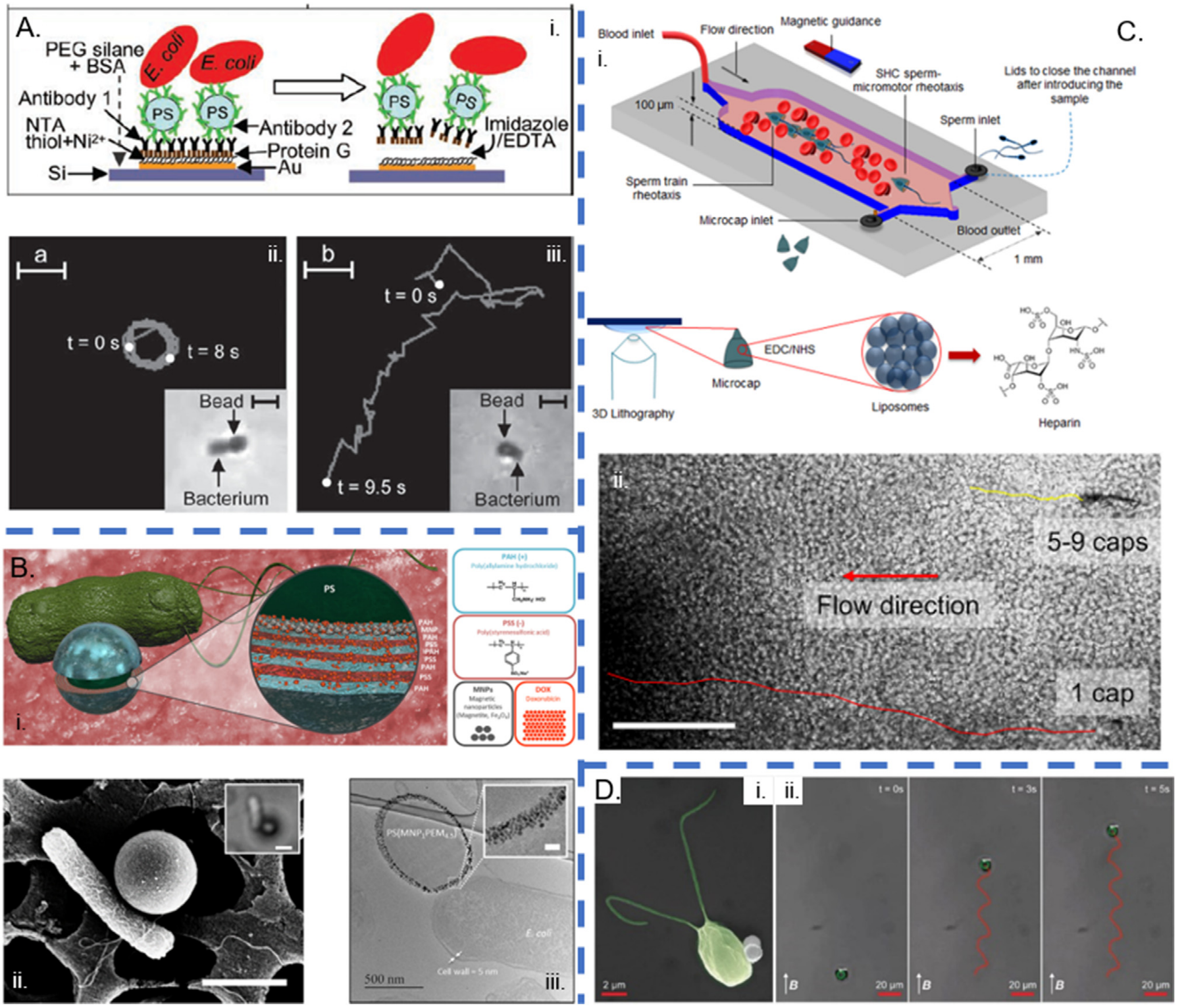

Fig. 5. (A) microrobots derived from *E. coli* for cargo delivery. (i). Schematic diagram depicting mechanism of bounding and releasing bacteria as transporter to carry cargo. (ii-iii). The motion of single bacteria—bead conjugates propelled by *E. coli* upon release from the substrate. (Reproduced with permission from Ref. [169]. Copyright 2011 (Wiley)) (B) SHC sperm micromotor. (i). Mechanism and constitution of the SHC sperm micromotors. (ii). Trajectory of an SHC sperm micromotor and a sperm train swimming against flowing blood. Scale bars: 100 μm. (Reproduced with permission from Ref. [192]. Copyright 2020 (American Chemical Society)) (C) Schematic, SEM (scale bar, 1 μm) and TEM (scale bar, 50 nm) images of bacteria-microrobots derived from *E. coli* MG1655 bacterium with PEM-MNP attached. (Reproduced with permission from Ref. [170]. Copyright 2017 (American Chemical Society)) (D) A certain kind of unicellular freshwater green microalga, Chlamydomonas reinhardtii, provide the propulsion energy. (i). Image of an example algal (C. reinhardtii) microswimmer in SEM (pseudocolored green). (ii). Track of microswimmer under 26 mT uniform magnetic field in 2D. (Reproduced with permission from Ref. [193]. Copyright 2018 (Wiley)). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

agent, it serving as a better cargo carrier when compared to other drug carriers that may be used [190].

Derived from bone marrow, neutrophils have chemotactic, phagocytic and bactericidal effects. Recently, this team has presented a neutrophil-based microrobot to deliver drugs to malignant glioma actively, which then can effectively inhibit the proliferation of tumor cells, compared to the traditional injection method that often is used [191].

Owning to their natural capacity for fertilization, sperm have an important role in the drug transportation field. As a powerful natural cargo delivery mechanism, sperm can target female oocytes accurately and promptly with a substance which is well encapsulated inside the membrane, preventing it from being destroyed by the immune system. Due to their bioactivity in the female reproductive system, sperm can swim inside tumors and transport drugs during the fusion between sperm and tumor cells, after the sperm release mechanism breaks the tumor walls [194—197]. To confirm the feasibility of sperm being used to cure gynecological disease, HeLa cells taken from cervical cancer patients have been used in several experiments. Using FITC-BSA to visualize the concept of protein-based drugs, an experiment on drug delivery that was carried out had demonstrated that sperm were both in solution and spheroids where HeLa sperm were cultured, which showed the ability of sperm to penetrate through tissue [197]. In the sperm-activated field, Schmidt's group has made a significant contribution by introducing many excellent ideas, such as a hybrid sperm micromotor capable of actively swimming against flowing blood (both continuous and pulsatile) and delivering a heparin cargo (see Fig. 5B) [192]. The group has developed a new strategy for the *in situ* selection and transportation of multiple motile sperm cells. The integrated system that they reported, consisting of a synthesized protein-based hyaluronic acid (HA) microflake and a magnetically driven micromotor, represents new thinking in the transportation of not only multiple motile sperm but also other biological cargos [198]. According to research reported by Wang [199], free swimming functionalized sperm micromotors (FSFSMs) can be described as intelligent self-guided biomotors with intrinsic chemotactic motile activity.

DNA, operating on a scale that is smaller than the cellular level, has shown important prospects for wide applications when used in nanorobotics. In addition to its benefits of biocompatibility, the unique structure of DNA provides abilities such as excellent sequence-based addressability, specific recognition capabilities and it can be originated directly. Therefore, the DNA self-assembly methods such as attaching DNA as flagella to cells, or appending DNA to polymeric or inorganic nanoparticles, permits an improvement of the bionic structure (DNA1, DNA2, Nanoparticles and DNA) [32,200,201], where DNA-based nanoparticles have stood out as a excellent platform for applications in sensing and imaging, for example. Moreover, autonomous DNA nanorobots have been proved to be valuable when used as well as a transporter mechanism. A DNA nanorobot performing and classifying cargos on the molecular scale has been created by fabricating nanorobots with a ‘foot’ and an ‘arm’. Due to the fact that this kind of nanorobot can be separated completely from the energy supply used when performing random walking, it shows qualities that will be particularly useful in future applications [202].

To solve the problem of transporting energy, scientists have devoted themselves to exploiting natural swimmers, most of which are a type of cell, but to modify their structure to ensure that they can carry drugs [203]. These natural swimmers can absorb energy from the environment autonomously to create movement, and so can be considered as a true propeller. Yasa, Erkoc et al. have recently reported a biocompatible biohybrid microrobot whose power comes from Chlamydomonas reinhardtii, a certain kind of unicellular freshwater green microalga. By using fluorescent isothiocyanate-dextran as the delivery cargo, they successfully simulated active cargo delivery, demonstrating this as a proof-of-concept. Through non-covalent interaction, magnetic spherical cargoes (1 μm in diameter) were connected to the surface of the microalgae and as a result no chemical reactions were needed (see Fig. 5D) [193].

7. Hybrid propulsion

Hybrid actuation can be defined as the propelling of MNRs by using two or more methods simultaneously, this being a promising approach due to its ready adaptability and today several research groups have made considerable progress on the combination of the magnetic approach with other methods. Magnetic actuation is the most commonly used method, which then creates a good foundation for developing hybrid actuation using biological, chemical or physical methods, such as optical or acoustic techniques. For example, Park et al. have proposed a hybrid actuated microrobot which, when combined with electromagnetic actuation, has been used in large, micro-range blood vessels and with bacterial actuation in smaller vessels, enabling the microrobot to track tumors in the large vessels in the bloodstream (see Fig. 6A) [204]. Also, the combination of the catalytic and magnetic mode has provided a speedy and convenient way to switch the source energy, and this has successfully solved both the fuel depletion and salt limitation problems and reversed the motion direction (see Fig. 6B) [205]. In the physical power field, a magneto-acoustic hybrid fuel-free nanomotor has been invented by Wang et al. to perform specific medical tasks [208] and a brief exposure of the hydrogel bilayer to light, where optical and magnetic techniques are used in combination, can enable the penetration of human tissue without any damage, for applications such as drug transportation and cell detection (see Fig. 6C) [206]. Furthermore, MNRs driven by the combination of electrical and other power sources is widely known to be very efficient and an example can be given of electrical and thermal actuation using an electrothermally activated SU-8 microgripper [209]. Actuation used in this way can be applied to cell manipulation and positioning in the normal environment, instead of using high temperature and pressure to do this. There are also some outstanding energy-combining studies which include the use of chemical power. In one way, a chemically driven hybrid micro-/nanomotor can also be counted as hybrid actuation and this was first achieved by Wang et al. presenting a chemically powered motor whose energy can be generated by three different fuels: base, acid, and hydrogen peroxide and this work has illustrated the autonomous switching between them without a loss of efficiency (see Fig. 6D) [207]. In conclusion, hybrid actuation can be seen not only to diversify the functionalities of MNRs, but also to improve adaptation and universality, enabling MNRs to operate across a range of different situations. Even with the higher cost associated with more complicated structures, this still is one of the most important directions that are developing in this field.

8. Outlook

MNRs have already overcome a wide range of technical difficulties with each of the propulsion methods considered. The improvement in the manufacturing process of untethered robots has gradually narrowed their size down to the nanometer level, and the use of a programmable paths and precise navigation has also enabled micro-swimmers to enter hard-to-reach places and release cargos into target locations precisely and autonomously [210,211]. Moreover, the invention of soft MNRs has made it more convenient for robots to shuttle between obstacles, without causing damage due to their soft nature [212,213].

However, a number of technological challenges must be overcome before robots can be widely used in practical medical or environmental applications, and a number of solutions are being worked on at present [46].

Biocompatibility and safety issues are both extremely important prerequisites for the use of MNRs in medical applications in vivo, which is why many current studies have been restricted to the theoretical or laboratory experimental level [210,214]. For example, when using chemically driven robots, many of the products of the chemical reactions used are toxic or cannot be fully broken down into substances that can be absorbed by the body [149,150], thus affecting the internal biological environment (such as the pH [142]), even causing irreversible damage or long-term side effects to the human body, this being the most critically important reason why they cannot be widely used in this way. Also, when certain substances do not break down in vivo, the removal of the waste produced is a serious challenge, because the robot could be used in any part of the body, rather than just the digestive system [215–217]. Current work [207,212] is focused on developing reactants and catalysts that can be completely degraded in the human body and that produce harmless substances. The first report on biodegradable microrobots was proposed by Nelson's group, in which a type of non-cytotoxic biodegradable hydrogel microswimmers was produced using two photon polymerization [156]. Besides, many attempts to modify human tissue as MNRs have been made with satisfactory results, as many examples mentioned above in chapter six. In addition, work is on-going to avoid creating harmful products or catalysts to maintain safety, since 100% discharge of such harmful products is not possible [218,219].

Another serious problem appears in locomotion and in obstacle avoidance. The environment seen in the body is far more

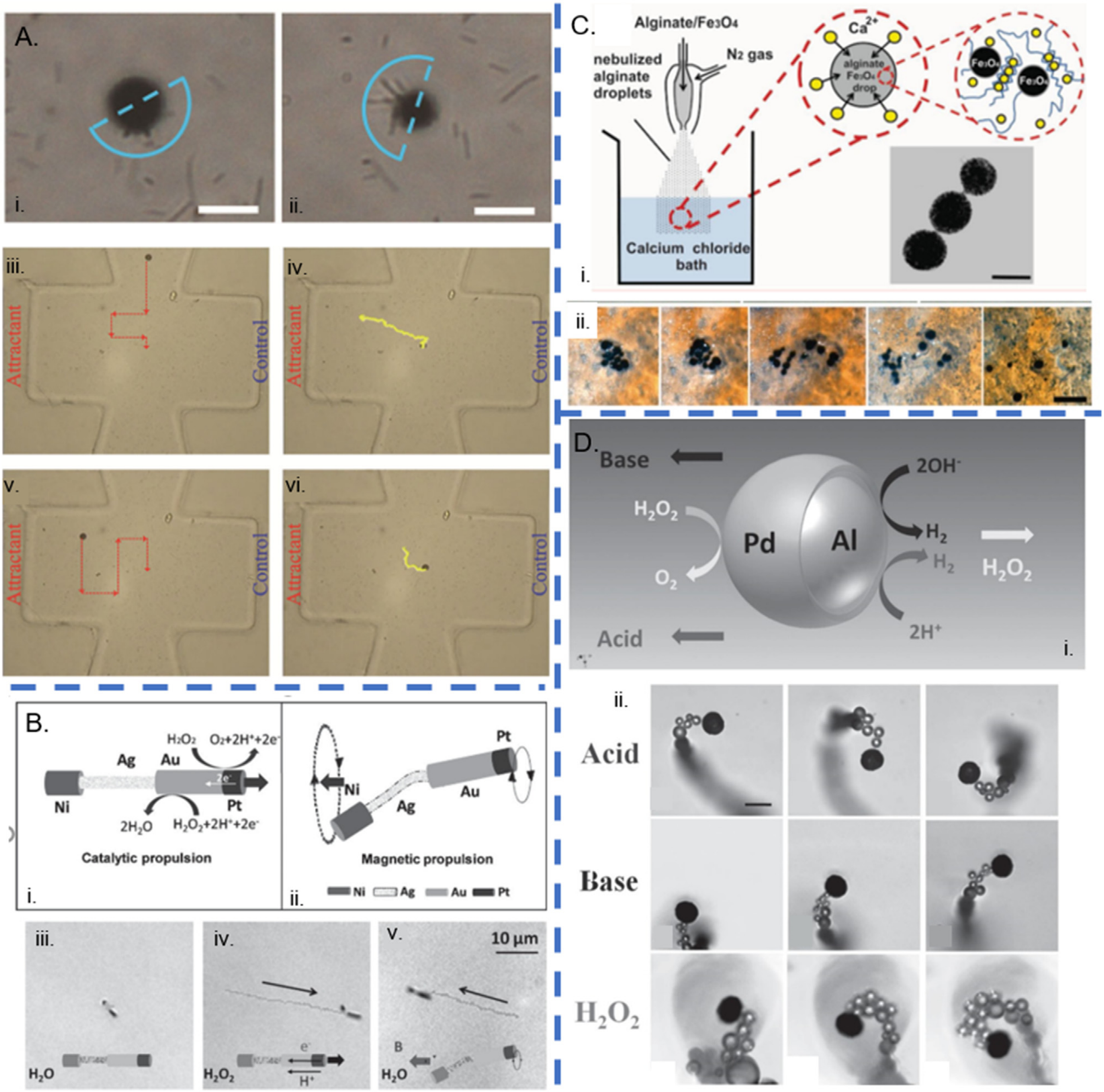

Fig. 6. (A) Brief introduction of magnetic microbeads. (i). Image of magnetic bead selectively attached to flagellate bacteria under optical microscope. (ii). Track of hybrid actuating magnetic microbeads utilizing an electromagnetic field and flagellate bacteria. (Reproduced with permission from Ref. [204]. Copyright 2015 (Wiley)) (B) Mechanism diagram (i-ii) of the parallel hybrid nanowire motor and the hybrid nanomotor (iii) moving under the catalytic (iv) and magnetic (v) field. (Reproduced with permission from Ref. [205]. Copyright 2011 (Wiley)) (C) Fabrication and optical image of magnetic alginate beads applied in cell delivery. (Reproduced with permission from Ref. [206]. Copyright 2014 (Wiley)) (D) Bubble propulsion used in Al/Pd spherical micromotors. (i). Schematic diagram of bubble-propelled Al/Pd spherical micromotors, which can be propelled using acid, base and hydrogen peroxide. (ii). Movement in three different fuels. (Reproduced with permission from Ref. [207]. Copyright 2013 (Wiley)).

complicated than that experienced in laboratory experiments, due to metabolic issues and thus the situation is difficult to predict and control [46]. Moreover, many hybrid curved surfaces such as the vascular wall (where there is a fluid-solid interface) make it harder to steer the direction and the speed of the MNR. As a result of this situation, any small deviation in the movement of the MNR could possibly damage it, or importantly the human environment in which it is being used, or even make the MNR deviate from its intended route. Therefore, taking advantage of the flexible nature that some MNRs offer, this can enable the shape of the device to be altered in a way to make it suitable for the environment in which it is to be used [212,213]. Also, in order to reduce the impact of the hybrid environment, a new way of moving in vivo has been developed using a 'claw' with a corresponding 'claw-like structure' adsorbed on the material along its route as it moves forward [92,220].

Additionally, the limitations of tracking methods have also made it harder to advance medically relevant technology in this field. When performing in vivo, it is critical that both MNRs and the surrounding internal environment should be monitored in real time, in a way that uses non-toxic materials [129]. Biocompatible nanomaterials and fluorescent quantum dots have been incorporated effectively with MNRs, operating at the micro or nanoscale

Table 1
Brief summary of the key advantages and disadvantages of the different propulsion methods.

Propulsion Way	Advantage	Disadvantage	Major literature references to the method (and date of publication)
Magnetic	Wireless, stable, external control device, non-toxicity and biocompatibility, advanced fabricating and controlling technology	Lack of accuracy and automation, hard to retrieve device in vivo	Tierno et al. [99], 2008; Gao et al. [53], 2010; Qiu et al. [91], 2014; Schamel et al. [59], 2014; Gaoet al. [56], 2014; Qiu et al. [66], 2015; Li et al. [52], 2018; Bozuyuk et al. [57], 2018.
Optical	Wireless, stable, easy to manipulate, energy transferring in various ways	Mostly applied in 2D, not working in vivo, strongly affected by environment, immature technology for medical area	Ibele et al. [103], 2009; Jiang et al. [116], 2010; Dong et al. [104], 2016; Wu et al. [118], 2016; Paven et al. [105], 2016; Zhou et al. [110], 2017.
Chemical	Convenient-to-get materials, various reaction types to adapt different environment, easy to design	Inevitable toxic substance or non-degradable product, causing damage in vivo, breaking homeostasis	Ismagilov et al. [136], 2002; Paxton et al. [162], 2004; Howse et al. [149], 2007; Kagan et al. [150], 2011; Liu et al. [151], 2011; Xu et al. [139], 2014; Magdanz et al. [140], 2014.
Biological	Better biocompatibility, natural propulsion method coming from creature itself	Difficult process of modifying, hard to control, limited fully developed types	Ouajdi et al. [172], 2011; Kolaczkowska et al. [189], 2013; Wu et al. [186], 2015; Park et al. [170], 2017; Chen et al. [199], 2017; Thubagere et al. [202], 2017; Xu et al. [192], 2020.
Hybrid	Better adaptability, biocompatibility, flexibility and durability with multiple energy sources and action posture	Hard to manufacture, complex structure and design, high fabricating cost	Chronis et al. [209], 2005; Gao et al. [205], 2011; Gao et al. [207], 2013; Li et al. [204], 2015; Li et al. [208],2015.

[221]. The public remains concerned about the safety of some current methods, such as positron emission tomography (PET), magnetic resonance imaging (MRI), computer-assisted X-ray tomography (CAT), or ultrasound imaging, in case they cause damage to human health [222–224].

A benefit of the use of such MNRs is the ability to achieve cluster control and cooperative behavior for use in the medical field, taking advantage of the small size of the devices [225]. However, this important major advantage that MNRs show has not been fully developed or enough attention been given to it, due to the limitations of current technology. Further, MNRs can easily work in groups and thus tackle complex missions and be used in complicated internal environments, allowing success in these specific operations. A number of groups are working on cluster control studies, this being one of the most promising future fields where MNRs can be applied [226–229]. A further important development lies in the combination of machine learning techniques and MNRs, which could be applied to train and thus optimize the navigation routes they use and allow precise shape deformations [230]. These advances have enormous potential to increase the adaptability of MNRs for use in vivo and thus to be applied with great effect in emergency situations [46] in the future.

Table 2
Brief summary of requirements for different applications.

Application	Examples	Major literature references to the method (and date of publication)	Requirement
Target Delivery	Pharmaceutical Drugs Biologics Living Cells Inorganic agents	R. Mhanna et al. [231], 2014 F. Striggow et al. [232], H. Xu [192], 2020 S Jeon [233], 2019 X. Wang [76], 2019	Highly target-orientated motion behavior with enough power, safely autonomous release, sometimes biodegradability or biocompatibility generating harmless substances
Surgery	Biopsy/Sample Collection Tissue Penetration Intracellular Delivery Biofilm Degradation	J. C. Breger [234], 2015 Z.Wu [235], 2018 W. Wang [26], 2014 L. O. Mair, citemair2017biofilm, 2017	Controllable and highly target-orientated motion behavior, special designs (such as deformable ones) to penetrate, grasp and hold with great force, sometimes biodegradability or biocompatibility
Diagnosis	Biosensors Isolation Physical Sensors	D. Kagan [200], 2011 Z.Lin [38], 2018 M. Pal [75], 2018	Similar to target delivery except for always biodegradability or biocompatibility generating harmless, detectable substances and strong combination ability with certain objects for treatment.
Biological	Optical Acoustic Magnetic Radionuclide	D. Akin [236], 2007 A. Aziz [40], 2019 X Yan, citeyan2017multifunctional, 2017 V. Iacovacci [44], 2019	Similar to diagnosis except for combined substances cannot be degraded by objects in vivo.

9. Conclusion

This article has looked at developments in this field over the past 20 years and it can be seen that considerable progress has been made in a number of different areas within it. The work has revealed that MNRs have great potential across a number of application areas and as a result are likely to provide society with a highly promising future in this field, taking advantage of the strengths of the technique, drawing on the flexibility, adaptability, robustness and accuracy that MNRs show. This article has looked at a number of different propulsion methods and examined many applications in these areas: the strengths and weaknesses of these methods have been discussed and conclusions drawn. Table 1 provides a brief summary of the key advantages and disadvantages of the different propulsion methods considered in the article, and Table 2 sums up related applications and addressed different requirements for micro-/nanorobots, as shown below. At last, challenges and technical limitations of MNRs has been discussed, as well as our forecasts about potential and possible future directions in this area.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Acknowledgements

This work was supported by the National Science Foundation of China (62173293), the Fundamental Research Funds for the Zhejiang Provincial Universities (2021XZZX021), and Zhejiang Provincial Natural Science Foundation of China (LD22E050007). Grattan acknowledge support from the Royal Academy of Engineering.

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