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NANOROBOTS PROVIDE A CONNECTION BETWEEN ROBOTICS AND NANOMEDICINE THROUGH APPLICATIONS THAT ENABLE THERAPEUTICS TO BE EXECUTED ON-DEMAND VIA REMOTE CONTROL VIA PROGRAMMABLE ENERGY INPUT

Moataz Dowaidar [1,2]

[1] Department of Bioengineering, King Fahd University of Petroleum and Minerals (KFUPM), Dhahran 31261, Saudi Arabia.

[2] Interdisciplinary Research Center for Hydrogen and Energy Storage (IRC-HES), King Fahd University of Petroleum and Minerals (KFUPM), Dhahran, 31261, Saudi Arabia.

Abstract

M-bots are small robots that can be actuated and localized inside the human body to aid in illness detection and treatment. Nanomaterials provide a link between robotics and nanomedicine through applications that allow for the on-demand execution of tasks via remote control via a programmable energy input. Microrobots are typically less than one millimeter in size, whereas nanorobots and M-bots are less than one meter in size.Micro- and nanoscale robots can be controlled by external fields for steering and propulsion. Using the GLAD method, nanoscale helical swimmer with a diameter of 200–300 nm and a length of only 1–2 m. capable of reaching speeds of up to 40 m s 1 when precisely controlled by a magnetic field. Ultrasonic field-related actuation may be used for a variety of m-bots, including metallic nanowire and tubular microagent propulsion, microbead rotation, and nanoparticle patterning.

1 Introduction

For decades, the idea of small robots that can be actuated and localized inside the human body to aid in illness detection and treatment has been popular. [1-7] Microrobots are typically smaller than 1 mm, whereas nanorobots are less than 1 m, in contrast to regular robots[8] that are bigger. When an object's size is lowered to the micro-/nanoscale, new problems arise, and micro-/nano-object propulsion in a liquid environment is not as simple as it is at the macroscale. M-bots can reach complicated and restricted locations within the human body, such as the distal of brain arteries and the bile duct, that are often inaccessible with existing minimally invasive medical devices and traditional robots, while being minimally invasive. For biomedical applications, a variety of micro- and nanoscale robots with various designs, kinds of functionalization, modes of actuation, and imaging techniques for localization and feedback have been reported. [9-14] Another form of nanorobot, known as a molecular motor, has also been created with demonstrated potential for targeted medication delivery. DNA nanorobots are commonly manufactured as folded structures with DNA aptamer-based locks, as is the case with most molecular motors. Certain proteins in the cells can mechanically unlock the locked DNA structure by recognizing the detecting strand, generally the aptamer, enabling the release of interior payloads for therapeutic reasons. [15-18] The targeting ability of DNA nanorobots is largely determined by the aptamer's protein recognition, whereas the targeting ability of programmed energy powered m-bots is determined by either energy supply methods or a combination of energy supply and site recognition.

Although molecular motors share some of the characteristics of robots, they vary from m-bots in that their primary role is conformational motion. The following elements of energy-powered m-bots are covered in this review: design, functionalization, actuation, localization, and application. Special locomotion methods should be devised to maneuver small robots in a low Reynolds number environment where inertia is insignificant compared to viscous force. Micro- and nanorobots can be classified as biological, artificial, or biohybrid based on the materials used in their construction. Biological m-bots are constructed from natural biological components and have a high degree of biocompatibility. Depending on the energy source, artificial m-bots may be self-propelled or powered by an external field. Self-propelled micro- and nanorobots obtain their driving power directly from the environment, and their propulsion systems may be classified as self-electrophoresis, self-thermophoresis, self-diffusiophoresis, and microbubble- based. [19] Although the self-propulsion of m-bots is appealing, the cytotoxicity of various chemical fuels in the surrounding environment has severely limited their practical uses in biomedical engineering. [20-24] External-field-propelled micro- and nanoscale robots, a form of m-bot that can be controlled utilizing a distant, external field for steering and propulsion, have the benefit of not requiring the presence of chemical fuels inside the fluid environment, as opposed to self-propelled m-bots. [25]

Magnetic fields, electric fields, light, ultrasonic waves, the Marangoni effect, and other external fields are examples. [26] Biohybrid m-bots combine the benefits of natural creatures, such as geometry, autofluorescence, and biocompatibility, with the multifunctionality of artificial m-bots that arises from the use of various materials. M-bots have a wide range of practical applications, including cargo manipulation,[27, 28] environmental remediation,[29-34], and targeted therapy,[35, 36], among others. [37, 38] Biocompatibility and therapeutic efficacy rely heavily on surface functionalization. To provide the surface of the m-bots with particular polymers, proteins, and quantum dots (QDs) for fulfilling diverse biomedical and environmental duties, certain functionalization techniques based on both physical absorption and chemical grafting should be selected. The ability to locate m-bots in real time, especially in vivo, is critical for biomedical applications. The localization of micro- and nanoscale robots has been studied using a variety of imaging techniques, including fluorescent imaging (FI), computed tomography (CT), magnetic resonance imaging (MRI), ultrasonic (US) imaging, positron emission tomography (PET), and single-photon emission computed tomography (SPECT). The resulting micro-/nanoscale robots can not only be monitored in real time in vitro and even in vivo thanks to the synergy of localization and navigation, but they can also be utilized for targeted administration and therapy in particular regions using vision-based control. Furthermore, as compared to static micro-/nanoagents, the addition of movement to the micro-/nanoagents may improve imaging contrast. The combination of medical imaging techniques and m-bot actuation creates a brand-new active instrument for locating particular locations and executing minimally invasive medical operations. [40, 39] Individual micro- and nanorobots may not be able to deliver enough medicines to entirely treat a disease in a practical setting. As a result, the synergy and collaboration of a group of micro- and nanorobots are critical, and the motion control of a group of m-bots differs significantly from that of individual m-bots. Individual m-

bots inside the swarming integration are not only impacted by their neighbors, but also by the external applied field/energy. In recent years, in addition to researching individual m-bots, researchers have focused on the motion control of swarms, which has numerous advantages for practical biological applications. [41-43] First, swarm motion and m-bot control have the potential to deliver huge quantities of medicines, cargo materials, or cells, as well as energy such as heat via photothermal and magnetic thermal conversion. Second, because to the accumulative effect, the swarming pattern as a whole may give considerably higher imaging contrast than individual agents at the micro- or nanoscale, aiding localisation. [44] As a result, the collective behavior of batches of m-bots and their use for in vivo administration are studied independently from the individual micro- and nanorobots. The design, functionalization, actuation, localisation, and applications of m-bots are all examined in this paper. The chemistry and geometry of the m-bots determine their design. The sorts of cargos/molecules that are either physically attached or chemically conjugated on the surface of the m-bots determine their functionalization. The bots may be driven utilizing magnetic fields, ultrasonic fields, light, electric fields, fuel, heat, and the Marangoni effect, and the actuation is systematically outlined according to the kind of energy used for propulsion. There are also hybrid m-bots and those that are pushed by the m-bots' collective activity. The collective behavior of m-bots is influenced by internal interactions between individual m-bots as well as propulsion under the applied energy field. The localisation of m-bots in vivo is explored based on the medical imaging techniques used, which are categorised as fluorescence imaging, MRI, US imaging, and radionuclide imaging. Diagnostics, isolation and cell growth, targeted therapy using peroral and injectable methods, thrombus ablation, and other bioapplications have been evaluated in terms of the applications, notably for in vivo usage of the m-bots.

2 Development of m-Bots

2.1 Chemistry and Structure

Biological, artificial, and biohybrid micro- and nanorobots are the three kinds. A micro- and nanorobot's typical size range from hundreds of nanometers to dozens of micrometers. Natural organisms are often found in biological and biohybrid m-bots, and they are extremely biocompatible; nevertheless, the difficulty in molding them is a significant restriction. Artificial m-bots are the bulk of the m-bots, which are created using either a top-down or bottom-up approach. Physical vapor deposition (direct deposition and glancing angle deposition), roll-up technique for the manufacturing of micro/nanotubes and helical microrobots, and 3D printing techniques such as direct laser writing are all top-down tactics used to make m-bots. [69] Electrochemical/electroless deposition, wet chemical synthesis, and the self-assembly process are some of the bottom-up techniques used to make m-bots. The review will go through all of the m-bots'manufacturing processes. The structural and component design of artificial m-bots are included in the design. Artificial m-bots can be classified as stiff or soft based on their structure, and as micro-/nanospheres, rigid/flexible nanowires, micro-/nanotubes, helical m-bots, and microbullets based on their appearance. [70-86] Rigid m-bots have been studied extensively for decades, but in recent years, the focus has turned to soft m-bots due to their significant benefits in interacting with the human body and adapting to unexpected environments when employed in biomedical applications. [87-97]

Active soft matter and smart materials are two subcategories of soft m- bots. The term "active soft matter" refers to soft-bodied m-bots made from polymers and organic components. The modulus and rigidity of soft matter are usually equivalent to those of genuine biological cells, tissues, and organs, making as-fabricated m-bots more biologically- like and therefore more appropriate for biomedical applications. During navigation, some soft- bodied robots can alter form. [98] When exposed to external stimuli such as heat, light, ultrasound, magnetic field, electric field, and mechanical force, smart materials are adaptable and changeable throughout their entire structure (such as spring-mass systems) or at predesigned hinges (such as segmented micro-/nanostructures with several soft joints)[99-101]. Li et al.[102] created a magnetic nanorobot with two soft connecting arms that can swim "freestyle" in low Reynolds number liquids. During motion, the degrees of freedom of these soft robots are far more than those of rigid robots.

The propulsion regime should also influence the component design of the m-bots. Magnetic materials such as Fe3O4, Ni, -Fe2O3, and FePt[28, 45, 54, 103] should be studied for magnetically operated m-bots. To achieve asymmetric bubble propulsion for bubble-propelled m-bots in a fuel-rich environment, catalytic materials such as Au, Pt, Ag, MnO2, TiO2, and enzyme may be required. Robots with anisotropic geometry or composition, also known as Janus particles, are widely used in self-propelled m-bots without bubble discharge. Currently, the design and production of micro- and nanorobots is based mostly on 20 elements, the majority of which are transition metals with a handful from the main group (the frequently used elements in m-bot design are highlighted with orange boxes). Design and fabrication may be accomplished in the future by experimenting with different multifunctional components. Biocompatibility and biodegradability are two important factors to consider. During the design stage, the biocompatibility and biodegradability of the micro/building nanomachine's components should also be met, which may be determined based on the elementary characteristics. Biodegradable materials are often selected throughout the design phase since they degrade gradually after usage and do not require any post-removal procedures. When used in moderation, these compounds can breakdown into noncytotoxic solutes in the biological milieu and cause no harm to the human body. In reality, only a tiny portion of the materials demonstrate biodegradability when restricted to the comparatively moderate environment of the living environment. Several biodegradable micro/nanomachines have been developed as building blocks/functional layers for in vivo applications, including inorganic materials such as Mg, Zn, and CaCO3, as well as organic materials such as polydopamine, polysaccharides, liposomes, and hydrogel gelatin methacryloyl,[104], which will be illustrated based on the different catalogs of applications in Section 6. Because of the many needs for actuation, imaging, and applications, the applied materials cannot always be kept biodegradable. In any case, while designing the micro/nanomachines, the building blocks should meet the minimal criterion of biocompatibility.

2.3 Robot Dimensions The size of the robots might be another key factor in biomedical applications because it directly influences the m-bots' ultimate orientation in vivo. Biological barriers such as the blood-brain barrier, vascular endothelial barrier, and glomeruli filter prohibit m-bots of particular sizes from passing through. [105] If the nanorobots are tiny

enough, the kidney removal procedure may be used to expel them from the body. Larger m-bots may be caught by immune cells in the circulatory system, such as monocytes, leukocytes, platelets, and dendritic cells, as well as resident phagocytes in tissues and organs. Kupffer cells in the liver, alveolar macrophages in the lungs, and B cells in the spleen are among the resident phagocytes, which can be found in a variety of organs. [106, 107] If you're looking for a unique way to express yourself As a result, while designing m-bots, the size of the micro-/nanomachines should be taken into account for clearance. Furthermore, the size of the m-bots can have an impact on their location and movements during remote control. Microscale robots and nanoscale robots move in very distinct ways. The interference produced by Brownian motion becomes more evident as the robot size lowers. The Brownian motion of the nanorobots causes random changes in direction of travel, making the trajectories far more chaotic and disordered than those of the microrobots. The trajectories are derived through directed actuation and, in some circumstances, should be examined using statistical analysis rather than intuitive judgment. [108]

3 m-Bots' Functionalization The functionalization of m-bots is a critical step in providing them with additional capabilities that allow them to do activities other than navigation. The functionalization method may be used in bioapplications for not only targeted delivery/therapy but also imaging and tracking (i.e., localisation) of m-bots in vitro and in vivo. Furthermore, the functionalization procedure enhances biocompatibility and prevents the immune system from mistaking m-bots for foreign items and fighting them, extending in vivo retention duration. To far, numerous functionalization methodologies and procedures for particular bioapplications have been investigated, using both physical absorption and chemical bonding to absorb and bind molecules of medicines, polymers, proteins, and QDs onto m-bots. [123-125]

Wang et al.[126] used a template-based approach to create a self-propelled Au/Ni/PANI/Pt microtubular device, which they then functionalized with a concanavalin A lectin bioreceptor for E. coli recognition. The m-bots were shown to be very successful for the simple real-time separation of E. coli in a fuel-enhanced environment and clinical samples, according to the researchers. Because of the dissociation of the sugar-lectin complex, the trapped E. coli could also be released from the tubular microrobots in a low pH solution. Aside from protein, grafting polymer onto m-bots can help them perform better in bioapplications. Due to the partially functionalized thermoresponsive PNIPAM polymer layers, Guan et al.[55] developed Mg/Pt-poly(n-isopropylacrylamide) (PNIPAM) Janus micromotors that show autonomous motion in simulated body fluids and blood plasma and can effectively uptake, transport, and affect the temperature-controlled release of drugs. Other polymers, such as PEG, have been frequently used to improve the biocompatibility of m-bots by functionalizing them. The researchers devised ways to wrap the micro-/nanodevices with cell membranes to improve biocompatibility and prevent the immune system from mistaking them for foreign substances and attacking them. [127-132] Bacterial membranes, on the other hand, can be used to coat m-bots in order to improve phagocytosis by phagocytic cells. He et al.[133] created chemotaxis-guided hybrid neutrophil microrobots using a camouflaging method combining drug-loaded mesoporous silica NPs with E. coli membranes, followed by phagocytosis by neutrophils due to their chemotaxis capabilities. This form of chemotaxis-guided hybrid neutrophil microrobot has

high cellular activity and motility, and it may be used for targeted medication administration via neutrophils. Wang et al.,[134] suggested a poly(3-aminophenylboronic acid)/Ni/Pt microtube functionalized by a boronic acid-based outer layer with an inner platinum layer to functionalize the m-bots with the cells on them. The outer boronic acid layer could recognize monosaccharides selectively, while the inner platinum layer served as a catalyst for propulsion. The microrobots that resulted were able to detect and bind to yeast cells and glucose, as well as transport them in the medium.

Maier et al.[135] used a self-assembly technique to bind the DNA flagella to the magnetic microparticles' flexible tails, making the microswimmer easily steerable by an external magnetic field. Inorganic functional nanoparticles may easily be attached to the m-bots, in addition to organic agents and organisms. Due to the fluorescent feature of QDs, Sanchez et al.[56] suggested tubular microrobots that were functionalized with QDs by electrostatic self-assembly for real-time optical observation.

4 Controlling m-Bots The actuation of m-bots is simply energy conversion, which converts different types of energy into kinetic energy, such as magnetic energy, electric energy, light energy, and chemical energy. M-bots' kinetic energy may be utilized to create a variety of motion patterns, including rectilinear, circular, and spiral motion. The m-bots can be classified as self-propelled or external field-propelled based on the forms of the provided energy. Water, H2O2 solution, and acidic solutions are commonly used to power self-propelled m-bots. Chemical-powered/self- propelled m-bots include those that are activated by a concentration gradient, self- electrophoresis, or bubbles. In comparison to chemical-powered m-bots, external field- propelled m-bots are driven by an external magnetic/electric/ultrasonic field and do not require chemical fuels inside the environment, making them considerably more appropriate for bioapplication. The introduction of the m-bot actuation based on the needs of biomedical applications will be the focus of the following sections.

4.1 m-Bots that are propelled by a magnetic field Magnetic field-propelled m-bots can convert magnetic energy into mechanical energy in the form of magnetic field gradient and magnetic torque, allowing them to perform tasks such as cargo handling and transportation, targeted/directed treatment, and environmental cleanup. Because low-intensity magnetic fields are considered safe for live beings, m-bots that are controlled and guided by external magnetic fields have a lot of potential for in vivo applications. In a magnetic field, the magnetic force (F) and magnetic torque (T) on a magnetic object may be represented as[137]. Following that, researchers devised a slew of new techniques, including glancing angle deposition (GLAD) [45, 144-151] direct laser writing Electrodeposition using a template [152-154] Natural-template approach To build the magnetic helical robots with smaller size[144] and oriental characteristics, researchers used a flow lithography integrated microfluidic spinning and spiraling system [157]. [122] Using the GLAD method, Fischer et al.[144, 146, 147] created a nanoscale helical swimmer with a diameter of just 200–300 nm and a length of only 1–2 m. It is the smallest helical robot yet built, and it can attain a speed of 40 m s1 when controlled accurately with a magnetic field. Zhang and Nelson et al.[45] devised a direct laser writing approach for fabricating helical microswimmers on a substrate one at a time, followed by Ni and Ti vapor deposition. Magnetic microswimmers are biocompatible and can be

operated at speeds of up to 180 m s1 in water. Park et al.[153] used electrodeposition on an anodic aluminum oxide template to create the Pt helical shape. After the e-beam evaporation method of Ti/Ni, Gao et al.[154] used spiral xylem vessel plant fibers as a natural template and manufactured microhelical swimmers. Yan et al.[155] employed a coprecipitation technique to construct biocompatible helical microrobots using Spirulina as the biotemplate. Zhao et al.[157] devised a flow-lithography integrated microfluidic spinning and spiraling system for the continuous production of helical microrobots. The length, diameter, and pitch of the helical microrobots could all be accurately adjusted using this approach. The fast-online gelation and polymerization by UV irradiation allowed the helical microrobots to be imbued with Janus, triplex, and core–shell structures with ease. The helical microrobots could be actuated not only in the manner of fuel-free magnetic rotation and corkscrew motion, but also in the manner of fuel-catalyzed bubble propulsion after spatially regulating the encapsulation of NPs in the helical structure. Although helical magnetic m-bots may be driven efficiently in a homogeneous rotating field, the design and production processes are difficult and time-consuming. Several research groups, including the ensemble of beads,[158-160] planar structures,[161-163] and even randomly distributed clusters, have created far simpler designs and structures that function effectively under a rotating field without any boundary in recent years. [104-106] These simpler designs and structures have a benefit over helical m-bots in that they can be mass manufactured owing to their basic designs and fabrication methods. In practice, magnetic micro/nanoagents may not be in a suspended condition and may interact with the restricted space limits. As a result, magnetic micro-
/nanoagents may be navigated using an external magnetic field; this is true even in a uniform scenario, in the form of tumbling/rolling, which takes use of boundary contact forces. Cappelleri et al.[167] used photolithography to create magnetic tumbling microrobots with a variety of geometrical forms that can negotiate complicated surface topographies with a precise trajectory. The microtumblers move forward by gripping the surface with friction. Not only can they tumble into valleys, but they can also ascend steep inclines. Apart from controlling and actuating individual microrobots, magnetic tumbling may also be used to swarm a group of m-bots (will be introduced in Section 6.6). This form of magnetic motion demonstrates its universality in terms of a wide range of magnetic objects and the application of different surface topographies.

4.2 m-Bots with Ultrasonic Field Propulsion When utilized as an external energy source for m-bots, ultrasound can provide noninvasive, on-demand motion control with a long lifetime and high biocompatibility. Ultrasound control and actuation of m-bots has so received a lot of interest in recent decades. [168-175] The acoustic radiation forces, which consist of a primary radiation force (responsible for m-bot migration) and a secondary radiation force, drive the m-bots suspended in the solution (responsible for the repulsion and attraction between the m-bots). [168] The ultrasonic field- related actuation may be used for a variety of m-bots, including metallic nanowire and tubular microagent propulsion, microbead rotation, and nanoparticle patterning. [168] Mallouk et al.[169] created a nanowire on the AAO substrate using template-assisted electrodeposition, and the nanowire was levitated into the node plane with a variable random motion speed using MHz frequency acoustic waves by varying the amplitude and frequency of the acoustic wave. The self-acoustophoresis process is ascribed to

the asymmetry of the nanorods in the composition/geometry, which can lead to unidirectional motion of the nanorobots. Ultrasound can also be used to regulate the speed of chemical-powered m-bots in a precise and reversible manner. The bubble-propelled microrobots respond to the ultrasonic field instantly for speed modification, according to Wang et al.[170]. The produced O2 inside the catalytic tubular microrobots is expelled instantly without the growth process, and the gas was propelled to the nodes/antinodes, forcing the microrobots to halt.

Nelson et al.[171] presented a flexible nanoswimmer with a stiff bimetallic head and a flexible tail, as opposed to rigid m-bots. Because it is difficult to create predicted standing waves in vivo, the flexible microswimmer may be pushed by the small-amplitude oscillation of its flexible tail in both standing and moving acoustic waves, which may ease in vivo applications of the US-driven m-bots. Apart from the m-bots' long-term actuation, the ultrasonic field might cause an immediate ejection of micro-/nanoobjects at ultrahigh instantaneous speeds, which could favor tissue barrier penetration. Wang et al.[176] created a controllable and strong microballistic instrument that enabled the loading and shooting of nanobullets like silica and fluorescent microspheres utilizing electrochemically manufactured microcannons and acoustic trigger action. The nanobullets were expelled at a very fast speed due to the spontaneous evaporation of the perfluorocarbon emulsions caused by the concentrated ultrasonic pulse. The technique not only provided a regulated means of launching nanobullets from a microstructure, but it also improved accessibility to target areas and increased tissue penetration for in vivo applications.

4.3 Light-Propelled m-Bots The actuation of m-bots based on light-sensitive molecules and atoms demonstrates light's unique qualities as an environmentally benign and renewable energy source. 177 and 178 In comparison to other external fields like magnetic, electric, and ultrasonic, the light field is often a highly confined field with a concentrated light beam, which is ideal for executing collaborative activities by a group of m-bots, as discussed in Section 4.7. [179-182] Because tissue penetration depth is limited, light-propelled micro-/nanomachines are ideal for diagnostic and therapy in close proximity to the skin. Janus spherical micro-/nanoparticles, rod/wire-like m-bots, tubular micro-/nanomotors, and other irregular structures with varied motion modes, including self-electrophoresis, self-thermophoresis, and self-diffusiophoresis, are all found in the structures of the m-bots. All motion produced by the light caused nonuniform gradient field (such as concentration, thermal field, and electric field) around the m-bots as a result of the energy conversion of external light energy to mechanical energy of the m-bots. The asymmetric structure of the m-bots or the nonuniform light field can both produce a nonuniform gradient field. We primarily focus on light-propelled m-bots with asymmetrical architectures since the nonuniform light field may be used to actuate particles containing light-sensitive components. Tang et al.[85] created light-propelled microswimmers known as Janus TiO2/Si nanotrees, which were made by partially growing TiO2 on a Si nanowire. A self-electrophoresis with photoelectrochemical reaction that creates anions and cations at opposing ends of the nanotree replicates the phototaxis of natural motile algae and allows the Janus TiO2/Si nanotree to perceive and orient itself to the illumination direction of the outer light source. Controlling the zeta potential of the photoanode allows the microswimmer to perform both positive and

negative phototaxis. [85] The visible-light-driven bismuth oxyiodide (BiOI) based Janus microspheres powered by photocatalytic processes described by Cai et al. are another example of self-electrophoresis microrobots. [192] The BiOI was chosen because of its small band gap (17 eV), which allows visible light to activate the components. The wavelength and strength of visible light may be adjusted to regulate the microrobots' propulsion. Self-thermophoresis can be used for effective m-bot propulsion in addition to self-electrophoresis propelled m-bots. He et al.[46, 61] created tubular-shaped polymer multilayer rockets using template-assisted layer-by-layer assembly and deposition of platinum NPs inside and gold shell outside, followed by functionalization of tumor-targeted peptide and antifouling polymer on the gold shell. The use of NIR light to rapidly initiate the motion of microrobots via self-thermophoresis has also been proven, as has the targeted identification ability and treatment of tumors utilizing the photothermal effect.

Another form of light-propelled m-bots uses light as a switch to stimulate photocatalysis, which allows the m-bots to produce gas bubbles and propel themselves. In comparison to self-propelled m-bots, this sort of m-bot requires external light energy, the motion can be switched on and off, and the speed can be controlled. UV-propelled TiO2-Au Janus microspheres and UV-driven TiO2 microtubes are two examples of light-triggered bubbling m-bots that have been described so far. Guan et al.[193] created a light-controlled bubble-propelled tubular TiO2 microrobot that produced O2 in the inner tube surface by photocatalysis of H2O2 when exposed to UV light. UV light may be used to cause the microtube to move at a controlled speed by changing the intensity of the UV light.

4.4 Self-Propelled m-Bots Fuel-dependent self-propelled m-bots transform chemical and biological energy from their surroundings into mechanical energy for autonomous propulsion. For the self-propelled m-bot system, several factors of the design should be examined. The structural design of the m-bots, as well as the components of the m-bots that are responsible for chemical/biochemical interactions, should be given special consideration. Bimetallic nanowires, tubular microjets, Janus micro-/nanospheres, and other m-bots are among the most common. [194-196] Motility is usually produced for self-propelled m-bots by either breakdown of a fuel by a catalyst or deterioration of the micro/nanorobots in a liquid environment. [194-199] A significant amount of the study in the literature on self-propelled m-bots focuses on the catalytic breakdown of hydrogen peroxide (H2O2). [30, 200-211] H2O2 may be degraded to water and oxygen for gas propulsion by integrating appropriate catalysts onto the m-bots, such as Au, Pt, and MnO2. [212, 213] Despite the fact that H2O2-fueled m-bots may attain significantly faster propulsion speeds than conventional motors, hydrogen peroxide is harmful to organs and tissues in the in vivo environment, limiting its use in biomedical applications. [214]

Because of the high reaction rate and variety of enzyme/fuel combinations available, enzyme-based m-bots offer up a whole new method for promoting biocompatible self-propelled m-bots for biomedical applications. Enzyme-based m-bots are a type of self-propelled m-bot that uses enzymes as catalysts instead of metals like Au, Pt, Ag, and MnO2, and exhibits excellent activity and biocompatibility. [215-218] Sanchez et al.[84, 219] created a self-propelled Janus nanorobot based on hollow mesoporous silica nanoparticles that were actuated by biocatalytic processes based on three types of enzymes placed on the Janus nanoparticles' surface, namely

catalase, urease, and glucose oxidase. The biocompatible enzyme-based active nanomotors presented intriguing promise for biomedical applications employing biologically benign fuels. [219]

Other studies confirmed that enzymes could propel themselves after being affixed to artificial micromachines such as carbon nanotubes[220] and tubular microrobots. [222, 221] Sanchez et al.[223] suggested bubble-free tubular nanojets driven by urea as the mild fuel in an enzyme-triggered biocatalytic process. Tubular nanojets are interesting for bioapplications because of their longitudinal self-actuation and good biocompatibility. Recently, Mao, Shen, and colleagues[224] produced nitric-oxide-driven hyperbranched polymide/l-arginine nanomotors. NO was produced by converting the amino acid L-arginine. In this field, the goal is to combine controlled propulsion, biological m-bots, and different benign fuels into a simple design that will allow the m-bots to be actuated and controlled in a range of biological conditions without requiring any changes to the m-bots. [225]

4.5 Other Types of Fields Propel m-Bots Aside from the actuation techniques listed above, numerous more ways for m-bot propulsion have been studied. These may demand a harsher environment and are not yet ready for in vivo testing. As a result, we've grouped them all together here to provide you a quick overview.

4.5.1 m-Bots that are propelled by an electric field Electric field-propelled m-bots must be conductive/semi-conductive or charged, much as magnetic field-propelled m-bots must contain incorporated magnetic elements in their constructions. Electrical actuation has the advantage of being non-contact and low-cost. The utilization of electrophoretic motion, electro-osmotic flow, and electrorotation to drive m-bots through DC and AC electric fields is developing as a novel wireless controlled and fuel-free actuation technique. [226-236] Due to Coulomb interactions, charged objects migrate toward the electrode with the opposite charge when a uniform DC electric field is provided. [227] M- bots may be activated in a controlled manner when an AC electric field is supplied. Due to dielectrophoretic forces, the AC electric field may also be used to actuate things at the microscale; the torque imposed on the items by the electric field generated dipole moments and electro-osmotic flow. 231; 230; The motion of microagents controlled by a DC electric field is generally simple, and the roundabout trajectory cannot adapt to the complicated fluid environment. In most earlier efforts, AC electric fields were used instead of DC electric fields for m-bot propulsion. [227, 228] Four phase-shifted AC voltages with a consecutive phase shift of 90° may likewise be used to electrorotate magnetic and nonmagnetic nanowires with carefully regulated rotational speed. 226-229; 236; Wang et al.[229] created poly(pyrrole)-cadmium (PPy-Cd) and CdSe–Au–CdSe semiconductor nanowires and used the electro-osmotic flow mechanism to actuate them under an external AC electric field, resulting in directional nanowire locomotion. Unlike electro-osmotic flow-driven micromachines, the electric field may also cause asymmetric bubble formation by triggering a redox reaction on the Janus particles' two sides. The micromotors may be constantly propelled by the released bubbles in both translational and circular movements. However, electric field-propelled m-bots' bioapplications may be limited owing to their low locomotion range, which is caused by the fast attenuation of the electric field with distance, and the electric

field may be incompatible[232] with highly ionic media like interstitial fluid and blood.

4.5.2 m-Bots Powered by the Marangoni Effect The Marangoni effect is a liquid flow motion induced by the gradient of interfacial tension at the interface between two distinct phases (e.g., liquid and air). The Marangoni effect is used to

actuate the visible scale's motors and droplets in most situations; nevertheless, the research is still useful for the design and production of next-generation m-bots, particularly for applications at the surface and interface of solid, liquid, and air. Propulsion via the Marangoni effect is most common at liquid/air or solid/liquid interfaces. [237-240] Baigl et al.[241] devised a system for controlling liquid movement and high precision droplet coalescence by actuating oil droplets on the water surface. The authors dissolved cis-AzoTAB in the solution because photoisomerization caused this molecule to change configuration during light irradiation, resulting in a change in the surface tension of the water solution. The liquid would flow toward the low liquid-tension area due to the local change in surface tension. As a result of the Marangoni effect, the droplet can be transferred on demand. According to Baigl et al., apart from the actuation of liquid droplets, the Marangoni flow and anti-Marangoni flow may be used to push solid objects and even liquid marbles consisting of both liquid and solid phases. [242] The translational motion of micro-objects (i.e., PS microspheres) over the surface of a liquid-crystalline thin film was demonstrated by Kurihara et al.[246]. The PS microsphere was actuated by UV and visible light irradiation of the surface, and the direction of motion was determined by the direction and position of the irradiation. The Marangoni effect might archive the collective motion of several small microrobots in addition to single microrobot actuation. In an H2O2 atmosphere, Zhao et al.[247] developed a Janus catalytic micromotor that could create O2 bubbles. Because of the evaporation-induced Marangoni flow surrounding the bubble, the micromotors moved collectively toward the bubble as it consolidated and expanded, finally forming an aggregation around the bubble's perimeter. Shi et al.[243, 244] and Sun et al.[243, 244] are two additional researchers who have proven the presence of Marangoni flow-induced propulsion of small agents. [245] As a result, the actuation of the items on the liquid surface is reliant on the liquid surface but independent of the objects. For the actuation of such small devices, Marangoni and anti-Marangoni propulsion are flexible methods.

4.6 Hybrid m-Bots

4.6.1 m-Bots with Multiple Energy Sources

Hybrid m-bots navigate and control themselves using two or more energy sources, extending their range of manipulation in complex and changing situations. [248-253] Wang et al.[253] created a flexible multisegmented Pt–Au–Agflex–Ni nanowire swimmer with catalytic and magnetic actuation by the Pt–Au and Au–Agflex–Ni sections, respectively. Hybrid m-bots with two energy sources can transition from catalytic to magnetic motion, allowing them to adapt more quickly to changes in the environment. Mallouk et al.[112] proposed a synthetic bimetallic micromotor that may be operated by a hybrid driving force of chemical fuel and acoustic field with both positive and negative rheotaxis properties. There have also been reports of m-bots that are powered by two different energy sources, such as UV and NH3. [254]

The motion and direction of hybrid m-bots can be controlled using external fields in addition to self-propelled and external field-steered hybrid m-bots, avoiding the usage of chemical fuels. [255] Wang et al.[249] created a magnetic/acoustic hybrid fuel-free nanorobot made up of an Au nanorod segment and a Ni-coated Pd helical structure that can be operated on-demand by either a magnetic or ultrasonic field. More crucially, this research showed that the m-bots' hybrid power actuation can achieve reversible swarming states and collective behaviors, which is difficult to do with a single propulsion mode. 4.6.2 Natural Microorganisms and Artificial Microdevices Hybrid Hybrid m-bots, which combine natural microorganisms with artificial microdevices, have also been produced in addition to artificial m-bots. [256-263] Sanchez et al.[256] created sperm- driven biohybrid microrobots that combine a motile sperm cell with a magnetic microtube. The sperm cell propels the micro-bio-robots, which are guided by an external magnetic field. Unlike previous chemically fuelled m-bots, this one does not utilize hazardous fuel. The same study group used 3D direct laser writing and subsequent Ni coating to create another sperm-propelled, helical micro-bio-robot that was operated and directed using a magnetic field. [257] The sperm-propelled micro-bio-robot might act as a helper in infertility treatments by delivering healthy but immotile sperm to an egg. They also discovered that these biohybrid microswimmers may be utilized as tailored medication delivery vehicles[259]. In this biohybrid microswimmer, the motile sperm cell not only propelled the swimmer, but also functioned as the carrier. The sperm and the hitting-induced release of the sperm cell for drug release were accurately guided by a 3D-printed magnetic tubular microstructure. Wang et al.[258] created an intelligent self-guided biomotor with chemotactic motile behavior by functionalizing different nanoscale payloads with sperm micromotors, such as quantum dots, doxorubicin hydrochloride drug-coated iron-oxide NPs, and fluorescein isothiocyanate-modified Pt nanoparticles. These micromotors can be used as targeted drug delivery carriers, with cargo transportation directed by the sperm micromotors' inherent chemotaxis. Furthermore, Sitti and Sanchez et al.[264] suggested bacterium biohybrid microswimmers to propel microtubes with motile E. coli in biological medium produced through adhesive bonding of E. coli with the inner surface of a microtube with a PDA layer. They revealed another bacteria-propelled biohybrid microswimmer that employed red blood cells as autologous cargo carriers for active and guided medication administration to improve loading-carrying efficiency, biocompatibility, and biodegradability. [268] Chemical bonding was used to attach the drug-loaded red blood cells and superparamagnetic iron oxide NPs to the motile bacteria, and an external magnetic field was used to direct the microswimmers. Following treatment, the bacteria can be destroyed using an on-demand light-activated hyperthermia procedure to keep the bacteria population in the organisms within a safe range.

4.7 m-Bot Collective Behavior The use of various energy sources to actuate and control single m-bots has been widely researched. However, in practice, a single m-bot may be constrained by its limited capacity and unable to achieve the desired results. The usage of multiagents may become necessary for completing the assigned tasks. There are two forms of mobility and control for multiagents: independent motion and dependent motion. The microagents are all independent without any restrictions of origin from their neighbors in the

multiagents' independent motion mode. As a result, their actuation is comparable to that of single m-bots. The research of the collective behavior and swarming motion of the m-bots has also been put on the agenda, inspired by natural collective behavior such as schooling of fish and swarming of starling birds. [270, 269] The collective behavior is significantly influenced by the distance between them. During swarming, short-range forces of attraction or repulsion, such as van der Waals attraction, electrostatic interaction, and steric repulsion, all function. Each unit creates a flow field that is felt by the units around it, resulting in alignment interactions. [36, 271-273] The micro-
/nanoagents in the collective motion are all interdependent and communicate with their neighbors in the form of attraction or repulsion, even with the inert micro-/nanoparticles in the efficient area. Because the short-range interactions between the active particles may not be strong enough to combine all of the particles when the m-bot concentration is low, more than one swarm pattern may be formed. The m-bots' collective mobility and swarm formation allow for the coordinated movement of disparate items, making it easier to carry and deliver material to specific locations. The effects of light on collective behavior have been widely researched. [274-276] The light- driven collective behavior of AgCl microparticles in aqueous solution under UV radiation was described by Sen et al.[274]. Diffusiophoresis was suggested as the cause of the collective motion. UV light induced AgCl to dissolve, resulting in protons and chloride ions. [275] Because protons have a significantly higher diffusivity than chloride ions, an electric field was created in the solution that operated on both the particles and any adjacent wall double layer. The electrolyte gradient causes a pressure differential, which causes a flow of liquid from the high-electrolyte-concentration area to the lower-electrolyte-concentration region, resulting in collective behavior. In addition, the work provided a nonbiological model for studying cell signaling and collective behavior. Palacci et al.,[276] called living crystals, exhibited the 2D collective behavior of Janus particles consisting of polymer microspheres with an antiferromagnetic hematite cube on one side with light activation. Dynamic living crystals were formed as a result of osmotically driven motion and steric hindrances. Buttinoni et al.,[277] provided an experimental investigation of a colloid suspension of Janus particles that are self- propelled by the heating (532 nm) of carbon-coated hemispheres in a combination of water and lutidine, as well as a thermally generated collective. Because of the self-trapping, a number of tiny dynamical clusters form at low densities and one large cluster appears at high densities in this system, which is explained in terms of a dynamical instability. SiO2–TiO2 micromotors,[278] Fe2O3 micromotors,[276, 279], and TiO2-Pt micromotors are among the photocatalytic and photosensitive m-bots being developed. [280-282], Sen et al.[278] created SiO2–TiO2 microparticles that can be built and destroyed reversibly under UV light. Under UV light, TiO2 produced O2, OH, and H+, according to the scientists, and the collective behavior of the particles may be attributed to osmotic propulsion or diffusiophoresis.

Recently, a magnetic field-controlled swarming motion was discovered. [283-286] After applying rotating and dynamic magnetic fields, the magnetite nanoparticles produced using the solvothermal technique may be assembled into a planar dynamic cluster and disassembled into scattered nanoparticles. For the assembly process, the final particle concentration can be

increased to 500 percent of the beginning concentration and lowered to 20% of the original concentration for the disassembly process. The reversible merging and splitting procedure is also possible with the constructed vortex swarm pattern. [283, 284] On uneven terrain, the swarming action is also effective. [283] The swarm pattern entity may be controlled to travel via curved and branching channels to the target place with high accuracy and minimal particle loss of less than 10%, which is far more efficient than the traditional tumbling motion. They also included dynamic bubbles for crosslinking and vertical axis stacking of the nanomotors, extending the magnetic swarm behavior from 2D to 3D. [271] Due to the dynamic dewetting and enhanced slip length induced by the constantly expelled small bubbles, the integral rotation and translation were improved when compared to 2D swarming movements without bubbles. Anisotropic swarming clusters can be created in addition to isotropic swarming clusters. Zhang et al.[287] used a programmable oscillating magnetic field to create a reconfigurable ribbon-like swarming pattern with dynamic-equilibrium structure in fluid. It is possible to achieve reversible elongation with a high aspect ratio, as well as controlled splitting and merging of subswarms. The scientists also proved that the ribbon-like dynamic swarming pattern may pass through a restricted channel and execute noncontact micromanipulation in the fluid while passing toward numerous targets with high access rates. They expanded the swarming behavior into 3D shape by using NIR light in addition to the planar swarming pattern. [288] They have stored and thoroughly examined the active production and magnetic actuation of microrobotic swarms in different biofluids under real-time surveillance by clinical imaging techniques such as US imaging to advance the biomedical applications of swarming m-bots. [288] The introduction of hybrid energies into a particle cluster can result in a more varied motion behavior. To simulate the vascular environment, Nelson et al.[290] combined the magnetic and acoustic fields and applied them to a particle system with boundaries. The superparamagnetic particles that formed numerous clusters inside the vasculature displayed rolling motion along the vessel's wall, according to the researchers. This novel motion behavior, which is based on the combination of various external energies, has the potential to transcend the limits of single-energy actuation and show promise in targeted treatments. Another hybrid energy-driven collective behavior of nanomotors was proposed by Li et al.[291]. The nanomotors' aggregation and separation were easily controlled using light and ultrasonic fields. The fluidic environment, such as viscosity, liquid mobility, boundary effect, and other contaminants, can readily affect the motion of the swarming m-bots. For further research into this subject, highly sensitive m-bots with reasonable structural and componential design may be required to overcome possible mobility inhibition in actual biological systems.

5 Detection of m-Bots M-bots must be able to locate themselves in order to be useful. The m-bots' position and objective inside the practical application system are critical for the m-bots' next-step navigation. The m-bots are typically located easily with an optical microscope for in vitro applications such as sensing, and the position information also gives the manipulator with the next step to control the m-bots as a feedback. Optical microscopy is inappropriate for in vivo applications such as tumor treatment and thrombus ablation, and additional imaging techniques should be investigated to observe the m-bots across the tissue. For m-bot localization, newer imaging techniques have been tried, including fluorescent imaging (FI), magnetic resonance imaging (MRI), ultrasonic imaging (US), computed

tomography (CT), positron emission tomography (PET), single-photon emission computed tomography (SPECT), and photoacoustic computed imaging (PACT). With vision-based control, tailored delivery/therapy at a predetermined place is feasible thanks to the synergy of imaging and motion control. Furthermore, due of the dynamic activity of the m-bots as opposed to the static m-bots, the added motion of the m-bots may improve image contrast. The visualization of moveable micro- and nanorobots has become a unique tool for targeting particular regions with high accuracy and performing certain medical activities in a less invasive manner thanks to enormous developments in biomedical imaging[39, 40].

5.1 Fluorescent Imaging Fluorescent imaging is a popular imaging method that may be employed in vitro and in vivo. In comparison to other imaging techniques, fluorescence imaging offers the benefits of using solely nonionizing radiation on the tissues and using very affordable probe materials. To identify cells at particular regions and unusual biomolecules, several fluorescent probes such as semiconductor quantum dots (QDs), fluorescent metal organic frameworks, and organic dyes have been created. [292-294] For minimally invasive deep tissue investigation, fluorescent imaging has been downsized and incorporated with catheterization and endoscopic devices. [295] The combination of m-bot actuation and control with fluorescent imaging facilitates m-bot localization and next-step actuation and broadens the bioapplication to active bioimaging and diagnostics due to these benefits of fluorescence imaging. Several fluorescent m-bots have been reported to achieve fluorescence-based m-bot tracking with precisely targeted imaging under visible locomotion using external fields to date. The fluorescent m-bots may be divided as autofluorescence-material based microrobots, dye-based microrobots, and quantum dot-based microrobots depending on the raw materials utilized for imaging. Parts of living organisms in nature have inherent fluorescence characteristics ranging from UV visible light to near-IR light .[296] These species' autofluorescence is caused by complex compounds that act as endogenous fluorophores. If autofluorescent organisms are utilized as a template for making m-bots, the as-prepared devices may inherit the fluorescent trait, allowing for real-time device tracking. The SU-8 photoresist is a fluorescent substance that emits light in the blue and green range. Steager et al.[119] used a photoresist and iron oxide NPs combination to print autofluorescent and biocompatible magnetic microrobots. The autofluorescent microrobots were operated by an external magnetic field and tracked in a dark field, proving the feasibility of fluorescence-based real-time microswimmer tracking. Natural autofluorescent organisms were utilized as a template in another case. Zhang et al.[64] used Spirulina as a template to coat iron oxide NPs using a dip-coating technique, resulting in a biohybrid helical magnetic microrobot. The resulting biohybrid helical magnetic microrobots have autofluorescence and can emit red light when illuminated with green light. In the subcutaneous tissue and intraperitoneal cavity of nude mice, the scientists performed real-time tracking and diagnostic sensing under the supervision of an external magnetic field combined with a fluorescence microscope.

Organic dyes, in addition to autofluorescence, are frequently employed as contrast agents for imaging biomolecules, cells, and organisms because of their widespread availability and small molecule size, which reduces the danger of steric hindrance, which can interfere with biomolecule function. [297] Organic dyes are used with m-bots for imaging because of their ease of conjugation, which helps anchoring. Servant et al.[44] suggested a near-infrared probe-

modified artificial bacterial flagellum microswimmer. An clear fluorescent signal was found in the abdominal cavity of a 4-week-old Balb/C mouse after the artificial bacterial flagellum microswimmers were injected into the intraperitoneal cavity. The red and yellow signals moved concurrently as the microswimmers were propelled toward the bottom portion of the mouse body, which was recorded in real time by the fluorescent microscope. The findings showed that in vivo targeted imaging under the supervision of an external magnetic field may be used to achieve whole-body fluorescence imaging using long-wavelength dyes.

Quantum dots, which are ultrasmall nanomaterials with sizes ranging from 1 to 10 nm, have been utilized in imaging as pure QDs and complexes that combine QDs with other nanomaterials/nanoparticles, including m-bots. High quantum yields, a broad absorption range, narrow and size-adjustable emission, and strong photobleaching resistance are only a few of the optical characteristics of QDs. [298,39]; For the first time, Jurado-Sánchez et al.[56] used a layer-by-layer self-assembly technique to create a microtube fixed with CdTe QDs that combined the optical characteristics of QDs with the autonomous movements of microrobots. Furthermore, the change in fluorescence was observed in order to assess the detection capabilities of hazardous organic and inorganic substances. However, QDs are typically cytotoxic, and the functionalization of QDs on m-bots can easily cause the fluorescence to be quenched, limiting the in vivo bioapplication of QD-based m-bots. Carbon quantum dots, which are QDs with high optical characteristics and biocompatibility, have been created for bioimaging applications to overcome the cytotoxicity. [300] Jurado-Sánchez et al.[301] placed graphene quantum dots onto Janus microrobots to create fluorescent microscopy trackable micromachines that may be used to identify bacteria endotoxins in a dark area. Fluorescence imaging-based m-bots offer the benefits of high sensitivity, high selectivity, and various characteristics, as illustrated by the three varieties of fluorescent m-bots listed above. However, they are frequently limited by low biological tissue penetration, making them unsuitable for deep-tissue imaging. Each luminous m-bot has its own set of restrictions. In autofluorescence-material based microrobots, the autofluorescence phenomenon is common in biological systems, which might interfere with microrobot signals, limiting the microrobots' in vivo applicability. Furthermore, autofluorescence-based microrobots have a nontunable emission range, which is often blue or green. The microrobots are inappropriate for deep tissue imaging due to the low wavelength of the produced light. The majority of colors used in dye- based microrobots are harmful to biological systems and can induce adverse effects. Furthermore, dyes like fluorescein, cyanine, and rhodamine have limited use in multicolor detection and long-term imaging. [302] The complicated functionalization process of m-bots using QDs, as well as the cytotoxicity of QDs for in vivo applications, are also limitations for QD-based microrobots. Long-wavelength fluorescent probes for deep-tissue imaging and the creation of biocompatible/biodegradable fluorescent m-bots for in vivo applications might be the focus of future research on these m-bots.

5.2 MRI

MRI is a very efficient method for localizing magnetic m-bots both in vitro and in vivo with good contrast for m-bots directed by the magnetic field. [303] MRI imaging of m-bots has numerous benefits over conventional imaging techniques, particularly CT imaging. To begin

with, magnetic resonance imaging (MRI) does not expose biological systems to ionizing radiation. Second, 3D section imaging may be achieved using MRI without the need for reconstruction. Third, as compared to other imaging techniques, MRI provides greater imaging contrast for soft tissues such as the bladder, as well as higher imaging resolution than CT. In general, MRI uses two types of imaging modes: longitudinal relaxation (T1-recovery) and transverse relaxation (T2-recovery). The T1 contrast agents provide brilliant contrast, whereas the T2 contrast agents produce dark contrast. As T2 contrast agents, superparamagnetic nanoparticles, which are widely utilized in the production of magnetic m-bots, are commonly used. Increases in the concentration of contrast agents and the degree of magnetization can

improve the contrast (applied external magnetic field). Magnetic m-bots are a form of external field actuated m-bot that may be driven by the magnetic field itself or by other methods but guided by the magnetic field, as shown in the actuation section. Magnetic m-bots, both natural and artificial, have been described for MRI viewing. Magnetotactic bacteria can align their bodies with a magnetic field outside of them. Magnetotactic bacteria have a chain of magnetosomes inside their bodies that cause them to organize themselves in an ordered way along the external magnetic field. [304] Under MRI, Martel et al.[305-308] examined the motion behavior of magnetotactic bacteria and discovered that they travel via the microvasculature for guided treatment. The authors created a medical interventional system that used MRI as an imaging technique to feed back locational information to the controller, which is in charge of real-time navigation of magnetotactic bacteria and artificial m-bots along pre-planned paths in blood vessels to perform targeted delivery tasks. They also demonstrated that the MRI platform can be used to track polar magnetotactic bacterial robots that are used to load goods. [309, 310] They proposed that utilizing MRI as an imaging tool, steerable magnetotactic bacteria may be used to treat cancer and thrombosis. Apart from natural magnetotactic bacteria, manufactured magnetic m-bots with magnetic propulsion were also observed under MRI. [64, 311] Zhang et al.[64] created biohybrid magnetite helical microrobots that can be examined using MRI in vivo as well as localized with fluorescence imaging. The application range of the particular environment for in vivo imaging-guided treatment has been expanded thanks to the dual mode imaging of biohybrid microrobots.

5.3 Imaging in the US US imaging is a technique for tracking micro-nanorobots via imaging. In comparison to other imaging techniques, US imaging offers real-time control of m-bots with instantaneous picture feedback, no negative health effects, cheap diagnostic costs, no need for contrast agents, and a high imaging depth of 10 cm for human tissue. In the case of other clinical imaging methods, such as CT and MRI, micromolar to millimolar contrast agents may be necessary for the identification of the target site, which might result in biological system adverse effects. The benefits of US imaging have sparked a lot of attention because of their potential for tracking m-bots.

Sanchez et al.[312] described self-propelled microjets controlled by an ultrasonic system, with constant ejection of O2 bubbles coming from a catalytic reaction on the inner platinum surface in the presence of H2O2 affecting the microjets' navigation. The magnetic field dictated the direction of travel of the microjets, and their motion routes were recorded by tracking the position of the microbubbles created by the microjets using a microscope and US imaging.

These two imaging modalities' microjet trajectories all show that the microjet may locomote in a pretty accurate manner, following preprogrammed closed-loop paths. In compared to microscopic imaging, the location accuracy of US imaging feedback is slightly lower. Apart from self-propelled microrobots, which are generally tracked by indirect imaging of microbubbles,[312, 313] some other studies, such as those conducted by Khalil et al.,[314], Peng et al.,[315], and Zhang et al.,[316-318], demonstrated the US imaging of microrobots actuated by the magnetic field without any bubbles. In addition, Scheggi et al.[319] developed soft miniature untethered grippers that respond to temperature and execute grabbing and releasing with 2D flat and 3D folded shapes, respectively. The magnetic characteristics of the tiny grippers were achieved by fabricating them from materials containing 3% Fe2O3. US imaging was used to navigate the gripper, delivering input along both the step and sinusoidal paths. The soft miniature gripper was demonstrated to be capable of grasping the bead (500 m) and transporting it along the planned path to its destination.

5.4 Imaging using Radionuclides Another imaging technique that may be used for deep tissue examination and imaging is radionuclide imaging (RI). Whole-body imaging is possible using RI, which has a far wider scan range than other imaging methods. It also has the benefit of having a high sensitivity.

[320] Scintigraphy, PET, and SPECT are all frequent RIs. Exogenous agents, like as radionuclides, are generally required for RI. In SPECT, the beam is directly emitted, but in PET, the ray production is indirect. The resultant beam may enter biological tissues and be imaged in real time to follow its progress. Based on the encapsulation of positron or gamma emitters, the actuation of m-bots was proposed utilizing RI inspection to receive feedback. Vilela et al.[67] used PET-CT to track 124I-functionalized bubble-propelled microtubes produced by template-directed electrodeposition followed by metal evaporation. The 124I microrobots' radiolabeling method, which involves chemisorption on the gold surface, is critical for PET-CT imaging, broadening the use of such microrobots from in vitro to in vivo. The authors utilized linear phantoms to show how PET coupled with X-ray CT could monitor the microtubes' position. The study looked into using widely accessible medical imaging tools to follow m-bots directly, making clinical microrobots a possibility in the near future.

RI has two major drawbacks: despite several safety restrictions, ionizing radiation exposure with X-rays and radionuclides is unavoidable. It is necessary to establish the appropriate dose that balances imaging performance with patient safety. [321] The study of m-bot propulsion using RI-based tracking is still in its early stages and has a long way to go. Other imaging technologies, such as PACT[322] and clinical optical coherence tomography (OCT)[345], are also incorporated for the in vivo tracking of the m-bots in addition to the above-mentioned imaging approaches. Gao et al.[322] created a type of m-bot capsule that can be monitored in real-time through the GI tract of a mouse using PACT, with high-resolution and rapid imaging speed. The PACT may be a promising option for the localization and tracking of m-bots inside tissue and organs with a depth less than 7 cm because to the restricted penetration depth of NIR.

The actuation of m-bots utilizing optical microscopic tracking for simultaneous feedback is an inexpensive alternative for in vitro use as well as nonbiomedical applications. When used in

vivo, however, the optical microscope is unable to penetrate the tissue and follow the bots. As a result, alternative medical and clinical imaging modalities that are appropriate for in vivo inspections must be utilized. Although research on micro-nanorobot imaging has developed tremendously, the area is still in its early stages. Researchers may need to focus more on this aspect of m-bot research than on the other aspects, such as design, functionalization, and actuation, because it is critical in realizing in vivo applications of the technology. Micro/nanorobot uses in vitro are extensively established, and a greater understanding of microrobot imaging will aid in the development of in vivo applications.

6 m-Bots Have Biomedical Applications

Despite the fact that a significant number of micro/nanomachines have been suggested in the last two decades, and their applications in diverse environments have been investigated, the in vivo usage of such devices has gotten little attention until recently. The gap between research and practical in vivo use of these technologies is steadily closing thanks to extensive study in this field. Micro-/nanomachines used in in vivo applications include those that have been used in real-life situations as well as those that have been studied in vitro and show promise for in vivo applications. In vivo applications such as diagnostics, cell isolation, guided cell growth, targeted delivery, and thrombus ablation can be addressed by combining these aspects ingeniously based on the above discussions regarding the design, functionalization, actuation, and localization of micro-/nanomachines. The majority of current micro/nano robotic systems for in vivo applications are used in organs/tissues.

6.1 Diagnosis, Isolation, and Cell Growth Guidance M-bots can be used for DNA/RNA sensing, biomacromolecule isolation and detection, and cell and bacteria isolation from biological samples. Diagnostics, isolation, and biosensing applications all require the functionalization of m-bots in this area. [225, 323-327] M-bots can capture, transport, and release biomacromolecules and cells for isolation purposes based on donor–receptor interactions. [328-332] Concanavalin With polysaccharides, lectins have a strong coupling effect. As previously stated, concanavalin-equipped microrobots Because of the polysaccharides on the cell surface, A lectin bioreceptors were effective in recognizing and isolating E. coli. [126] Boronic acid interacts strongly with monosaccharides. Wang et al.[134] proposed a boronic acid-functionalized microrobot that can recognize and bond with the monosaccharide on the yeast cell, allowing yeast cells to be transported in media. The anti-carcinoembryonic antigen monoclonal antibody (mAb) and the carcinoembryonic antigen are another example of donor–receptor interactions (CEA). In 95% of colorectal, gastric, and pancreatic cancers, the antigen CEA is overexpressed. Zhang and Wang et al.[57] developed an in vitro strategy for cancer cell isolation using mAb-grafted microrobots to selectively bind and transport cancer cells. They also proposed ultrasound-actuated nanorobots made of gold nanowires cloaked in a mix of red blood cell and platelet membranes[333]. The nanorobots are endowed with some appealing biological capabilities, such as adhesion and anchoring to Staphylococcus aureus bacteria and neutralization of -toxins, thanks to the intrinsic functional proteins on the hybrid membranes. The nanorobots that were actuated by ultrasound moved like naturally occurring motile cells and were nonadhesive to blood vessels. These bots showed a lot of promise in terms of applications like biological threat isolation and removal for detoxification.

The micro-/nanomotors can also be used for sensing and diagnosis. MiRNA is a well-known biomarker that can be used for both diagnosis and treatment. The detection of intracellular miRNA is important in clinical diagnosis because abnormal miRNA expression is an indicator of many conditions. In 80 percent of tumor tissues, for example, miRNA-21 is found to be overexpressed. Zhang and Wang et al.[334] developed a nanomotor-based strategy for intracellular biosensing and detection of the miRNA-21, which is expressed at the single cell level in intact cancer cells. A gold nanorod wrapped the DNA (ssDNA)/graphene-oxide (GO), which shows a quenched fluorescence signal due to the – interaction between GO and dye-labeled ssDNA. The ssDNA@GO-coated gold nanowires can be actuated to penetrate intact cancer cells using an ultrasound field. Because the dye-ssDNA was replaced by miRNA-21, the internalized nanomotor in cancer cells showed an intracellular "OFF–ON" fluorescence switching. The authors used cancer cell lines, such as MCF-7 and HeLa cells, to show that miRNA-21 can be detected intracellularly at the single-cell level. According to previous research[335], the level of expression in MCF-7 cells is much higher than in HeLa cells. The authors then propelled the ssDNA@GO-modified AuNWs with US field under incubation (10 min) and discovered that the fluorescence intensity of the MCF-7 cells is 44 times higher than that of the HeLa cells, proving the accuracy of their strategy for miRNA detection in different cell types. Such micromotor-based sensing suggests the possibility of real-time intracellular miRNA expression monitoring and cancer diagnosis. Berns also achieved notable microrobot-guided cell growth in addition to cell transportation and release. [336] Using a light-actuated spinning microsphere, the authors developed an optics-based system to guide and control the direction of individual nerve fiber growth. The rotating micromotor generated a localized microfluidic flow with a shearing force of 0.17 pN, allowing the nerve cells' growth direction to be controlled. Another recent discovery shows that the delivered micromotors can also induce neural stem-like cell differentiation by converting ultrasonic energy to an electrical signal in situ via the piezoelectric effect. [337] These micromotor-guided cell growth and differentiation could lead to axon regeneration in vivo, which could help with brain and spinal cord repair.

6.2 MicrosurgicalProcedures Minimally invasive surgery (MIS) is traditionally performed by attaching a tethered tool to the targeted site in the body, which is usually equipped with a light source, tiny cameras, and mechanical grasping, cutting, and suturing devices. [338] However, getting the tethered tool to the targeted area is difficult, and because the tools aren't collapsible, they can't be used on tissues with small interspaces. To address the drawbacks of tethered MIS tools, several companies have developed and commercialized wireless capsules that include light sources, tiny cameras, batteries, a radio transmitter, and an antenna. [339] These tetherless capsular devices are centimeter-sized and may be incompatible with small vessels and channels in the body. Furthermore, most commercial capsules with no external moving parts are only suitable for imaging and sensing, with mechanical operations being difficult to achieve. The well- developed field of remotely actuated micro-nanomachines filled a gap in the microscale world for deep-tissue therapy by achieving mechanical motion and operations at the microscale.

Tetherless cell microgrippers made of biocompatible and bioresorbable silicon monoxide and silicon dioxide were created by Gracias et al.[340] for gripping single cells. The release of residual tension allows the cell to grab without being tethered. They also suggested a photolithographically manufactured tetherless thermo-biochemically actuated microgripper with magnetic characteristics. The microgripper was hundreds of micrometers in size and traveled magnetically to regions of the body that tethered or wireless-capsule devices couldn't reach. The microrobots' thermo-responsive polymer hinges rendered them stiff at low temperatures, keeping the star-fish-like tool flat and open, yet softened and closed to grab the tissue when the temperature was increased to body temperature. Following that, the microgrippers' in vivo MIS was achieved by a delivery procedure using a catheter and a camera for localisation. The authors[341] demonstrated that the microgrippers could extract tissue samples from live pigs' actual organs and hard-to-reach areas. A magnetic catheter tip could collect up to 95% of microgrippers containing the acquired tissues. The complete recovery of the microgrippers and the removal of the cytotoxicity of such micromachines may be the subject of future study. Furthermore, the more efficient localization techniques might be used to guide microrobots in real time, replacing catheter and camera-based imaging systems with restricted imaging scope and resolution. Magnetic microrobot-based MIS might potentially be utilized to treat eye diseases. Because the vitreous body of the eyes is transparent, real-time microrobot localisation in vivo may be accomplished very easily with an optical microscope. Nelson et al.[342-344] suggested an invasive, wirelessly controlled, and powered microrobot for use in ophthalmology. Magnetic materials such as CoNi with an Au and PPy coating are used to make these microrobots. These may be injected into the eye without sutures, coupled with a hyaluronic acid solution, and precisely guided by a five-degree-of-freedom external remote magnetic field. [343] Microrobots can be accurately guided to regions of the eyes that are difficult to reach with conventional instruments. For the procedure, the microrobots may arrive in the posterior section of the vitreous cavity, near to the retina. However, depending on the implantation time of the microrobots, long-term implantation of the microrobots may cause optic nerve inflammation and irreversible detachment of the retina. Inflammation was verified by histopathologic investigations. Fischer et al.[345] recently created a swarm of slick micropropellers that can be magnetically activated to pierce the vitreous body (a dense macromolecular matrix) and reach the retina following surface functionalization with a perfluorocarbon coating to reduce drag. These findings showed that microrobots might be a good fit for next-generation MIS for the eye, as well as targeted delivery and diagnostic procedures by implantation in the posterior portion of the eye. Microrobots, on the other hand, may not be ideal for long-term implantation and should be withdrawn as soon as possible after therapy since tissues in the eye are generally more sensitive than those in the surrounding area.

6.3 Targeted Treatment Micro- and nanoparticle-based medication delivery methods have been intensively studied during the last few decades. [346-354] Micro- and nanoparticles provide several advantages in drug administration, including a regulated release rate, a large loading capacity, and quick renal clearance. [355-361] Nanoparticle-based drug delivery, on the other hand, frequently relies on the circulatory system and lacks adequate drug targeting and barrier penetration for highly localized therapeutic drug delivery. To improve the targeting

capabilities of passive particles, they may need to be functionalized. Active matter and m-bots that may be actuated autonomously or pushed by external fields offer distinct benefits in directed/targeted medication delivery from single cells to local tissue/organ delivery in this regard. [362-367] M-bots have been used to deliver targeted treatment to several sick regions via the peroral route (for the gastrointestinal tract) or intravenous injection (via the circulatory system). [368-370]

6.3.1 Gastrointestinal Disease Drug Delivery Mg- and Zn-based m-bots are the most promising of all the m-bots for peroral medication delivery. Mg/Pt-PNIPAM Janus microrobots were proposed by Guan et al.[55] for the effective absorption, transport, and temperature-controlled release of medicines. The microrobots' autonomous mobility allows for targeting, while the thermoresponsive PNIPAM hydrogel layers allow for on-demand release. Wang et al.[81] created an enteric microrobot with a magnesium core and an enteric polymer covering on the outside. The Mg core permits autonomous propulsion in intestinal fluid, while the enteric polymer covering keeps the microrobots from being dissolved in the stomach's acidic environment until they reach the nonacidic environment of the intestines. For site-specific delivery, the suggested microrobots can achieve optimal biodistribution and improved retention in the gastrointestinal system. The active administration of antigens for oral vaccination might be achieved using this technique.

[371] When mice were given the micromotor toxoid orally, the micromotors reached the stomach, where the enteric coating prevented the motors from degrading in the low pH environment. Within the digestive tract, antigenic material retention and uptake improved. Wang et al.[372] also discovered Mg-based microrobots that can neutralize stomach acids autonomously and temporally by depleting protons through the self-propulsion of Mg Janus particles in the gastric fluid. As a result of the local pH shift produced by Mg neutralization, the medicines placed within the surface of the pH-responsive polymer layers are autonomously released. The stomachs of mice treated with Mg microrobots (5 mg) had uniformly distributed fluorescence intensity created from the medicines loaded across the whole stomach, showing that the microrobots could actively adapt to the stomach environment and dissolve the pH-sensitive polymer for drug release. Because the PS microparticles could not modify the stomach pH and initiate the breakdown of the pH-responsive polymer for drug release, the fluorescence intensity was as low as the DI water control group. They[373] combined the active Mg-based micromotors with other inactive excipients and disintegration-aid additives from a pharmaceutical strategy to create micromotor tablets that comprised the encapsulated micromotors in the pill matrix with other inactive excipients and disintegration-aid additives. The micromotor pill platform efficiently shielded and transported the active micromotors to the stomach, according to the in vivo investigation. The micromotors with the loaded cargo were able to drive efficiently in the stomach fluid and release the medicines in a concentrated way after being freed from the pill matrix. When compared to the DI water, fluorescent silica pill, and free fluorescent Mg/TiO2/PLGA/chitosan micromotor groups, the fluorescent Mg/TiO2/PLGA/chitosan micromotor pill produced the strongest signal, indicating that the micromotor pill improved the transportation, dynamic release, and retention of the micromotors in the mouse stomach. These findings suggested that combining conventional tablets with active micromotors might be a promising method for in vivo motor-based medication

administration. Due to their biodegradable nature, which creates harmless by-products and does not need the microrobots to be separated after the delivery process, Mg and Zn based microrobots[374] show high promise for functioning as active drug delivery carriers. They also created in vivo therapeutic microrobots for active drug administration in a mouse model for the treatment of a stomach bacterial infection, using clarithromycin as the model antibiotic for the simulated Helicobacter pylori illness. The actuation of drug-loaded Mg-based microrobots in the gastric medium with a substantial reduction in the bacteria burden in the mouse stomach indicated efficient antibiotic delivery when compared to passive drug carriers.

6.3.2 Drug Delivery for Cancer Treatment Tumor-targeted treatment has sparked a lot of interest in the scientific community. [47, 376-382] [47, 376-382] [47, 376-382] Using various magnetic fields, Pané et al.[382] produced FeGa@P(VDF-TrFE) core–shell magnetoelectric nanowires that can be pushed for targeted drug delivery. Melt-wetting of the AAO template is followed by FeGa electrodeposition to create FeGa@P(VDF-TrFE) core–shell nanowires, resulting in P(VDF- TrFE) nanotubes. The anticancer medication paclitaxel may be loaded onto the nanowires after surface functionalization with polydopamine. After applying alternating magnetic fields to create the magnetoelectric effect, the loaded medication can be released for on-demand killing of cancer cells. The actuated m-bots may potentially be used to provide intensive drug release. Sánchez et al.[376] created urease-modified mesoporous silica-based core–shell nanorobots that were actuated by enzyme catalysis for active drug transport and delivery to the target locations. After 6 hours of treatment, the nanorobots achieve a four-fold increase in medication release compared to their passive counterparts. Because of the increased drug release kinetics and ammonia generation caused by the catalytic breakdown of urea, the authors discovered that active DOX-loaded nanorobots have a greater effect on HeLa cells than passive carriers. Bladder cancer is also being treated using a similar method. [381] The elevated urea content in the bladder serves as the fuel for the nanomotors' self-actuation. In comparison to most self- propelled m-bots, the new m-bots that use enzymes as catalysts have a lot more in-vivo application potential.

6.3.3 Cell Delivery with a Specific Purpose The microrobots were also able to transport different cells to specific locations. [67, 74, 383, 384] Choi et al.[111] used a 3D direct laser writing approach to show tailored cell delivery. Magnetic and biocompatible characteristics were added to the cage-like microrobots by coating them with Ni and Ti layers. They discovered that the HEK 293 cells quickly attached, moved, and proliferated across the scaffold of the 3D microrobots after being grown in 3D. An external magnetic field gradient can be used to push microrobots loaded with cells. The suggested microrobots can be used for in vivo cell micromanipulation using targeted microrobots.

Sun et al.[116] suggested a biocompatible burr-like microrobot that can easily load cells onto a 3D framework through 3D cell culture and release them at the required location. Subcutaneous injection in the dorsum of nude mice with FI tracking confirmed the in vivo release of stem cells by these types of microrobots.

6.4 Thrombus Ablation Blood clots obstructing blood arteries is one of the main causes of mortality worldwide. [382, 385] Thrombosis can occur in a variety of places, and depending

on where the thrombus forms, it can result in ischemic stroke, coronary infarction, pulmonary embolism, and other complications. For thrombus ablation, two methods have been developed: thrombectomy and thrombolysis. Thrombectomy is a procedure that includes manually removing a thrombus from a blood artery using a catheter. It does, however, have a number of contraindications, and the catheter may not be able to reach arteries of tiny diameter. Thrombolysis is another drug-induced lysis method for eliminating blood clots. The US Food and Drug Administration has authorized tissue plasminogen activator (tPA) as one of the thrombolysis medicines (FDA). It can accelerate the conversion of plasminogen to plasmin, and the plasmin produced can break up thrombus by binding to fibrin on blood cells. Thrombolysis is usually done by an intravenous injection, and the dosage of tPA should be kept under 0.9 mg kg1 to avoid potential adverse effects including internal bleeding. The carriers' ability to deliver tPA quickly and effectively after the development of thrombosis is critical for therapy. Researchers have pushed the use of m-bots in the effective elimination of thrombus in recent years due to the rapid growth of the field of m-bots. [132, 385-391] With the use of thrombolytic medicines, macroscale helical robots with lengths in the centimeter range were used to drill holes into the blood clot via mechanical rubbing. Sitti et al.[386] confirmed and replicated the findings in an in vitro model, finding that mechanical rubbing with helical robots resulted in a clearance rate three times higher than chemical lysis using streptokinase. Furthermore, ultrasonography guidance can be used to follow the removal process. Zhao et al.[385] created active nickel nanorods to guide and improve tPA-mediated thrombolysis directly. The findings showed that employing magnetic-field induced hydrodynamic convection, nanorobots can improve tPA thrombolysis speed. The in vivo trial on a mouse also confirms the efficacy of thrombolysis. Engelhard and colleagues[388] used a revolving permanent magnet to spin and translate iron oxide NPs inside the vessel, allowing medicines to be delivered to the thrombus location in a stationary flow. Hest et al.[389] built erythrocyte membrane-cloaked Janus polymeric microrobots for thrombus ablation that were operated by near-infrared (NIR) laser light. Because of the asymmetric distribution of Au on the surface of these biodegradable and biocompatible microrobots, they can generate a local thermal gradient under NIR irradiation, causing “on/off” controlled motion of the microrobots via the self-thermophoresis effect generated by the control of the irradiation source. In thrombolysis photothermal treatment, therapeutic microrobots performed well. Due to the locally enhanced flow impact, nanorobot-guided treatment may need fewer dosages of tPA than standard techniques that entail intravenous administration of the medication. [392] As a result, the risk of tPA side effects may be decreased to some level. In addition, the m-bots can create a thrombin-inhibiting layer that prevents the thrombus from regenerating.

6.5 Wound Regeneration A wound is an injury to the body that can be caused by abrasions, lacerations, punctures, avulsions, or animal bites and can be caused by a variety of external factors such as accidents, aggression, or surgery. It generally includes the organ skin breaking and perhaps causing harm to the underlying tissues. The majority of people will have an open wound at some point in their lives. It is critical to treat wounds properly since improper treatment raises the risk of bacterial infection. Minor wounds can be treated at home, but larger or deeper wounds should be treated by a doctor. The hemostasis phase, inflammation phase,

proliferation phase, and remodeling phase are the four stages of wound healing that may be distinguished. Skin glue, medical sutures, laser welding, and other methods are commonly used to seal and treat open wounds. However, these techniques are either intrusive or need a considerable period of rehabilitation following therapy, affecting the patient's everyday activities. Topical medication delivery for wound healing has also been explored using nanoparticles, nano scaffolds, and other nanomaterials. [393-395] The outward flow of blood, however, makes passive administration of medicines into injured deep tissue during bleeding difficult. The creation of m-bots has provided a novel method for enhancing healing despite external blood flow. When wounds are treated using micro-/nanomotors, the blood clotting velocity is lowered, and the wound healing rate is increased, compared to traditional treatment procedures. Furthermore, unlike traditional sutures, nanomotor-based healing methods are less intrusive and do not cause additional damage. [358]

Kastrup et al.[359] suggested self-propelled micromotors composed of carbonate and tranexamic acid that can move against the blood flow and traverse through aqueous solutions at a maximum speed of 1.5 cm s1. The increased delivery of the coagulation enzyme and the staunching of severe bleeding were verified using three animal models: mouse liver, mouse tail, and pig femoral artery. When the micromotors were loaded with thrombin, they were able to penetrate deeply into the wound, decrease blood loss, and shorten the bleeding period. The device has a wide range of therapeutic applications and might be used to treat postpartum hemorrhage, which is one of the leading causes of mother death during childbirth. He et al.[360] formed high-controllability magnetic field- and NIR-actuated Janus micromotors for wound repair and hemostasis using laser beam irradiation. As the wound's temperature rose, the collagen fiber melted, and as the temperature dropped, a collagen film formed, closing the open wound. The enhanced, laser-controlled stopping of bleeding was demonstrated using micromotor aided wound welding on beef liver, beef flesh, and chicken meat.

In the case of severe and large trauma, such as postpartum bleeding and war wounds, active micromotor-based hemorrhage stopping is critical. Exsanguination puts the patient at danger of hypovolemic shock and death. Fast-acting active administration of the hemostatic substance to the deep, difficult-to-reach lesion using micro-/nanomotors enhances the chances of saving the patient's life.

6.6 Swarming m-Bot Biomedical Applications The m-bots' swarming behavior not only broadens our understanding of self-assembly behavior in natural living systems, as discussed in Section 4.7, but also highlights the potential for applications such as multiple cargo manipulation,[273, 396, 397] antidiffusion by swarming m-bots,[287] enhanced delivery capability,[398] reconfigurable adjustment of the energy delivery [401] The in vivo swarming behavior of biological and artificial m-bots has been researched and explored, and it has been confirmed that the collective motion behavior of micro-/nanoagents may typically achieve improved medication and energy delivery. Martel et al.[398] investigated the magnetoaerotactic and collective migration behavior of magnetotactic bacteria (MC-1) and applied this to the transportation of drug-loaded nanoliposomes into the hypoxic regions of the tumor for cancer therapy by combining the enrichment of MC-1 in oxygen-depleted hypoxic regions of the tumor with magnetic field guidance. Peritumoral injection of MC-1 into a tumor

xenograft in mice revealed that directional magnetic fields can significantly concentrate MC-1 in the tumor's core hypoxic area. At 4 and 6 cm, which is at the heart of the tumor, the MC-1 stained with antirabbit FITC- labeled secondary antibodies has a greater density than at the border region. A directional magnetic field can be used to anchor multiple liposomes with medication loaded in them to the MC-1's surface for targeting to the tumor core. With this type of collective motion by magnetic field, the targeting ratio in the tumor may reach up to 55 percent, according to the findings. The findings showed that swarms of microorganisms with magnetoaerotactic activity may effectively increase the therapeutic index of different nanocarriers in hypoxic tumor areas. Zhang et al.[399] devised a new method for achieving an adjustable energy dose by combining a reconfigurable swarm of ferromagnetic particles with a radio-frequency alternating magnetic field and remote and nonintrusive magnetic field management. The benefit of this technique is that the initial particle dosage may be kept low to prevent toxicity to the surrounding cells and tissue. Under the influence of a magnetic field, the local particle concentration may be adjusted throughout a large range. After the alternating magnetic field treatment, the shrinkage of the particle swarm pattern would result in a larger localized temperature rise, whilst the swelling of the particle swarm pattern would result in a lower temperature rise. Furthermore, the swarm pattern movement of the particles may be readily regulated for targeted energy distribution to specific locations. With a relatively low starting particle dosage, the magnetic particle swarm offers a novel way to achieve improved targeted tumor therapy. Wang et al.[401] explored ultrasonic-driven axial propulsion and spinning of the Au rod within live HeLa cells without the need of chemical fuels. A cluster of Au rods may be used to align the cells in an ultrasonic field, and the internalized Au rods demonstrated active motion in the cells. The scientists speculated that nanomotors with ultrasonic propulsion might provide a novel tool for mechanical stimulation and intracellular organelle manipulation to stimulate live cells.

7 In-depth analysis of state-of-the-art in-vitro applications In vivo applications of micro-/nanomachines, such as microsurgery, medication administration, cell transport, thrombus ablation, and wound healing, have so far shown early effectiveness in animal models such as mouse, rat, and pig organs and tissues. Three types of propulsion modes, magnetic field, bubbles, and light field, are highly common and favored for the steering and actuation of micro-/nanomachines in real-world applications. Other means of propulsion are still in the in vitro and ex vivo stages of research. Magnetic field-based propulsion and bubble- based propulsion can be used on both superficial and deep tissues, however light field propulsion is exclusively used for superficial treatments like wound healing. In terms of imaging in vivo therapy methods with micro-/nanomachines, researchers have previously used endoscopy, FI, and MRI to pinpoint the location of the small robots and the nidus. For diverse purposes, several imaging methods are generally utilized. Endoscopy may be used to image micro/nanomachines in vivo ducts, and FI is a strong option for high-resolution superficial localisation of tiny robots. For deep tissue imaging, MRI is a good localization approach. Micro/nanomachine distribution methods usually include catheter delivery from a mouse, oral administration, IV injection, topical application, and in situ injection. After treatment, the majority of real-world applications did not go through a post-clearance process. However, following treatment, the bubble-propelled micro/nanomotors

based on Mg and CaCO3 progressively dissolved in the stomach and wound site with low cytotoxicity, requiring no additional removal procedures. Other proposed uses for micro-/nanomachines, such as hyperthermia, micromotor guided brain healing, infertility, and cancer detection, have not effectively translated the in vitro and ex vivo models to the corresponding in vivo instances. The magnetic field is still the most prevalent propulsion method, and US propulsion is becoming significant for in vitro and ex vivo applications. We expect the US propulsion to be effectively utilized for in vivo instances in the near future. The potential and relevance of each of these propulsion modes may be rated based on how frequently they are used in in vivo applications: US field > magnetic field > light field > bubble Because the attractive force between the magnetic m-bots and the magnetic field is inversely proportional to no less than the fourth power of the distance, the magnetic propulsion system's short effective actuation range may pose a significant challenge for translating these methods from the lab to human clinical cases. In terms of m-bot localization, the BF is the most commonly utilized imaging mode for in vitro and ex vivo experiments, whereas the FI is the most generally used approach for real in vivo testing. For in vitro/ex vivo applications, BF, FI, and US imaging are also being developed.

Self-propelled micromotors made of carbonate and tranexamic acid, according to Kastrup et al.[359], can move against blood flow and traverse aqueous solutions at a maximum speed of 1.5 cm s1. Three animal models were used to verify the enhanced delivery of the coagulation enzyme and the stoppage of severe bleeding: mouse liver, mouse tail, and pig femoral artery. When the micromotors were loaded with thrombin, they were able to penetrate deeply into the incision, reducing blood loss and cutting down on bleeding time. The gadget might be utilized to treat postpartum hemorrhage, which is one of the main causes of maternal mortality during childbirth.

He et al.[360] used laser beam irradiation to produce high-controllability magnetic field and NIR-actuated Janus micromotors for wound healing and hemostasis. The collagen fiber melted as the wound's temperature rose, and a collagen film developed when the temperature decreased, sealing the open wound. Micromotor-assisted wound welding was used to demonstrate improved, laser-controlled bleeding stopping on beef liver, beef flesh, and chicken meat.

Active micromotor-based hemorrhage stopping is important in the case of severe and massive trauma, such as postpartum bleeding and battle wounds. The patient is at risk of hypovolemic shock and mortality as a result of exsanguination. The use of micro-/nanomotors to deliver a fast-acting active hemostatic material to a deep, difficult-to-reach lesion improves the odds of saving the patient's life. Biomedical Applications of Swarming m-Bots Swarming behavior of m-bots not only expands our understanding of self-assembly behavior in natural living systems, as discussed in Section 4.7, but also highlights the potential for applications such as multiple cargo manipulation,[273, 396, 397] antidiffusion by swarming m-bots,[287] enhanced delivery capability,[398] reconfigurable adjustment of the energy delivery system.[401] The in vivo swarming behavior of biological and artificial m-bots has been studied, and it has been proven that the collective motion behavior of micro-/nanoagents may generally enhance medicine and energy distribution. By combining the

enrichment of MC-1 in oxygen-depleted hypoxic regions of the tumor with magnetic field guidance, Martel et al.[398] investigated the magnetoaerotactic and collective migration behavior of magnetotactic bacteria (MC-1) and applied this to the transportation of drug-loaded nanoliposomes into hypoxic regions of the tumor for cancer therapy. The use of directional magnetic fields to concentrate MC-1 in the tumor's core hypoxic region was discovered after peritumoral injection of MC-1 into a tumor xenograft in mice. The MC-1 stained with antirabbit FITC-labeled secondary antibodies shows a higher density at 4 and 6 cm, which is at the core of the tumor, than at the border area. Multiple liposomes with medicine loaded in them can be anchored to the MC-1's surface using a directed magnetic field for targeting to the tumor core. According to the findings, the targeting ratio in the tumor may reach up to 55 percent with this sort of collective motion by magnetic field. Swarms of microorganisms with magnetoaerotactic activity were shown to successfully improve the therapeutic index of several nanocarriers in hypoxic tumor regions. By combining a reconfigurable swarm of ferromagnetic particles with a radio-frequency alternating magnetic field and remote and nonintrusive magnetic field control, Zhang et al.[399] established a new technique for generating a changeable energy dosage. This method has the advantage of allowing the initial particle dosage to be maintained low in order to avoid toxicity to the surrounding cells and tissue. The local particle concentration may be changed across a wide range when a magnetic field is applied. The shrinking of the particle swarm pattern after the alternating magnetic field treatment would result in a higher localized temperature rise, whereas the swelling of the particle swarm pattern would result in a smaller temperature rise. Furthermore, the particle swarm pattern may be easily controlled for targeted energy distribution to specified areas. The magnetic particle swarm is a unique approach to provide enhanced targeted tumor treatment with a relatively modest beginning particle dose. Without the use of chemical fuels, Wang et al.[401] investigated ultrasonic-driven axial propulsion and spinning of the Au rod within living HeLa cells. In an ultrasonic field, a cluster

of Au rods could be utilized to align the cells, and the internalized Au rods showed active motion in the cells. Nanomotors with ultrasonic propulsion, the researchers hypothesized, may provide a unique tool for mechanical stimulation and intracellular organelle manipulation in living cells.

7 In-depth examination of cutting-edge in-vitro applications Microsurgery, medicine delivery, cell transport, thrombus ablation, and wound healing are just a few of the in vivo uses of micro-/nanomachines that have showed early promise in animal models including mouse, rat, and pig organs and tissues. In real-world applications, three types of propulsion modes, magnetic field, bubbles, and light field, are widely used and preferred for steering and actuation of micro-/nanomachines. Other propulsion methods are still in the in vitro and ex vivo stages of development. Although magnetic field and bubble-based propulsion may be utilized on both superficial and deep tissues, light field propulsion is only employed for superficial therapies such as wound healing. Researchers have previously employed endoscopy, FI, and MRI to locate the site of the small robots and the nidus while imaging in vivo treatment techniques with micro-/nanomachines. Several imaging techniques are often used for various reasons. Endoscopy may be utilized to scan micro/nanomachines in vivo ducts,

and FI is a viable alternative for high-resolution superficial robot localization. MRI is a useful method of localisation for deep tissue imaging. Catheter delivery from a mouse, oral administration, IV injection, topical treatment, and in situ injection are common micro/nanomachine distribution techniques. The majority of real-world applications did not go through a post-clearance process after treatment. The bubble-propelled micro/nanomotors based on Mg and CaCO3 gradually dissolved in the stomach and wound site with low cytotoxicity after treatment, needing no further removal operations. Other suggested applications for micro-/nanomachines, such as hyperthermia, micromotor assisted brain repair, infertility, and cancer detection, have failed to successfully convert in vitro and ex vivo models to in vivo scenarios. The magnetic field is still the most common propulsion method, although US propulsion is gaining traction in in vitro and ex vivo studies. In the near future, we expect US propulsion to be successfully used in in vivo scenarios. Each of these propulsion modes' potential and significance may be assessed based on how frequently they are utilized in in vivo applications: field in the US > magnetic field in the US > light field in the US > bubble in the US The magnetic propulsion system's short effective actuation range may pose a significant challenge for translating these methods from the lab to human clinical cases because the attractive force between the magnetic m-bots and the magnetic field is inversely proportional to no less than the fourth power of the distance. The BF is the most often used imaging mode for in vitro and ex vivo investigations, whereas the FI is the most commonly used technique for genuine in vivo testing. BF, FI, and US imaging are also being developed for in vitro/ex vivo applications.

8 Summary

M-bots are small robots that can be actuated and localized inside the human body to aid in illness detection and treatment. Nanomaterials provide a link between robotics and nanomedicine through applications that allow for the on-demand execution of tasks via remote control via a programmable energy input. Microrobots are typically less than 1 mm in size, whereas nanorobots M-bots are less than 1 m in size, in contrast to larger regular robots.M-bots can reach complicated and restricted locations, such as the distal brain arteries and the bile duct, that are often inaccessible with existing minimally invasive medical devices and traditional robots. Micro- and nanoscale robots can be controlled by external fields for steering and propulsion. The ability to locate m-bots in real time, especially in vivo, is critical for biomedical applications. M-bots have a variety of practical applications, including cargo manipulation, environmental remediation, and targeted therapy. Biocompatibility and therapeutic efficacy rely heavily on surface functionalization. To provide the surface of the m-bot with particular polymers, proteins, and quantum dots (QDs), certain functionalization techniques based on both physical absorption and chemical grafting should be selected. The chemistry and geometry of the m-bots determine their design.

The cargo/molecules physically attached or chemically conjugated on the surface of the bots determine their functionalization. The bots may be driven by utilizing magnetic fields, ultrasonic fields, light, electric fields, fuel, heat, and the Marangoni effect. There are hybrid M-

bots as well as those that are propelled by a collective activity.The localisation of the m-bot in vivo is being explored based on the medical imaging techniques used. Diagnostics, isolation and cell growth, targeted therapy using peroral and injectable methods, thrombus ablation, and other bioapplications have been evaluated. Li et al. created a magnetic nanorobot with two soft connecting arms that can swim "freestyle" in low Reynolds number liquids. The degrees of freedom of these soft robots are far higher than those of rigid robots. Magnetic materials such as Fe3O4, Ni, -Fe2O3, and FePt [28, 45, 54, 103] should be studied for magnetically operated m-bots. To achieve asymmetric bubble propulsion for a bubble-propelled m-bot in a fuel-rich environment, catalytic materials may be required. The size of the nanorobots may be a key factor in biomedical applications. Biological barriers such as the blood-brain barrier, vascular endothelial barrier, and glomerulus filters prohibit m-bots of particular sizes from passing through. The size of the M-bot's location can have an effect on its location and movements when controlled remotely.M-bot functionalization is a critical step in providing them with additional capabilities that allow them to do activities other than navigation. M-bots can be used to improve biocompatibility and prevent the immune system from mistaking them for foreign substances and attacking them. The actuation of m-bots is simply energy conversion, which converts different types of energy into kinetic energy, such as magnetic energy, electric energy, light energy, and chemical energy.

M-bots' kinetic energy may be utilized to create a variety of motion patterns that could lead to new applications. M-bots that are controlled and guided by external magnetic fields have a lot of potential for in-vivo applications. They can convert magnetic energy into mechanical energy in the form of magnetic field gradients and magnetic torque, allowing them to perform tasks such as cargo handling and transportation, targeted/directed treatment, and environmental cleanup. Using the GLAD method, Fischer et al. created a nanoscale helical swimmer with a diameter of just 200–300 nm and a length of only 1–2 m. It is the world's smallest helical robot, capable of reaching speeds of up to 40 m s 1 when precisely controlled by a magnetic field.In practice, magnetic micro/nanoagents may not be in a suspended condition and may interact with the restricted space limits. Ultrasonic field-related actuation may be used for a variety of m-bots, including metallic nanowire and tubular microagent propulsion, microbead rotation, and nanoparticle patterning. This form of magnetic motion demonstrates its universality in terms of a wide range of magnetic objects and the application of different surface topographies.

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