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REVIEW

Micro(bio)robotics: design and applications

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Abstract
Microrobots are motile microsystems constructed using physical, chemical and biological components for operations with respect to definite applications. In the present review, we have discussed the various aspects of microbiorobots, their history, and design. While designing a microrobot, two critical parameters (and their varities)- actuation and sensing affect the different micromanipulation techniques to be employed (Magnetic, Optical, Electric, fluidic, or acoustic). The controlling and actuation system (Vision-based or Force-sensing) selected for the specific application can dictate the fabrication type to be used for manufacture the microrobot. The type of propulsion systems, Powering system, and mobility in a complex environment, and applicability of the microrobot further influence the controlling parameters. Presently, microbiorobotics have applications in biomedical and environmental engineering. In this review, we have analyzed various aspects of microbiorobot design, fabrication, and applications that can help future works in nanosciences and microbiorobotics.

Keywords Biomedical applications · Fabrication · Microrobot/s · And micromanipulation

1 Introduction

A microrobot is a motile microsystem arranged physically, chemically, and biologically to attain its actuation for a definite task. Engineering microrobots can incorporate ancillary robotic attributes such as sensing, mobility, and vision control [1]. There are three classifications of microrobots based on their construction and actuation sources: (i) Cellular microrobots consist of cellular components actuated biologically to evince an anticancer effect, (ii) Synthetic microrobots consist of an artificial material, pattern, and element which are actuated either physically or chemically and (iii) Bio-hybrid microrobots are composed of cellular and synthetic (artificial) components that is propelled conventionally using biological or synthetic systems [1, 2]. The chronology of microrobot research is summarized in Table 1.

The scope of this review lies in the challenges and limitation of existing methods in designing and constructing viable microrobots. When cellular microrobots are used for biomedical application, they face challenges such as ethical clearance, regulation of nutrient transport for survival, maintenance of cell lines, limited life span (in vivo), and complexity of operating them for the desired task. To overcome these challenges and drawbacks, microorganisms-based robots, that is, microrobots can be a helpful solution. They provide the advantage of being small in size (μm in range), thus, have on-board motility for their propulsion, survive in harsh conditions, and have easy maintenance compared to cellular microrobots. Using safe microorganisms (generally regarded as safe or GRAS) can enhance the applicability of microbes in this field. GRAS is defined as general safety recognized via scientific procedures depending upon the type of applications that are available and accepted in the form of scientific principles, methods, data, and that are published and approved by the Food, Drug, and Cosmetics Act (FDA) [3]. The microorganisms, or microbial products, are only GRAS if it is a general recognition based on the view of qualified experts to evaluate their safety measurements [4]. So, the present review shares details about the history of microrobots at different length scales, their design parameters, and their biomedical application for future exploration.

The miniature robots can also be classified based on their different length scale and functions as millirobots, microrobots, nanorobots, and mesorobots. Millirobots are mobile microsystems containing untethered motile components with

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Table 1 History of robots at the different length scales

Year	Milestone	Reference(s)
At the centimeter scale, significant milestones (Millirobots)		
1959	Feynman conceived the idea of miniature machines and their feasibility in his lecture on 'There is plenty of room at the bottom.'	[9]
1999	The development of a crawling millirobot with inbuilt computing and power	[5]
2000	Micromechanical flying insects robot	[10]
2001	Under FDA authorization, the first capsule endoscope for medical application was employed in clinical trials. The crawling mechanism and on board precise drug distribution have been introduced	[5]
2004	A solar- powered crawling robot	[11]
	Two explanted frog semitendinosus muscles are used to power a swimming appliance controlled by an embedded microcontroller	[12]
2005	Cardiomyocytes implanted on a micromechanical device made of silicon (walking bio-hybrid microrobot)	[13]
2007	Cardiomyocytes were linked to a polydimethylsiloxane (PDMS) framework to construct a swimming crab-like bio-hybrid robot	[14]
	Pattern alignment of a monolayer of Cardiomyocytes on PDMS thin film can be easily formed to synthesize a highly customized series of bio-hybrid microrobots	[15]
2008	Synthesis of a novel autonomous 2.4 g crawling hexapod (RoACH) millirobot with on-board actuators, power, and control having flexible mechanisms	[16]
2009	The self-contractile dorsal–ventral-tissue (DVT) was excised, cultured, and optimized to correspond to mammalian cardiac tissue in lepidopteran species (*Ctenoplusia agnate*) having actuators with piller-deflecting functions at room temperature stable for 90 days without medium replacement	[17]
2012	A bio-hybrid robot with similar muscle architecture and body shape to a Jelly fish was constructed utilizing appropriate cell patterning to exhibit similar motility to the real animal	[18]
2012	Using excised dorsal vessel tissue (DVT) of inchworm, an autonomously moving polypod microrobot (PMR) was constructed	[19]
2013	An artificial biologically insect scale (80 mg) flapping wing-robot	[20]
	An atmospheric-operable bioactuator (AOB)/microdevices fabricated by coupling insect dorsal vessel tissue (DVT) with microtweezers in a capsule with a finite amount of culture medium	[21]
2016	A bio-hybrid system that consists of tissue-engineered analog of batoid fish, such as skates and stingrays, that follows a phototactic guidance (optogenetics)	[22]
At the submillimeter scale, significant milestones		
	Untethered Microrobot	Reference(s)
2003	A novel surgical microrobot inspired by bacterial (*E.coli*) swimming propulsion for *in-vivo* application to destroy kidney stones as a function of minimally invasive medical intervention	[23]
2004	"Auto-mobile chips" and/or "auto-mobile beads"	[24]
2005	A flexible artificial flagellum constructed through a series of colloidal magnetic particles (MPs) join through DNA with coupled red blood cells (RBCs)	[25]
2006	Electrostatic, untethered, two actuators-based MEMS microrobot	[26]
2006	Untethered nickel microbiorobot driven by electromagnetic fields	[27]
2006	Laser-powered, thermally actuated, steerable untethered locomotive devices	[28]
2007	Untethered 1.5 mm diameter ferromagnetic beads were demonstrated in vivo as milli-robots or devices having controls and tracking in a living swine carotid artery using clinical magnetic resonance imaging (MRI)	[29]
2009	An untethered microrobot actuated by a pulsed electromagnetic field having "stick–slip motion"	[30]
2011	Optically driven bubble microrobot	[31]
2012	Microrobots are driven directly by the transfer of momentum from a directed laser spot. Light sailboats: laser-driven autonomous microrobots	[32]
2012	Fuel-free (off-board approach) locomotion of spherical magnetic Janus motor driven by magnetically induce temperature gradient (thermophoresis), e.g., hyperthermia, and drug carrier for anticancer therapy	[33]
2013	Micro-bio-robot development driven by sperm flagella was developed (Spermbots)	[34]
2013	Microdrillers composed of tubular Ti/Cr/Fe having sharp ends and can be used for ex vivo mechanical drilling of swine hepatic tissue	[35]
2015	Artificially motorized sperm cells are delivered to oocyte for fertilization	[36]
2015	A micro propeller actuated through magnetism can navigate through gastric mucin while immobilized/functionalized with urease that mimic *H. pylori* propulsion in the stomach lining	[37]

Table 1 (continued)

Year	Milestone	Reference(s)
2015	Magnetic helical microswimmers surface-functionalized with lipoplexes (loaded with plasmid DNA or pDNA) to generate functionalized artificial bacterial flagella (f-ABFs) used for targeted single-cell gene delivery to human embryonic kidney cells (HEK 293)	[38]
2015	Stimuli (thermo-magnetic) responsive hydrogel-based actuators, self-folding soft microgrippers for biomedical (surgical) application in soft robotics	[39]
2016	Use two-state (magneto-aerotactic) migrations of magnetotactic bacteria, *Magnetococcus marinus* strain MC-1, for transport of drug-loaded nano-liposomes into hypoxic regions of HCT116 colorectal xenografts	[40]
2016	Medibots: dual action as cellular microsurgery with drug-rehabilitation	[41]
2016	A stimulus (pH) responsive hydrogel-based soft microrobot/microdevices actuated through an electromagnetic actuation system (EMA) for targeted delivery of therapeutics agents such as anticancer drug PCL-DTX	[42]
2017	Biohybrid magnetic robots (BMRs) for image-guided biodegradable therapeutic inventions	[43]
2018	Multifunctional superparamagnetic/catalytic microrobots made up of iron oxide/polymer Janus particles partly coated with platinum (PM/Pt) microrobots for cell manipulation, anticancer doxorubicin (DOX) therapeutics loading, and transportation to breast cancer cells	[44]
2018	Burr-like porous spherical microrobot for capturing and targeting cells	[45]
2018	Intravascular microrobots/magnetic drilling actuators (MDAs) are devices for the treatment of circulatory system disease, e.g., removal of thrombus	[46]
2018	Magnetically actuated peanut-shaped colloid motor for non-contact fluidic manipulation and patterning of cells in an autonomously controlled manner	[47]
2018	1st (novel) slippery micropropeller (a microvehicle for intravitreal delivery) drives through the eye's vitreous body to reach the retina	[48]
2019	Catalytic antimicrobial robots (CARs) for biofilm eradication by "kill-degrade-remove"	[49]
2019	Micron-sized magnetic hair-derived robots (hairbots) for autologous cargo carriers for guided drug delivery, bioimaging (ultrasound contrast agent), and untethered osteogenesis	[50]
2019	3D-printed, biodegradable microrobotic swimmers for theranostic cargo delivery and release	[51]
2019	Ex-vivo generation of medium-induced swarms and their targeted deliveries in the bovine eyeball	[52]
2019	The inclusion of radioactive compounds in soft thermoresponsive magnetic microrobots for single-photon emission computed tomography imaging (SPECT)	[53]
2019	Multi-spectral optoacoustic tomography (MSOT) is a new tool for localization and monitoring a single rotating microobjects (components of a medical microrobot) in hard-to-reach target sites	[54]
2019	Magnetically actuated scaffold-type microrobots as a tool for precise stem cell delivery and transportation in vitro, ex vivo, and in vivo	[55]
2020	"Walking micromachines (microrobotic scalpels) for cancer cells microsurgery	[56]
2020	CIP@T-Budbots is a plant-based magnetic microrobot for "kill-n-remove" biofilm	[57]
2020	Leukocyte-inspired multifunctional microrollers for targeted active cargo delivery and control navigation inside the blood flow	[58]
2020	Non-invasive, magnetic micromotor-assisted zygote intrafallopian transfer (ZIFT) by capture with the help of micropropellers such as helix, and spiral, transport and release the cellular cargo	[59]
2020	Hybrid sperm micromotor for heparin cargo delivery through continuous and pulsatile blood flow	[60]
2021	Biocompatible, biodegradable, and ultrafast Aqua sperm micromotor derive from African Catfish *Clarias gariepinus,* B.1822 for efficient destruction of biofilm from medical devices such as catheters	[61]
2021	A biohybrid magnetic helical microrobots (BMHMs) composed of *Spirulina*, MnO_2, and Fe_3O_4 nanoparticles for application in removal of Pb (II) from wastewater	[62]
2022	The biohybrid magnetic nanoparticle loaded probiotic *Escherichia coli Nissle* 1917 (EcN microrobot) with triple perceptivity such as magnetic, hypoxia, and thermal logic circuit engineered into bacteria to control the biosynthesis of reporter gene mCherry and NDH-2 enzyme incorporated into EcN@MNP for targeted anticancer therapy	[63]
2022	Magnetically controlled, biodegradable microrobot composed of spherical gelatin meth-acrylate (GelMA) for targeted stem cell delivery	[64]
2022	A microrobot consist of magnetic nanoparticles and hydrogel for targeted delivery of therapeutics via trans catheters for *invivo* hepatic chemoembolization with real time imaging using X-ray, and Ultrasound for hepatocellular carcinoma	[65]
2023	A magnetic microrobot constructed via neurospheroid also called as Mag-Neurobot which form structural and functional connection with an organotypic hippocampal slice (OHS) for targeted cell delivery	[66]

Table 1 (continued)

Year	Milestone	Reference(s)
2023	Microalgae *Chlamydomonas reinhardtii* based engineered biohybrid microrobot functionalization via two step method using vancomycin derivative 7 as a linker and a thiol as a reducing agent for successful killing of gram positive organisms such as *Staphylococcus aureus* and *Bacillus subtilis*	[67]

dimensions of 1-150 mm. They are influenced by macroscale forces such as bulk, buoyant, and inertial forces to their artificer [5]. While microrobots have their untethered motile components having all aspects in the range of 1-1000 µm their mechanics are greatly influenced at the microscale due to the consequences of physical forces because, for microrobots, bulk forces are insignificant in contrast to surface area and circumference-related forces (adhesion, friction, drag forces, viscous forces, and surface tension) [5, 6]. Nanorobots, being at the nanometer scale, focus more on biomedical applications. Their small size, and high surface-to-volume ratio make it feasible to be used in healthcare as it enables minimum invasive strategy instead of untargeted chemical and radiotherapy surgeries [7]. Untethered mesorobots have a length scale between nanoscale (1–100 nm) and microscale (10 µm to 1 mm), also micro-nano-robots. At this mesoscale level, most steering forces, such as diaelectrophoretic force, magnetic force, and radiation pressure, are essential for their proper function [8].

2 Microrobot design

Designing a microrobot requires a set of Sensors, actuators, micromanipulators, powering systems, and control strategies [2, 68, 69]. The present section explains how a microrobot is designed and executed for the targeted application.

2.1 Sensor

A microrobot requires a sensor that responds to such stimuli to perform a predefined task. In a Bio-hybrid microrobot, microbes can be considered an "actuator-sensor processing unit." Motile microorganisms exploit sensory-based responses collectively known as "taxes," either positive or negative towards/away from the source [68]. The different taxis mechanisms (Fig. 2) used by the microrobotic system are; Magnetotaxis, aerotaxis, thermotaxis, [2, 5], phototaxis, chemotaxis, galvanotaxis, and pH gradients [69].

2.2 Actuators

Actuation is crucial for synthetic (artificial) devices to communicate with their surrounding habitat and to perform the task for which they are designed. There are many actuation techniques established, but still, for many applications, directional locomotion is the challenges that affects the evolution of robots at a minute length scale especially having bio-hybrid nature. To overcome this issue and achieve maximum actuation of robots at a minute scale (e.g., millirobots- greater than 1 mm and microrobots- less than 1 mm), the bacteria-powered microrobots (bacteriabots, biohybrid robots) construction was envisaged [70]. Bacteriabots include the fusion of live motile (flagellated) bacteria with synthetic compounds such as liposomes, magnetic nanoparticles (MNPs), magnetosomes, and polymersomes [6, 71]. The system can incorporate the non-identical functional components: cargo and carrier. The cargo is an ingredient to be moved and feasibly released in an alternate manner, while the carrier is a tool that conducts the motion of a microbiorobots that carries the cargo, which is of interest that is linked to its surface. Essential features of carriers include-size and shape, cargo loading and release, degradability, and deformability. Size and shape are considered critical elements responsible for the propulsion and cargo (drug) loading execution of bacteriabots. Two significant characteristics of microorganisms need to be taken into consideration while designing a microbiorobot: (i) surface properties that direct the cargo attachment and (ii) their motility potential, i.e., whether the attachment of cargo can move from one place to the targeted [6, 71]. A list of reported actuators is summarized in Table 2.

2.2.1 Propulsion/motility

There can be any one of the Directional control-based taxis methods (Fig. 1). Deterministic, Environmental, and Autonomous directional control encompasses the planned, programmed, and attraction-dependent deterministic taxis of the microbiorobot. Assisted directional control uses fixed path directions for taxis, and multi-taxes directional controls (switched/simultaneous) uses external influences (Photo/magnetic/nutritional) can enhance the taxis of the microbiorobot) [68].

Deterministic directional control It allows microrobots to recognize the planned trajectory (Fig. 1) under an external physical controller, such as a magnetic field to generate magnetotaxis. Other taxes-based systems include *Spirulina plantensis* based magnetic microrobot (Table 1) used for environmental applications for the removal of Pb [62]. Other taxes based on deterministic control include, galvanotaxis,

Table 2 Microorganism/s based microrobot/s, Actuators and their targets/biomedical applications

Microrobot	Sensors/actuators	Specific target	Reference(s)
Attenuated *Salmonella typhimurium* attached to polystyrene microbead	Chemotaxis	Tumor spheroids	[72, 73]
Micro-swimmers (e.g., *E.coli*) for targeted active drug delivery	Chemotaxis/Magnetic field-based steering	Tumor tissue	[74]
Magnetotactic bacteria (*Magnetococcus marinus*) loaded with liposomes containing anticancer drug	Aero-taxis-based self-steering, remote magnetic steering	Colorectal xenografts in mice, drug diffusion into the hypoxic region of a tumor	[40]
Serratia marcescens attached polystyrene or polydimethylsiloxane to form bacterial carpets	Chemotaxis	Automobile beads/chip, targeted therapeutics delivery	[24, 75]
Vibrio alginolyticus	Chemotaxis/surface swarming as actuators	Bio-motor	[76]
Bacillus subtilis (microscopic gears)	Oxygen gradation	Redox environment	[77]
Gliding bacterial species (*Mycoplasma mobil*)	Rotatory motor as actuator	NA	[78]
Protozoa (*Paramecium caudatum*)	Negative Galveno-taxis as micromanipulator or actuator	Micromanipulation of genes, cells	[79]
Vorticella convallaria	Chemotaxis Cilia as a micro actuators	Microfluidic applications (a collective ciliary motion for enhancement of mixing in a continuous fluid)	[80, 81]
Tetrahymena pyriformis (eukaryon)	Magnetotaxis, Phototaxis, galvenotaxis/ Magnetically steered micro transporters	Transport engineered microstructures	[82–84]
The strain of algae, such as *Chlamydomonas reinhardti,*	Phototaxis steered	Antibiotic delivery to successful killing of gram positive organisms	[67, 85]
Magnetotactic cocci (MC-1)	Microwave/Magnetic gradient	Breast cancer	[86]
Actuators based on explanted whole-muscle tissue			
Cardiomyocytes drive hydrogel micropiller	Cardiomyocytes	In situ drug screening on microchip	[87]
Swimming robot	Living muscle tissue with embedded microcontroller	Muscle prosthesis	[12]
A cellular micropump on chip	Cardiomyocytes sheets	μ-TAS (micro-total-analysis system)	[88]
Skeletal muscle on a chip	Optical stimulation (optogenetics)		

NA- Not Available

magnetotaxis (*Magnetococcus marinus* strain MC-1), and electrophoretic mobility based microrobot (*Serratia marcescences*) [89].

Environmental and autonomous directional control Flagellated bacteria have been used as a source of propulsion as they offer advantages such as the ability to convert chemical energy into motion, movement at low Reynolds numbers, which are efficient parameters to be used with artificial microsystems such as microrobots [75]. The taxis based sensory systems included in environmental directional control are chemotaxis and aerotaxis (positive as well as negative). An example of negative aerotaxis while using an anaerobic bacteria-based microrobot in the tumor hypoxic region for targeted drug delivery is one of the illustrations of environmental directional control. Another method to control the motion of bacteria-based microrobots is oxygen gradients, as described in [90]. This postulates that bacterial programming is feasible as oxygen microbubbles to be used as environmental directional control or autonomous directional control while integrating inside a bacterial artificial system, referred to as oxygen programming as represented by [91], and it is illustrated in Fig. 1 as autonomous directional control. The autonomous microrobot should be operated without any external control. Therefore, the embedded program includes the bacterial flagellum motor as steering control for the propulsion of the microrobot at the target site.

Assisted directional control It was first described by [29, 92] for application in tumor targeting. The capillaries of the tumor angiogenesis network are the solitary route to deliver therapeutics carried by magnetotactic bacteria to the targeted tumor and are below the spatial imaging modalities. There is no information regarding the type of path followed; therefore, a close-loop servo is not suitable to be used as

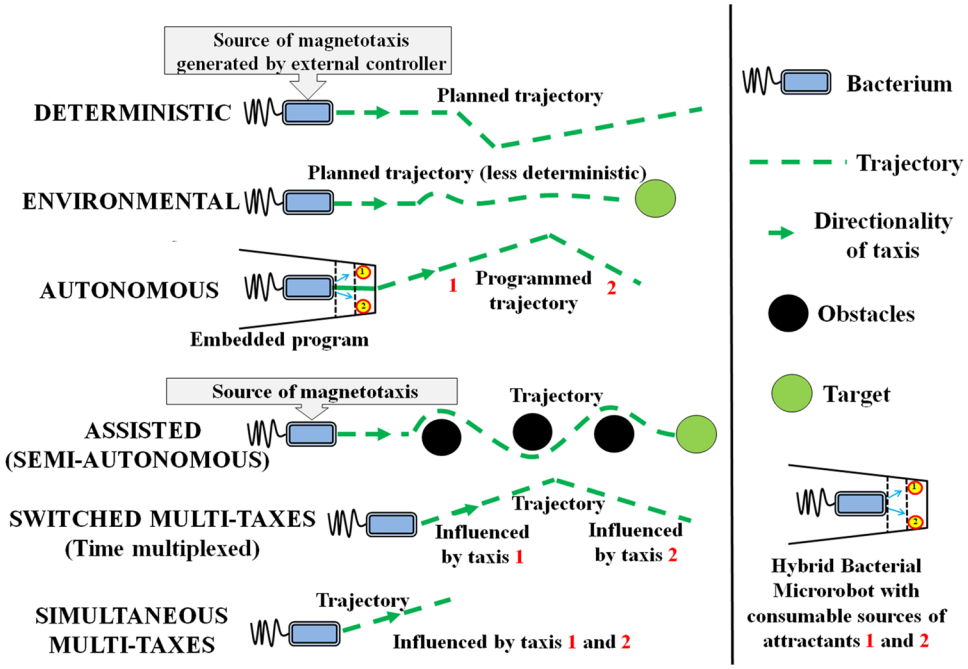

Fig. 1 Schematic representation of taxis and structure based directional control methods from a source to the target site (including the various directional, stimulated, autonomous and assisted taxes seen in construction of micro-bio-robot)

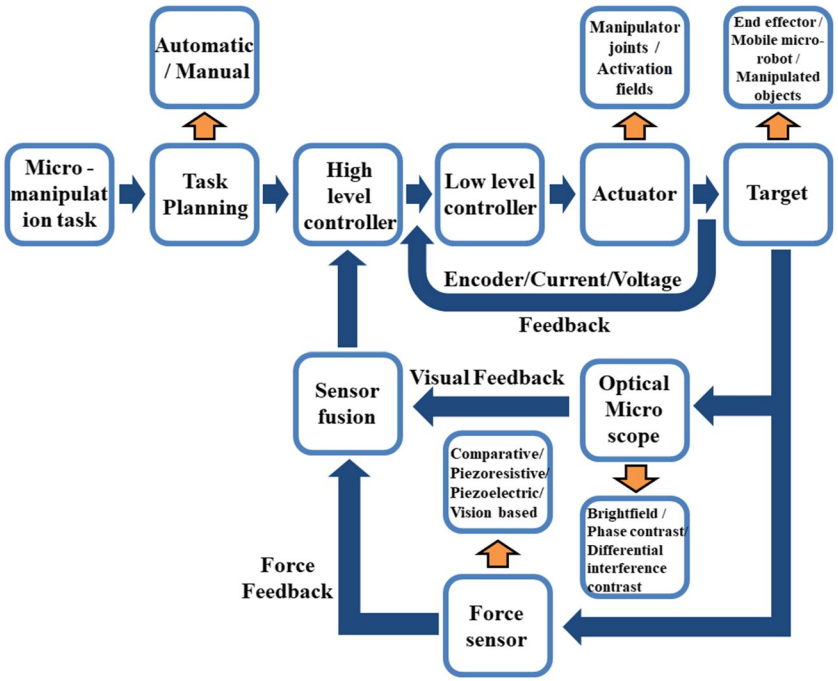

Fig. 2 General control diagram/components of microrobot and their interactions: For robotic control, micromanipulation of desired architecture is achieved either by manually (with human input) or automatically (with a task planner). A high-level controller receives visual or force feedback which enables desired input for the actuators while Low-level controller receives sensor feedback from actuators and controles it to follow the desired input

a steering control. To avoid the obstacles (fluidic environment) in the path of the target and seek the path-finding (PF) strategy for enhancing tumor targeting as well as therapeutic delivery [68].

Multi-taxes directional control Incorporation of more than one taxis (multi-switchable) into a single microrobot brings advantages when used in the treatment of hard-to-reach areas, such as the hypoxic region. An example of multi-taxes directional control is *Magnetospirillum magneticum* AMB-1, which responds to magnetotaxis along with phototaxis. They respond to light from 400–700 nm, which is independent of wavelength, and magnetotaxis [93]. Another example of multi-taxes directional control includes magnetotactic MC-1, which switches between magneto-aerotaxis for the tumoral interstitial fluid microenvironment. Their migration path was guided by decreasing the oxygen concentration to reach the hypoxic region [94].

Actuators of microbiorobots can be classified into two broad categories: (1) Application-oriented non-scalable actuators (dealing with the biological system with no or negligible modification) and (2) General purpose scalable actuators (that are constituents of cells or cellular extracts that can be fabricated into desired dimensions to achieve a function at a bottom-up approach). The application-oriented non-scalable approach can deal with micro- (as motile microorganisms, motile cells) and macro-scales (explanted whole muscle tissue) while the biological system cannot be scaled down or up in size, but they attain their actual task proficiently. Thus these actuators can be used for specific applications at the microscale (targeted drug delivery, motile cells on Lab-on-chip) and macroscale (swimming robots or fluidic pump) instead of performing the general functions [70]. The need for bottom-up approach is to enhanced flexibility using single cell fabrication for definite microrobot architecture. This includes cardiomyocytes based microrobotic system fabricated on PDMS as swimming crab-like microrobot, walking bio-hybrid microrobot (Table-1), and application to be used in tissue engineering.

2.3 Robotic micromanipulation:

The manipulation procedures used at the microscale for the fabrication of components and payload delivery at the desired (target) locations require precise actuation and control over adhesion forces (Fig. 2) [95]. Micromanipulation requires: micro-manipulators (tools for manipulation at micro-scale), external physical fields (fields used to generate actuation such as acoustic, magnetic, optical, electrical and fluidic fields), and end-effectors (apparatus integrated with micromanipulators to communicate with micro-objects to perform functions such as to pull and locate, for example, single ended probes (micropipette and Atomic Force Microscopy (AFM) probes) and microgrippers) to carry out actuation at the microscale [69, 96].

2.3.1 Field-driven micromanipulation

Magnetic micromanipulation Magnetic micromanipulation has several advantages, such as high specificity, precision, deep tissue penetration, and untethered control [96]. It can be classified into gradient-based and torque-based micromanipulation.

a) Gradient-based micromanipulation:

The magnetic gradient can be produced either by electromagnetic coils or permanent magnets. When an electric current is applied to an electromagnetic coil, it generates a controllable magnetic field gradient to drive a magnetic object. Thus when permanent magnets are used to generate the magnetic gradient, significant features such as less heat generation and a strong magnetic field are observed. Controlling the location and direction of these permanent magnets generate a non-uniform (heterogeneous) magnetic field flux, resulting in a magnetic gradient. Gradient magnetic fields actuate many devices (lengths between centimeters to sub-millimeter) to perform the task such as intraocular microrobots for retinal surgery [97], imaging using MRI based Magnetic Navigation (MRN) for catheterization to release microrobots in coronary arteries [98], and biopsies, intraembryonic navigation for mechanical measurement in mouse embryo [99]. Gradient-generated forces become less (pico-newton) when the size of manipulating objects is reduced to micro- and nano-scale in accordance to 'the scaling law' (magnetic forces (F) increase with the magnetic dipole moment (m) and decline with the third power of the object's size so the manipulated magnetic objects must be placed together with magnetic coils or magnetic poles).

b) Torque-based micromanipulation:

Magnetic torque guides the direction of the magnetic micro object dipole to orient with respect to magnetic field. Torque relies significantly on the magnetic flux density (B) and a strong magnetic field (Helmholtz coils). Thus it generates a time-variant magnetic field and actuates micro-objects on a 2-D surface (a surface walker or surface roller) and helical micro swimmers in 3-D space. Here, an object moves forward when the roller (walker) is larger and rolls on the surface, creating friction (between the two) and, thus, movement. A helical microrobot consists of magnetic head with a helical tail- which moves the object forward or backward when a rotating magnetic field is applied. Torque-based micromanipulation has many applications, such as magnetic swarm control, drug delivery, cell delivery [100], and gene delivery [101]. When microrobots carry the cargo (liposomes or bacteria), they perform intelligent task such as the delivery of biological agents following environmental changes such as pH, temperature, pressure, and light [6].

Magnetic gradient and torque can also be applied together for performing micromanipulation tasks. Magnetic gradient produces a control magnetic field which makes the micro object find actuation while magnetic torque is exerted on a micro object to deform or move to carry out propulsion.

Optical micromanipulation: Optical micromanipulation using focused laser beams (to generate an optical field for manipulating micro-objects, e.g., micro-particles) has been used in several disciplines, such as cell manipulation, micro-assembly, and biophysical characterization. Micro-particles generate two different types of forces, namely- (i) Gradient force generated by an electric field of focused laser beams

and (ii) Scattering force associated with the optical strength of the light that passes on particles. Equilibrium between these two forces brings micro-objects like micro-particles to the targeted location. It uses visual surveying to track/control the location of micro-object at the equilibrium apex. The application of optical micromanipulation includes- target cell sorting (embryonic stem cells, yeast cells, sperm cells) and transport of the cells. In tissue engineering and cell mechanics, where nano-newton forces are essential for mechanical measurements &/or stimulation, optical manipulation becomes restricted due to the scaled-up power of the laser that can rupture the manipulated object. Low force output is applied to overcome this issue, but it poses the challenges such as thermal and Brownian convulsions for robotic optical micromanipulation [96].

Electric micromanipulation: When microparticles are dispersed in fluid, they react with either AC/DC electric fields through numerous mechanisms. When applied to a DC electrical field, polarizable particles can move towards the oppositely charged electrodes through electrophoresis, while the particles, when applied an AC electrical field, exhibit diaelectrophoretic force on the particle. The manipulation of particles (objects) from the nanoscale to the microscale can be done by managing the frequency, field strength, and electrode configuration. When the electric force is applied directly to the micro/nano particles in AC/DC electrokinetics, it may guide the particle to swim across the workspace resulting in transportation and redistribution of the dispersed particles. However, the electric force applied to the manipulated objects dramatically relies on the electrical properties of the apparatus, such as size & shape. The application of electric micromanipulation includes; DNA analysis [102], measurement of single-cell mechanics [103], micro-assembly of nanowires and nanoparticles (NPs) [104], and manipulation of microbeads.

Fluidic micromanipulation: In fluidic micromanipulation, a fluidic field can be produced by rotating magnetic field-based microrobots (rod-shaped), oscillating piezoelectric actuators, and controlling the flow rate inside a micropipette. A rod-shaped microrobot generates vortices and can be controlled by a rotating magnetic field with visual feedback to manipulate protein crystals [105]. The flow rate is controlled inside the micropipette for rotating, delivering, and laying down the biological cells [106]. It is difficult to control and quantify the forces applied to the manipulating object during the manipulation process because of inappropriate fluid dynamics modeling.

Acoustic micromanipulation: It is a robust and non-invasive approach that uses sound waves to manipulate the objects- which are manipulated and moved either in minimal pressure domains (acoustic pressure nodes) or maximum pressure domains (acoustic pressure antinodes), relying on the firmness and compressibility of the object. The object manipulated by using sound waves is attained by regulating the pressure nodes. Acoustic waves and tweezers have a function, such as capturing and manipulating specimens such as microparticles and biological cells without direct communication. Due to this, it has critical applications in various disciplines like medicine, engineering, chemistry, and biology. Acoustic tweezers are used for diagnostic applications such as purifying viruses and circulating tumor cells from the blood [107]. It also cultivates engineered tissue inside a microfluidic chip by clustering the cells [108]. It is beneficial for swarm control because it has various pressure nodes to capture the micro-object for manipulation.

2.4 Fabrication of microrobots

Different types of reported fabrication techniques have been enlisted in Table 3.

2.4.1 Sacrificial Layer

For surface micromachining, water-soluble polymers are used as a sacrificial layer. The importance of this layer as it prevents any structural damage that occurs during the release of microstructures into a fluidic chamber [118]. The essential properties required for the water-soluble sacrificial layer are a) homogenous films after spin-coating; b) Water-soluble films before and after photolithography; and c) insoluble films in organic solvents before and after photolithography. Dextran and poly (acrylic acid) (PAA) are used as sacrificial layer for the fabrication. However, poly (vinyl alcohol), a water-soluble sacrificial polymer, has already been reported to be used for microstructure

Table 3 Lists of fabrication techniques with their dimensions

Dimensions	Fabrication techniques	References
1-D	Lithography • Optical/photolithography • X-ray lithography • Particle beam lithography • Ion beam lithography	[109–112]
2-D	Soft lithography • Micromolding method • Microfluidic method • Spin coating	[113]
3-D	Microstereolithography • Deep x-ray lithography • Two/single-photon Microstereolithography	[114, 115]
	• Laser assisted bio-printing (LAB)	[116, 117]

fabrication, while PAA and dextran have some essential characteristics required in the process. Characteristics of a sacrificial layer, such as the thickness of the film, rely on the viscosity of material, i.e., the polymer's molecular weight, concentration, and speed of the spin-coating process. One of the limiting factors for the surface micromachining process is that it requires exposure to an aqueous solution before taking it off. To overcome these limitations, chemical treatments help modify the water solubility of the film. E.g., the side chain of PAA contains Na^{+}—carboxylate groups, Na^{+} is exchanged for Ca^{++} ions that cross-link the PAA chains, and a water-insoluble PAA-Ca^{++} polymer is developed. Some other bivalents ions are also used, such as Cu^{++} in $Cuso_4$ and $CuCl_2$, but trivalent ions cannot perform the function [119].

2.4.2 Soft-lithographic techniques

Preparation of Polymer solution: Poly (Lactic-co-Glycolic Acid) (PLGA) solution derived from 85/15 PLGA in chloroform is used to attain the sufficiently required concentration. The concentration of the solution depends on the type of microfabrication technique (Fig. 3) used, such as 5%-20% [113].

Steps for microfabrication of silicon master The method involved the following steps:

EPON-SU8 photoresists were spin-coated onto the wafers of Silicon 100, which were baked further just enough to remove remaining solvents and exposed to UV light from the mask-enabling side of the mask-aligner situated at the bottom. After the exposed photoresist (SU-8 developer) was developed, wafers were baked. By mixing the commercially available catalyzer and pre-polymer at a ratio of 1:10, degassed (under vacuum to eliminate bubbles) and cast on the master (keeping further in vacuum to avoid bubbles). Curing of PDMS was done by baking at 65 °C for 2 h. After cooling to RT, the silicon master can be separated by peeling the PDMS. The mold is washed with 70% ethanol and further sonicated before use [113].

Optical/photolithography: It uses UV light as a source of radiation. The substrates used for photolithography are glass, silicon, and quartz. After selecting an appropriate substrate for fabrication, it requires a coating of light sensitive polymer called resist. The resist can further be classified into two broad categories: positive and negative tones. The difference lies in the solubility after the exposure. The exposed areas of negative resist remain insoluble, while the opposite is true for positive resist. To fabricate biological devices, negative resists are most frequently used (e.g., SU-8) because of their biocompatibility and great thickness, which is suitable for the construction of microfluidics [110, 112]. Figure 3 shows the microfabrication of a polyethylene glycol (PEG)-based scaffold. The scaffold is constructed layer by layer by polymerization of PEG-hydrogel upon UV light exposure, followed by washing off the unexposed pattern.

X-ray lithography (XRL): XRL is a potential 1-D microfabrication technique and high aspect ratio (HAR). In India, Indus-2 and beamline-7 are radiation sources for soft and deep XRL. It provides low surface roughness and vertical side walls which is ideal to be used for fabrication of microfluidics channels. It requires high precision to perform the lithography process. The X-ray mask consists of X-ray transmitting (transmission > 90%) and absorbing material (absorption > 80%). The mask membrane contributes the structural support to the entire mask therefore it must be used as a low atomic number (low-Z) i.e. X-ray transmission. The selected absorbing material (such as gold) has a high atomic number (high-Z) [120]. As shown in Fig. 3, XRL is a three-step micropatterning procedure, namely, lithography, electroplating, and molding. The X-ray mask is developed by using ultraviolet (UV) maskless photolithography [111]. XRL offers advantages in corresponds to HAR in the construction of microfluidics channels because it is not easily to be produced with other techniques, fabrication of microdevices in small batches, and application includes lab-on-a-chip, biosensors, biomedicine, biochemistry (in terms of microfluidics) for manipulation of environmental sample, synthesis and patterning of novel materials [109].

Stereolithography: It was used to fabricate 3D structures by utilizing a light source (UV light) for photopolymerization. It can further be classified into two broad categories: single photon stereolithography and two- or multiphoton stereo lithography (Table 3). The application of stereolithography includes prostheses, implants, surgical procedures, regenerative medicine, integration with medical imaging technologies such as MRI and CT to enhance disease diagnosis, scaffolds, and tissue engineering [115]. This procedure consists of a laser source, liquid photocurable resin, a computer device, and a deflection mirror (Fig. 3). First, a 2D structure pattern was developed, followed by a 3D structure developed layer by layer. Stereolithography offers advantages such as high resolution, rapidness, high cell viability, and rapid polymerization. The primary challenges associated with stereolithography are the need to fabricate multimaterial 3D structures in translational biomedical applications and the scarcity of biocompatible and biodegradable liquid polymer resins [114].

Laser assisted bio-printing (LAB): It utilizes laser as a source of energy. The device consists of a pulsed laser, a scanning mirror, a material (titanium, silver, or gold) that can absorb the laser with biological material, and a collecting substrate. The pulse of the laser (IR or UV) falls on an absorbing material, resulting in the evaporation of liquid

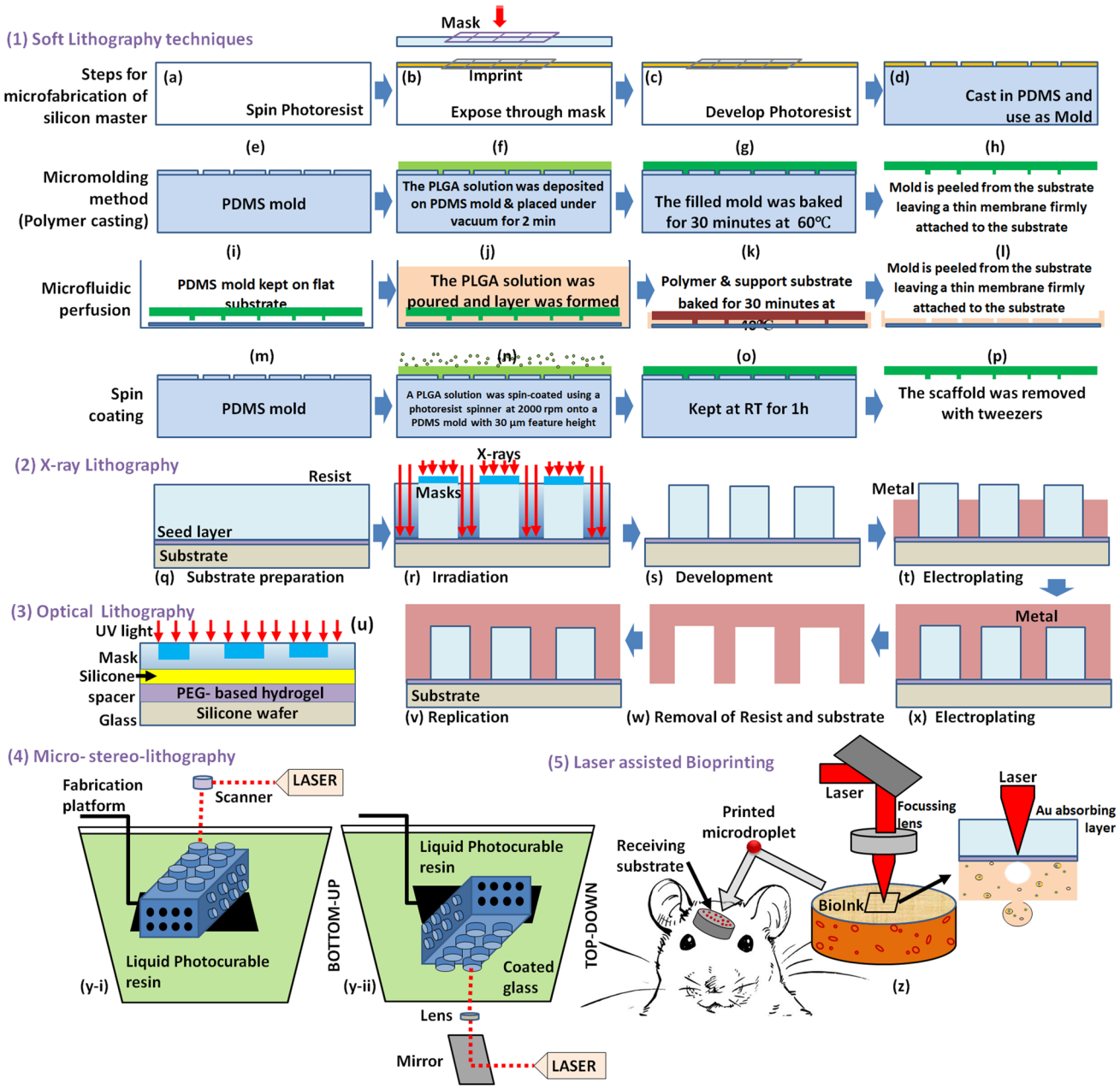

Fig. 3 Schematic illustration of Microfabrication methods (1) Soft-lithographic techniques (2) X-ray lithography (3) Optical lithography (4) Laser assisted bioprinting (5) Stereolithography. Subfigures (a-e) microfabrication of silicon master, (e–h) Micromolding method for polymer casting, (i-l) microfluidic perfusion (m-p) spin coating

biological materials, and reaches the target receiver in the form of a substrate (Fig. 3) [117]. The resolution of LAB varies from microscale to picoscale, but it is affected by various parameters such as the energy level of the laser pulse, the type of biological materials along with their thickness and rheological properties, substrate wettability, printing speed, and the organization of the structure [116]. LAB offers various advantages like being contact and nozzal-free, nozzal free so no clogging issue while printing; control over ink droplets resulting in precise delivery of objects; printing with high resolution and a higher cell survival rate while minimizing the shear stress compared to other printing techniques [114, 121]. However, a few limitations associated with LAB include its high cost and time consumption.

2.5 Powering Microrobots

Microrobots require fuel to perform any task depending on the driving force, which is essential for propulsion. It is classified into two different categories:

2.5.1 On-board (Scavenged power) approach

Most microrobots can use the onboard chemical and bio-chemical substances (nutrients) as fuel/power [122] while others utilize mechanical energy such as vibration from the surrounding environment [123, 124]. One way to power a medical microrobot is to extract chemical energy from the surrounding environment, for example, biofuel cells can work in an environment where pH is neutral, and temperature is low as 20 to 40 °C. To power, an *invivo* microrobot nutrient/fuel such as glucose [124] and blood can be used [123]. On-board (scavenged power) is a potential approach for millirobots, as many commercial components are available which are not feasible for microrobots. Thus, mobile microrobots usually use an off-board approach [5].

2.5.2 Off-board approach

An alternative approach to power a mobile microrobotic system is an off-board approach which includes a transmitted power strategy controlled externally. The most frequently used method is the magnetic field [124]. To generate electricity for powering a microrobot, one can utilize time-varying magnetic field to induce a current, while another method uses low-frequency and quasi-static magnetic fields to apply force and torque to magnetic materials [123].

2.6 Control of microrobots

In robotic micromanipulation, force, location, and vision are frequently used modalities for feedback control. However, which control strategy to use depends on the type of microrobot (single or swarm of microrobot) and target application (in vivo biomedical application, environmental application). Although challenges are associated, it is difficult to devise novel structures on the microscale (capsules and crawling microrobot) [125, 126], difficult to control multiple objects simultaneously [127], and difficult to fabricate individuals with distinct properties [128].

2.6.1 Vision-based robot control

A control method using vision sensor feedback information for micro-robotic control is known as Visual servoing (or VS). In microbial microrobotics, visual servoing include optical microscopes (Fig. 2) which provide high resolution (0.2 μm), narrow field of view, and low-depth-of-fields. It frequently requires an eye-to-hand motif as the camera is attached to the microscope [96].

2.6.2 Force sensing and control

It is used for closed-loop micromanipulation procedures. The force sensors are articulated with the micromanipulator and end effectors (target/microrobots). The function of force sensors is to measure a force at levels of micronewtones and/or below. However, the major drawback of most field-driven micromanipulation is the need to integrate force sensors into/adjacent to the object of need (target/microrobots) [96].

3 Essential biomedical applications/functions of a microrobot

3.1 Targeted therapy:

A microrobot is an excellent choice to distribute biological and chemical substances at constraining areas with different forms of energy.

- For targeted drug delivery, microrobots can be used to elevate the concentration of payloads in a definite region of a subject, thereby reducing the side effects [129, 130].
- Brachytherapy is a procedure that involves placing a radioactive substance known as a radioactive seed onto nearby undesirable cells, such as in tumors. The death of cells can result from radiated energy of radioactive substances close to the cells [95, 123].
- Hyperthermia and thermoablation is a procedure that is used to deliver localized heat energy for the destruction of an undesirable cell such as a tumor. Hyperthermia requires controlled heating for the long term at temperatures ranging from 41–46 °C for targeted cell disruption [131]. While thermoablation involves the temperature exceeding up to 50 °C till cell death, the most favorable methods for wireless delivery of heat energy are by i) using ultrasonic resonating mechanical structures and ii) using high-frequency magnetic fields [95, 123, 130].

3.2 Material removal

Microrobots being smaller in size, can be used as a tool for material removal. Following are the two different methods to accomplish the task by microrobots:

- Ablation is a procedure that includes the excludes material from an object's surface that is achieved by scraping. To perform the ablation task, microrobots utilize a rotary motion, for example, plaque eviction from the endothelium of blood vessels [132].

- Microrobot utilizes vibrating mechanical structures to radiate ultrasonic pressure waves to eradicate an object like a kidney stone, known as ultrasonic ablation [23].
- Microrobots can also perform a task such as a biopsy/excision. To conduct an ex-vivo study, a microrobot recollects the tissue sample and is removed from the body. The sample can be analyzed in situ when both procedures combine with remote sensing technology [95, 123, 130].

3.3 Controllable structures

Microrobots themselves are used as a stagnant system whose locations are tractable.

- Scaffolds' function assists cell physique on which simulated organs can be developed, blood vessels and nerves can be regenerated [133, 134].
- Stents are an apparatus that keep channels open by enabling blood circulation through a clogged vessel. The microrobot is solely used as a stent that moves across the desired location to complete the task [135].
- Occlusions can be present deliberately to prevent blood flow either during a transitory period or permanently. Microrobots can assist in occlusions by obstructing the blood flow that nourishes abnormal cell masses such as a tumor [136].
- Microrobots can also be used as temporary or permanent implants: E.g., electrodes used for brain stimulation [95, 123].

3.4 Telemetry

Microrobots are used in telecommunication to transfer particular information from a source that was hard or not feasible to get it. The methods used to transfer this information are visible light, radio, and ultrasound. Telemetry application involves remote sensing and marking.

- Microrobots execute the task for remote sensing by transmitting the physical signal, such as oxygen concentration or binary signal, in the presence of an object, e.g., blood and cancer [63].
- The marking application tracks microrobots and is used with remote sensing to localize unknown incidents, such as bleeding [123].

4 Applications areas

After completing a successful fabrication procedure at a minute-length scale with fundamental functions, a microrobot facilitates to performance of medical interventions throughout the body.

4.1 The circulatory system

The circulatory system contains the heart and blood vessels, which carry blood throughout the body. Every body area is accessed through blood, so it is a crucial application point for wireless microrobots to perform various tasks [95, 123, 130]. Some of the applications are given in Table 4.

4.1.1 Cancer treatment

Microrobots receiving higher attention for targeted drug delivery to cancerous cells due to characteristics such as smaller size, propulsion strength at low Reynold numbers, minimal invasiveness, wireless control, and deep penetration in the tumor hypoxic region, which make is suitable candidate for cancer treatment. *Magnetococcus marinus* strain MC-1 travelled via magneto-aerotaxis containing drug SN-38 nanoliposomes for anticancer effect against the tumor hypoxic region of HCT116 colorectal xenografts [40] (Tables 1, 4). Park et al., 2018 [137] constructed a degradable hyperthermia microrobot (DHM) using iron oxide magnetic nanoparticles, 5-fluorouracil (5-FU) as anticancer drug, poly(ethylene glycol) diacrylate (PEGDA) and pentaerythritol triacrylate (PETA) as a polymer matrix for controlled release of therapeutics with hyperthermia treatment Table 4.

4.2 The central nervous system

The central nervous system contains the brain, Cerebrospinal Fluid (CSF), and spinal cord. The potential applications of the untethered microrobots are in hard-to-reach areas such as deep brain stimulation (DBS) and neural prostheses. Microrobots can be used as an implant for long periods. To avoid craniotomy, microrobots should be introduced at the site of lumbar puncture and can be steered to the brain for intervention [123].

4.3 The urinary system and the prostate

The potential application of microrobots in the urinary system includes the treatment of nephrolithotomy [23] and prostate cancer [151]. The advantage of using microrobots is to enhance the effectiveness of prostate techniques and lower the feasibility of nerve damage [123].

4.4 Ophthalmology

The importance of microrobots in ophthalmological research relies on precise minimal invasive diagnosis of eye diseases and its treatment. He et al., 2020 [146] construct a stady-hand-eye-robot for retinal microsurgery. The robotic features include tool to measure sclera forces, real time substitution, controlled commands to avoid undesirable circumstances,

Table 4 Biomedical applications of microrobot

Areas of microrobotics applications	Application	Reference/s
Circulatory system	Act as a stent to maintain blood flow	[46]
	Act as occlusion to intentional nutrition starvation	
	Carry electrodes for electrophysiology	
	Administration and therapy for aneurysms	
	Thrombolysis	[132, 136]
	Targeted drug delivery	[138, 139]
	Rational atharectomy	
Oncology	Physical therapy by degradable hyperthermia microrobot	[137]
	Photodynamic therapy	[140]
	Nanoliposomes	[40, 141]
	Nanomotor based Immunotherapy for glioblastoma	[142]
	Urease powered Nanomotors for targeting 3D bladder cancer spheroids	[143]
Biomedical engineering	Tissue engineering	[134]
	Nerve engineering	[144]
	Re-growth of vessels and skin substitute	
	Development of biosynthetic organ and bone	
Embryology	Amniocentesis and cordocentesis	[145]
	Temporary tracheal occlusion	
	In vivo assisted fertilization	
	Gamete Intrafallopian Transfer (GIFT)	
	Embryo implantation	
	Clear urinary obstruction	[23]
	Ablation to prevent hydrops	
Ophthalmology	Temponading agent in retinal therapy	[146]
	Intraoccular procedures	[147]
	Optical oxygen sensor	
	Targeted retinal drug therapy	[148]
	Luminance quenching for retinal health	
GI tract	Capsule endoscopy	[149]
	pH responsive microrobot to heal *Helicobacter pylori* infection	[150]

Application of microrobot in the circulatory system, Oncology, Biomedical engineering, Embryology, Ophthalmology.

and auditory feedback. A wireless sensor device which is controlled by external magnetic field to measure intraocular oxygen concentration of living eye (Table 4). Another magnetic microrobot used for retinal drug delivery to remove the retinal occlusion via minimal invasive method [148].

4.5 The ear

Microrobots can be used in the inner ear that involves semicircular canals and cochlea. The complication with the current surgical procedures of cochlear implants includes infection, trauma, and partial paralysis. To overcome the present issues, wireless (untethered) microrobotic techniques play an essential role [123]. The potential use of stem cells in cochlear hair cells generations is responsible for natural hearing, while microrobots can deliver differentiated stem cells to the targeted location [123, 152].

4.6 Embryology

Microrobots can be key to future fetal surgery with possibilities in Clearing urinary obstruction, ablation to prevent hydrops, Amniocentesis/cordocentesis, and temporary tracheal occlusion processes (Table 4) [123]. Intratubal transfer via medical microrobots (μGIFT) offer advantages such as less time consumption for ex vivo culturing for in vitro fertilization (IVF) treatment as excessive washing steps with human manipulation caused oxidative stress [145].

4.7 Tissue engineering/biomedical engineering

The tissue engineering discipline deals with biology, engineering, and synthesis (materials & methods) to develop a viable alternative approach. The microrobotic characteristics

include restoration, sustaining, or enhancing the function of human tissue (Table 4) [95, 153].

4.8 Capsule endoscopy

A capsule endoscope is a tiny swallowable camera pill used for endoscopic investigations of the patient body. These are being used clinically as passive devices which work as drive-throughs in the gastrointestinal tract. Efforts are made to modify the capsule endoscope as an active robot for enhanced diagnosis and therapy. Magnetic micromanipulation has been reported for localizing capsules/millirobots with magnetic actuation [129, 149].

4.9 Micromixer: (Microfluidic system & microassembly)

Micromixers are extensively used in a microfluidic system for drug delivery, biochemical analysis, and sequencing of nucleic acids. To perform complex chemical reactions such as Lab-on-chip (LOC), Micromixers must be integrated into the system. It can work as a stand-alone device with functions at the microscale to understand the transport mechanism. Micromixers can be used as sensor to detect ammonia in environmental monitoring. T-mixer is driven electrokinetically are used for performing enzyme assay. Micromixers are used as surface-based biosensor, in freeze-quenching techniques. Apart from being used as a sensor and in analysis, a Micromixer can be further used as an apparatus for dispersing immiscible liquids to form microdroplets. It can work as a particle separator based on concentration gradient or diffusion coefficient [154].

4.10 Biofilm eradication

Biofilm can form on biotic surfaces (mucosal and teeth), abiotic surfaces (catheters and implants), and hard-to-reach areas [49, 57], which ultimately laid to detrimental effects such as continual infections and therapeutic complications. Biofilm also obstructs the water channels and crevices in commercial environments. A bacterial community that forms biofilm secretes extra polymeric substances (EPS) (matrix), such as exopolysaccharides and amyloid proteins, that contribute to adhesion and aggregation, leading to biofilm architecture. This framework acts as a scaffold that obstructs antibacterial drugs, thereby protecting bacterial cells (in biofilm). Thus the current issue regarding biofilm in antimicrobial approach is limited as they cannot address the biofilm network, its properties such as drug resistance, and its ability to re-establish biofilm if bacteria and debris from the matrix are not removed. By using robotic systems/functionalized micro-robots [49, 57], a new approach was developed to eradicate biofilm by "kill-degrade-and-remove"[49] and/or "kill-n-clean" mechanisms. Catalytic antimicrobial robots (CARs) were developed that perform multiple tasks, such as killing the bacteria using chemicals, degrade the biofilm matrix, and removing the biofilm from surfaces by using physical methods such as magnetic actuation. The magneto-catalytic potential of CARs greatly depends on iron oxide nanoparticles (NPs) with inherent enzymatic activity, such as peroxidase.

The nanoparticles (NPs) catalyze the H_2O_2 (hydrogen peroxide) to produce free radicals that act as antibiofilm fragments, while dextranase and/or glucanase can break down extracellular glucan molecules, thereby enhancing the EPS degrading activity. Two different millimeter-scale CARs were developed, namely, helicoids and vanes. The helicoid CARs removed the biofilm clog via the drilling process, while vanes CARs were removed from enclosed glass tube curved walls. CAR systems were used to remove the biofilm from a hard-to-reach area such as the isthmus (area between the root canal of a tooth) [49]. T-Budbots are magnetotactic microrobots acquired from mesoporous tea buds of *Camellia sinesis* [57]. The microrobot delivers pH-dependent encapsulated drugs such as ciprofloxacin (CIP), thereby eliminating biofilm and free-floating microbes. This microrobot (CIP@T-Budbots) establishes the "kill-n-clean" approach by following steps such as i) microorganisms killed by CIP antibiotic-loaded on CIP@T-Budbots, ii) interrupting biofilm network for enhancing the antibiotic (CIP) concentration, and iii) using magnetic field-based actuation to remove degraded biofilm matrix [57].

4.11 Micromagnetofluidics

It deals with magnetism and microfluidics, providing systematic and productive application for Lab-On-Chip (LOC) devices. Magnetism offers the untethered operation of devices, while microfluidics requires the least amount of samples with high throughput outcomes, for example, droplet-based microfluidic devices for single-cell manipulation, and characterization of biological macromolecules [130].

4.12 Image guided therapy

To design a microrobot/nanorobots for image-guided therapy few critical measures should be taken: i) to navigate the complex biological environment with feedback control, the locomotion of micro/nanorobots must be tracked in vivo for that minimal invasive real-time imagine is beneficial. ii) such miniature devices should be biodegradable after executing the desired task. They must be removed from the body without causing any side effects, and iii) while executing the desired task, the miniature device should not affect the healthy cells and must encounter the effect of abnormal cells such as tumors [43]. Real-time imaging is

the prime and most important to track microrobots inside in vivo conditions. The techniques to device a miniaturized robotic system are, Magnetic resonance imaging (MRI) [155], Magnetic particle imaging (MPI) tracer [155, 156], X-ray computed tomography (CT), Photoacoustic computed tomography (PECT) [155, 157], single photoemission computed tomography (SPECT), Ultrasound (US) imaging [155], fluorescence imaging [43], and combined imagine techniques such as MR/CT.

Magnetic particle imaging (MPI) was first proposed in 2001. It detects the signal from MPI tracers (superparamagnetic nanomaterials) which are generated through a fast-moving magnetic field-free region (FFR) [155, 156]. However, this technique is used to visualize swarming micro/nanorobots rather than individual ones [155]. Fluorescence-based in vivo imaging by using magnetized *Spirulina plantensis* (MSP) bio-hybrid microrobot performs multiple tasks such as remote sensing ability, image-able, biodegradable, and anticancer potential that is better suitable for image-guided therapy [43]. X-ray-CT and SPECT belong to ionizing techniques with wavelengths ranging from 10–100 nm have high (depth) tissue penetration and spatial resolution, but radiation will harm the living tissues [155]. However, these many techniques are used for imaging purposes, but micro/nanorobots are small in size, so their clear vision using currents techniques is still challenging.

5 Challenges

When used as a cell-based microrobotic system, Red Blood Cells lack the potential for immanent sensing and ability cross biological barriers. Therefore, there is a necessity for additional membrane conjugation on the RBCs to perform tasks such as targeted drug delivery. When neutrophils are used as a leukocyte-based drug delivery system, the main limiting factor is the short life span of five days in circulation and a few hours after isolation. Another prime limitation associated with neutrophils is the internal loading of drugs in the cells with risks of degradation (function as a macrophage), limited steering control over mobility, and unconstrained delivery of payloads [6]. The magnetic micropropellers used for the delivery of targeted therapeutics in digestive system poses a significant challenges as Gastrointestinal track secrete excessive mucus (glycoprotein mucin) and acidic environment present major obstacles. The designed micropropellers have been tested into biological media which are non-Newtonian, and non-homogenous [37, 158]. Some synthetic microrobots react with bodyfluids when used for biomedical application leads to changing the surrounding environment causing potential side effects. The challenges associated with Millirobots are effective propulsion in harsh fluids, integration of more than two components as per the target application, targeted anchoring, uncontrolled increase in size increment, structure of the robot cannot be modified after the fabrication, downstream process after task completion [159], and controlled release [160]. Crucial criteria while designing microbiorobots are- optimum locomotion conditions at the microscale, propulsion strength, taxis behaviors to sense the target microenvironment, immunogenicity of microorganisms, interaction with the synthetic (artificial) substrate, and swimming behaviors in complex body fluids before performing the fabrication of microrobots [2]. When microbiorobots are used for *invivo* applications, body temperature plays a crucial role. Microorganisms such as *E.coli* and *Salmonella typhimurium* can increase their swimming motility at 37 °C while the same is not true for a few strains of magnetotactic bacteria (MTB), which eventually limits the effectiveness inside the body after 30–45 min [161–163]. In vivo applications of microbiorobots requires overcoming the challenges such as the effectiveness of pharmacokinetics, biocompatibility, toxicity, retention, and biodistribution, which are of extreme concern [2].

6 Conclusion

Microrobotics has gained tremendous scope and applications in biological systems. It has been reported for use in environmental remediation to biomedical application and thus has innumerable future uses. Investigations into application of bio-based microrobots have opened a plethora of new applications than those envisaged before. Present manuscript reviews various aspects of microrobotic uses of biological systems. From history of microrobotics to their fabrication, manipulation, actuation and control mechanisms have been discussed. Applications of micro(bio)robots in various therapies, remediation (of chemicals and biofilm) and biomedical engineering have also been discussed in detail here. The review thus helps in future fabrications, manufacturing and application dimensions of bio-based-microrobots.

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