

Review

Targeting biofilm infections in humans using small scale robotics

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The eradication of drug-resistant microbial biofilms remains an unresolved global health challenge. Small-scale robotics are providing innovative therapeutic and diagnostic approaches with high precision and efficacy. These approaches are rapidly moving from proof-of-concept studies to translational biomedical applications using *ex vivo*, animal, and clinical models. Here, we discuss the fundamental and translational aspects of how microrobots target the infection sites to disrupt the structural and functional traits of biofilms and their antimicrobial resistance mechanisms. We emphasize current approaches of mechanochemical disruption and on-site drug delivery that are supported by *in vivo* models and preclinical testing, while also highlighting diagnostics potential. We also discuss clinical translation challenges and provide perspectives for development of microrobotics approaches to combat biofilm infections and biofouling in humans.

Current antibiofilm strategies and limitations

Biofilms are highly structured microbial communities causing nearly 80% of human infections, requiring extensive and costly treatments that are complicated by increased antimicrobial tolerance [1,2]. These biostructures form on natural (teeth, mucosa, skin, and organs) and artificial surfaces (implants, catheters, and devices) in the human body, often in hard-to-reach locations, leading to long lasting refractory infections and drug resistance causing millions of deaths and billions in economic loss annually [3,4]. Current antibiofilm modalities have demonstrated limited success in the treatment of these persistent microbial communities. Microorganisms in biofilm are often encased by a protective matrix of **extracellular polymeric substances (EPS)** (see Glossary) and develop chemical and shear tolerance mechanisms rendering traditional antimicrobial approaches ineffective [5–8].

Antibiofilm strategies (disease- and procedure-dependent) have a common goal to either prevent microbial binding/colonization and subsequent biofilm formation or removal of established biofilms. Preventive measures include the use of biofilm-resistant materials or application of antimicrobial surface coatings [9–13]. However, given the unique characteristics of biofilm-forming microbes, such as being able to produce EPS and physically attach, persist, and develop drug tolerance and immune subversion, these modalities have demonstrated limited long-term efficacy [1,14,15]. To eliminate an established biofilm, chemical (antibiotics, antiseptics/disinfectants) or enzymatic approaches (EPS degradation) for microbial killing or dispersal, mechanical disruption for physical removal, or a combination thereof have been used in clinical settings. Notably, current chemicals have limited ability to diffuse within formed biofilms or kill the embedded microbes, leading to reduced efficacy [1,5,8]. Conversely, dispersal of biofilms should be paired with systemic antimicrobials and/or active microbial killing to avoid the potential complications of seeding new infections or causing sepsis by the released microbes [5].

Highlights

Microrobotics can address current limitations to control biofilm infections, and advance diagnostic and therapeutic modalities, in the face of increased antimicrobial resistance.

Versatility in design and control of microrobots combined with targeting and detection capabilities, physical disruption and *in situ* drug delivery provides ample opportunities to improve the standard of care against biofilm infections.

Opportunities for clinical translation and feasibility in complex physiological environments and different anatomical niches without harmful effects, mitigating the need for invasive surgical intervention.

Biocompatibility and biodegradability of materials used in microrobotics is critical toward regulatory approval and clinical implementation.

Potential solutions for microrobotics to overcome technical and regulatory hurdles for practical development and commercialization.

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Biofilm treatment and removal are further complicated by physical confinement, microscale operational spaces, and complex geometries. For instance, biofilms formed on implants are typically confined to a thin surface layer following complex contours of the implanted device, while root canal infections occur within complex internal anatomical structures. The curved anatomy of these canals with a narrow lumen (often at submillimeter scale) makes it challenging for clinicians to completely remove the biofilm. Thus, inaccessible surfaces provide major treatment challenges, leading to incomplete biofilm removal with the risk of regrowth and infection persistence.

To address **biofilm recalcitrance** and physical accessibility, small-scale robots can provide innovative solutions for mechanochemical disruption of biofilms and drug delivery with high precision and efficacy (Figure 1A–C). Small-scale robots with dimensions ranging from nanometers to millimeters represent a distinctive strategy for addressing the current unmet challenges in the field of biomedicine (Box 1). Owing to their small size, robots can navigate through physical and biological barriers to target narrow and hard-to-reach places [16–20]. With their unique features, including untethered, remote control, reconfigurability, adaptability, and multifunctionality (from modifications with antimicrobials and drug loading/release to shear force generation), small scale robotics provide new practical strategies against biofilm infections. Furthermore, the size, shape, rheology, and mobility can adjust to particular conditions (known as **physical intelligence**) while automation can be exploited using simple algorithms and control, including optimization through artificial intelligence (AI).

These robotics functionalities provide unparalleled design options and flexibility for multitasking for biofilm eradication that cannot be achieved using current modalities (Figure 1B–D), allowing access to confined workspaces with precise targeting, on-site drug delivery combined with mechanical disruption, and real-time pathogen detection and diagnostic sampling [19,21]. Details about robotics principles and additional applications can be found in other excellent reviews [17,22–24]. Here, we focus on how small-scale robots (microrobots) can be tasked to address challenges associated with biofilm infections.

Design and control principles

Microrobots (Box 1) are primarily categorized by their actuation mechanisms which can be intrinsically powered, externally powered, or a combination of both. These actuation methods do not require any on-board source of energy or electronic components, wirelessly tackling the biomedical challenges where the workspace is restricted.

Intrinsically powered

Achieving locomotion that enables access to hard-to-reach areas is the first step to targeting biofilm infection. Intrinsically powered robots can harness energy from fuels (ionic or chemical) present in their environment for autonomous motion, eliminating the need for external energy. This self-propulsion is achieved via built-in energy conversion mechanisms, either from chemical reactions or microorganism-induced mobility [25].

The chemically powered approach includes electrochemical-driven ion gradients, bubble generation/enzyme-powered catalysis using chemical substrates (fuels), and combinations thereof [26]. In general, this type of robot is inspired by **Janus particles**, where the motion of these robots is governed by a fuel gradient [27]. For example, nanorod-like robots can propel forward through the ion concentration gradients generated by electrochemical reactions at their two ends (Figure 2A) [28]. The self-propulsion can also be induced by catalytic reactions where a chemical energy source is used [29]. Noble metals like Pt and Au or semiconductors like TiO₂ and ZnO are popular catalysts for H₂O₂ oxidation, producing oxygen bubbles for self-

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propulsion (Figure 2B) [30]. The autonomous motion of microrobots can mechanically disrupt the EPS, enhancing the accessibility and exposure of antimicrobials to the cells embedded within biofilms [31–33]. However, creating robots capable of sustained, autonomous movement using fuels in the environment remains an important challenge for catalysis-driven mechanisms to target biofilm infections.

Microrobots have been designed to use bioavailable sugar fuels like glucose by incorporating nanomaterials with glucose oxidase activity [34], but their use is limited to the air–liquid interface and requires high concentrations of glucose and oxygen [35]. Enzyme-functionalized microrobots, employing urease-driven propulsion, have been developed to improve mobility in biological fluids. For instance, an iridium/silica robot can be powered by self-diffusiophoresis in a urea solution whereby the iridium end creates solute molecules, inducing fluid flow from the silica end, pushing the robot forward (Figure 2C) [36]. However, the short working durations and limited mobility of these catalysis-based robots in biofluids hinder their widespread applicability.

To improve the movement of microrobots in biofluids, living organisms like algae can be integrated to form microorganism-powered microrobots (Figure 2D) [37]. Such robots possess self-propulsion based on flagella motion, making it an active drug delivery platform in biological settings. However, microorganisms must be kept in an environment supplying critical nutrients with suitable chemical conditions for survival.

In general, intrinsically powered microrobots hold the potential to address the limited ability of chemicals to access the microbes within formed biofilms. Despite improved mobility and functionality, lack of controlled directionality or speed of self-propulsion remains a critical drawback for precise biofilm targeting in the human body.

Extrinsically powered

To improve the directionality of the motion of the robot, various external power sources have been used to actuate microrobots, including acoustic, electric, optical, and magnetic fields and their combinations (Figure 2E) [38,39]. Magnetic fields stand out because of their distinct advantages, including untethered, fuel-free operation circumventing conventional propulsion challenges; penetration through biological tissues without cellular damage; versatile types and configurations of magnets providing highly tunable approaches for dynamic control of microrobots; retrievability of magnetic robots for safety; flexibility for combinations with other actuation methods; and easy integration with a wide range of materials. Magnetic fields can be also exploited to generate mechanical forces, trigger drug-release or induce thermal effects for antibiofilm applications and diagnostic sampling [40,41]. Given the well-recognized advantages of magnetic fields, we focus on magnetic microrobots for biofilm infection control.

Fundamentally, a magnetic field can exert **magnetic forces** and induce **magnetic torques** on a magnetic robot, effectively providing methods for remote control and navigation (Figure 2F). The use of magnetic force and torque depends on the type of robots and applications. By manipulating the magnetic field parameters, including field direction and magnitude, fine control over the movement, position and behavior of microrobots can be achieved. Magnetic forces and torques can produce a controllable shear force for biofilm physical disruption [42,43]. While magnetic force is mainly effective for direct biofilm removal, magnetic torques can also be effective for drug delivery applications by taking advantage of high controllability in positioning microrobots over a large workspace. For example, microrobots can be loaded with antimicrobial agents and directed towards biofilm infection site where the drug can be release in situ, increasing precision and efficacy. Furthermore, magnetic torque enables twists, turns, and spins, and the

Glossary

Biofilm recalcitrance: the resistance of biofilms to antimicrobial agents, such as antibiotics, disinfectants, or the host's immune system, originating from the complex physical and biological properties of the extracellular polymeric matrix as well as drug resistance/tolerance mechanisms arising from microbial cells residing within.

Extracellular polymeric substances (EPS): exopolysaccharides, fibrous and globular proteins (including extracellular enzymes), lipids, and nucleic acids as well as host-derived biomolecules. This matrix functions as a multifunctional scaffold, providing support, protection, and nutrition to embedded microbes while forming the essential structure of biofilms.

Ferrofluids: nanoscale magnetic particles suspended in a carrier fluid, creating dynamic structures via interactions of IONPs under magnetic fields to either create single entities that mimic biological systems, such as the diverse shapes of cells or swarm-like behavior.

Isthmus: a narrow gap (on order of hundreds of micrometers in width) between the root canals inside the tooth where bacterial biofilms often form. Currently available methods are unable to physically access or disinfect this anatomical region.

Janus particles: particles with two different sides where these particles can be asymmetrical in regard to material chemistry or shape to generate self-propulsion.

Magnetic force: a force that arises when the magnetic dipole moments of the robot respond to the magnetic field gradient, propelling the microrobot in the direction of increasing magnetic field intensity.

Magnetic torque: the rotational force experienced by a magnetic dipole when it is subjected to a magnetic field. This torque tends to align the dipole of the robot with the magnetic field direction.

Medical device: as defined by the US Food and Drug Administration (FDA), is based on risk level and intended use. Class I includes low-risk devices with well-understood and simple designs, subject to the least regulatory control. Class II includes moderate-risk devices with moderate complexity, warranting closer regulatory scrutiny. Class III includes high-risk devices that typically support or sustain human life, are

induced rotational motion stirs surrounding fluids which can enhance the diffusion of antimicrobials while imposing mechanical stress on biofilms [44].

Magnetic materials, readily available in different shapes and sizes, can serve as building blocks or be incorporated with biomaterials (e.g. hydrogels) and living cells to design a variety of microrobots. Such versatility offers multiple avenues for reconfigurability and control that can be tailored for specific antibiofilm applications. Despite the many advantages of magnetic control, there are some limitations. For instance, certain magnetic materials, such as nickel, are not biocompatible, with the potential to induce undesirable immune responses or introduce toxicity within the biological environments [19]. A common approach to make biocompatible magnetic microrobots is to embed polymers like hydrogels with magnetic nanoparticles to create composites. However, this often results in uneven particle distribution and has a volume limit of magnetic particles which can affect performance, reducing the effectiveness of the magnetic guidance [45]. The choice between soft magnetic and hard magnetic materials is crucial as it determines the magnetic property of the composite microrobots. Soft magnetic materials magnetize easily but demagnetize with weak magnetic fields, with their response largely influenced by microstructure and particle distribution. Hard magnetic materials offer a strong magnetization, due to their large magnetic hysteresis. However, fabricating microrobots with these materials requires postproduction magnetization under strong fields, complicating the manufacturing scalability [46]. Finally, the current magnetic control systems are bulky with multiple electromagnets [17], which requires miniaturization to broaden clinical applications.

Microrobots can be generally divided into individual robots and collectives of many units (known as swarms or aggregates) [38,47]. We focus on microrobots containing magnetic materials which have been more widely adopted for biofilm treatment.

Individual robots

Magnetic nanoparticles can be used directly as the primary building blocks or be incorporated as the magnetic filler in polymer matrix using 3D-printing or 3D-molds to fabricate microrobots [48]. Helical robots (Figure 2G) have gained attention for their potential in eradicating biofilm by leveraging shear forces from their motion toward constricted locations [49]. These microrobots can be tailored to exhibit specific functionalities, such as rigidity for drilling biofilm clogs and responsiveness to external factors (e.g. pH and light) by incorporating responsive materials for controlled drug delivery [48].

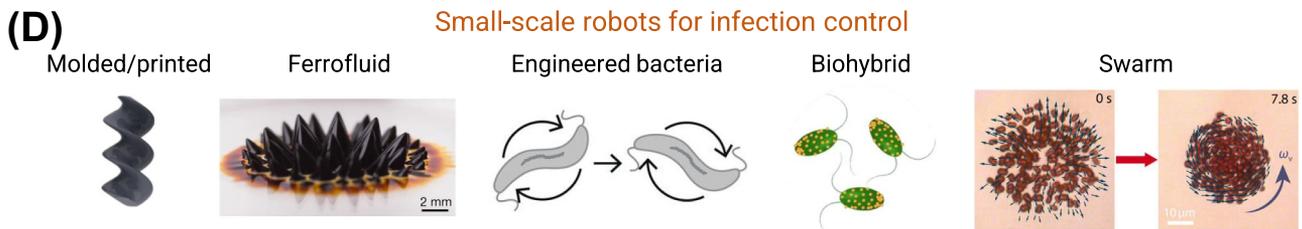
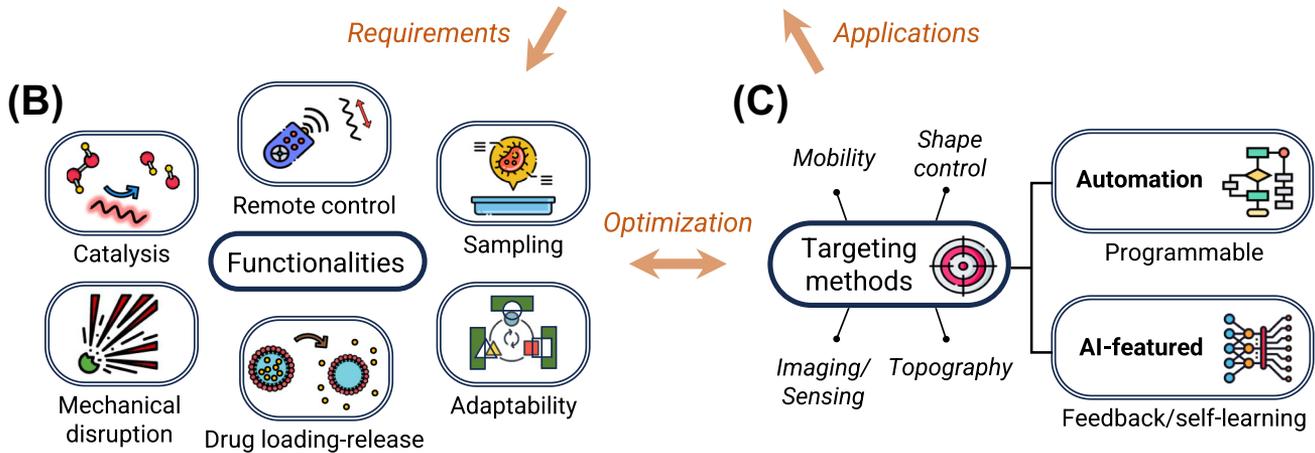
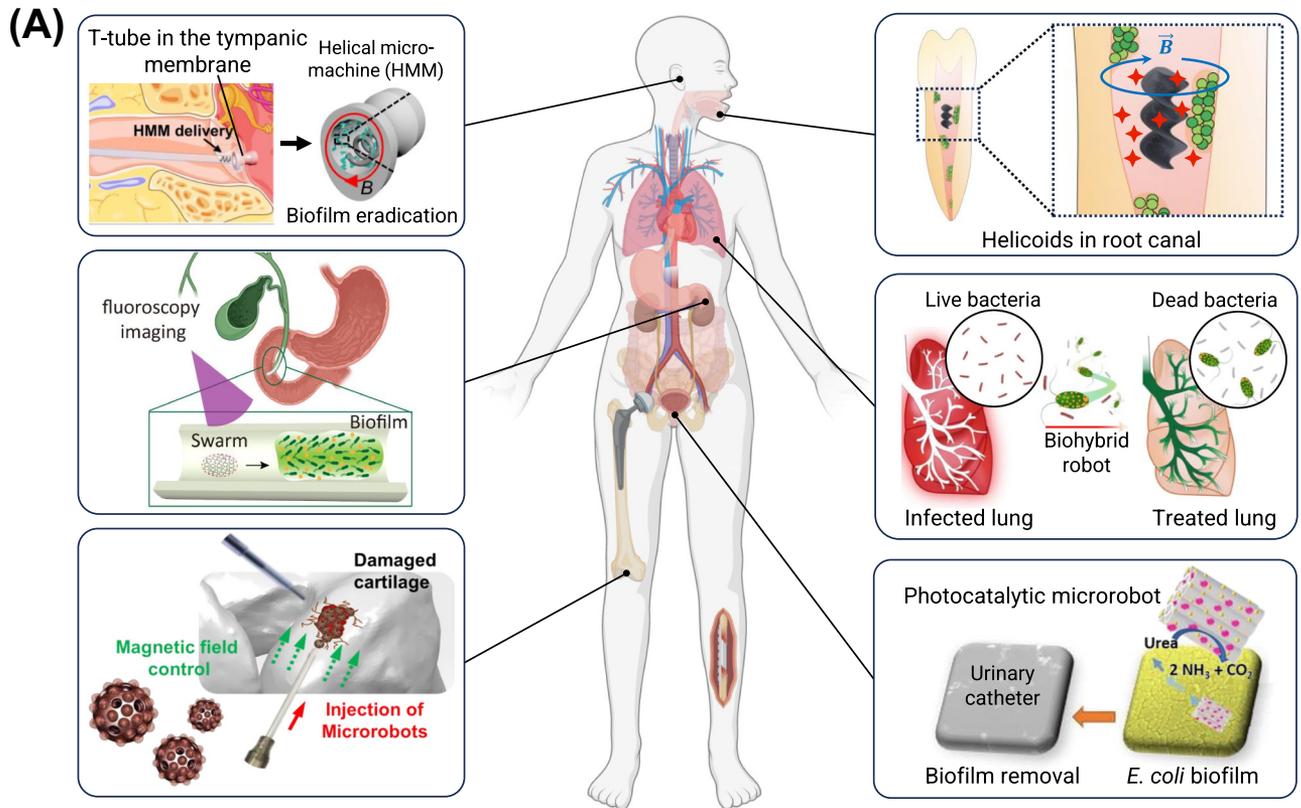
Collectives

Drawing inspiration from natural swarms like bird flocks or fish schools, robotic collectives can tackle complex tasks beyond the scope of individual robots. These collectives have a high surface-to-volume ratio, higher resistance against fluid shear, enhanced drug delivery capacity, and greater capability to reach target sites [50,51]. For example, nanoparticles can gather and form chain-like structures that break due to **viscous torque** when interactions weaken and then re-form as interactions strengthen (Figure 2H) [52,53]. Through magnetic manipulation these chains grow into a large ribbon-like swarm that can be split into smaller chains to pass through narrow spaces, reassembling afterwards to reach the target as a collective unit. Such programable functionality potentially allows effective navigation and transport of cargos to target sites [52]. Furthermore, magnetic field-directed assembly of iron oxide nanoparticles (IONPs) can create superstructures that exert high shear forces, enabling mechanical disruption of biofilms [42]. Recently, dynamic electromagnetic fields were employed to generate bristle-like superstructures that can extend or retract across micro-to-centimeter scales and adjust their shape, length, and stiffness, enabling them to enter narrow spaces to scrub and kill biofilms *in situ* [43].

implanted, or present potential risks beyond general controls' mitigation.

Physical intelligence: physically encoding sensing, actuation, control, memory, logic, computation, adaptation, learning and decision-making into the body of an agent.

Viscous torque: the twisting force that results from the resistance in a rotating system (robot) when it's moving through a viscous fluid. In essence, it's the force the robot needs to overcome due to the resistance to flow and viscosity of the fluid when a robot is turning or rotating through it.



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Box 1. What is an antibiofilm microrobot?

The definition of what constitutes a robot can be complex particularly at small scales, considered here as millimeter scale and below. Robotic systems larger than 1 cm typically integrate actuation, control and sensing on-board in a single entity. Due to technological limitations at smaller scales, systems for power, computation and tracking are commonly moved off-board. At scales from micrometers to millimeters, the element that is being controlled is commonly referred to as the (micro)robot and is part of a larger robotic system. Most often, this robot has a specified functionality in terms of shape (e.g., helicoid), chemistry (e.g., catalytic), composition (materials) or behavior (individual or swarm). We understand that individual nanoparticles with controlled actuation could be considered robotic, but for treatment of biofilm infections robotic systems tend to integrate higher levels of functionality. Since this review covers the progress and potential of microrobotic systems as applied to biofilm removal and human infection, the functionality of the system is an important element of defining what constitutes an antibiofilm microrobot. Thus, systems that enable (i) controlled behavior (as individual or swarms) to navigate towards the target area to access the biofilm infection site, and (ii) exert antibiofilm functionalities (physical or chemical degradation and killing of the microbes, drug delivery, sampling retrieval or pathogen detection for diagnostics) are those we consider to have microrobotic characteristics (Figure 1).

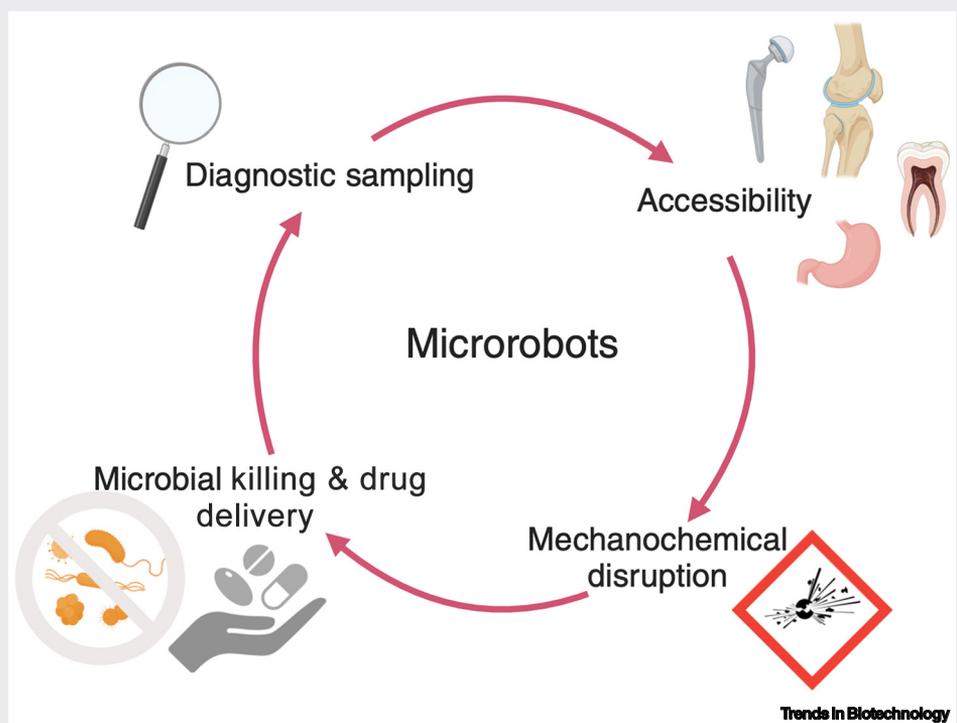
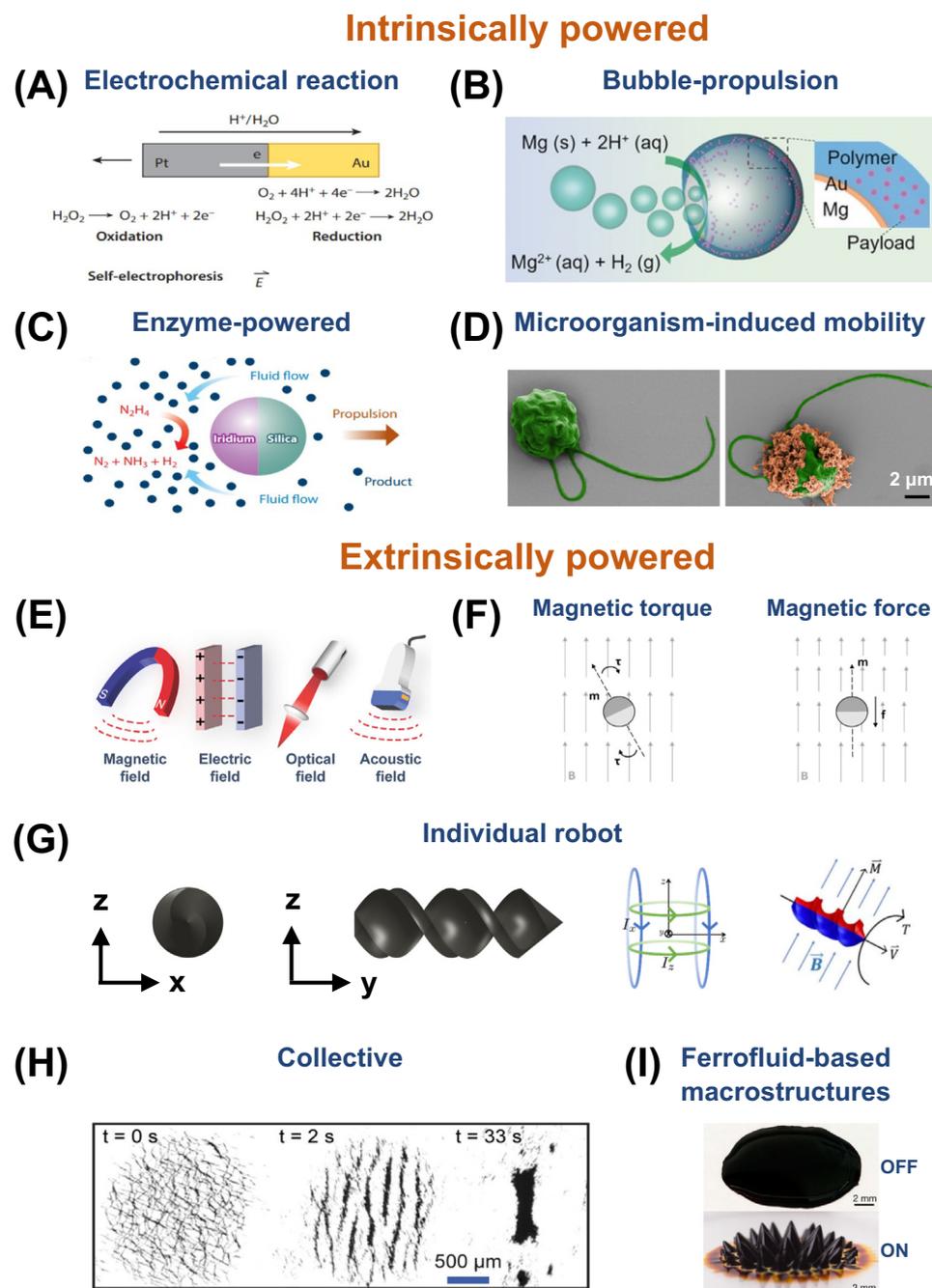


Figure 1. Microrobotic-based approach for targeting biofilms.

Ferrofluids

Owing to their remarkable reconfigurability and deformability, magnetic liquids termed **ferrofluids** can be potentially used to develop multifunctional robots for targeting biofilm [54,55]. For example, a hierarchical magneto-responsive composite surface infused with ferrofluid can undergo various multiscale topographical reconfigurations using magnetic field gradients, leading to self-assembly of the infused lubricant from micro-to-millimeter scale, and

Figure 1. Diagram depicting the unique features, types and use in targeting biofilm infections across the human body by microrobots. Inaccessible surfaces and difficult-to-reach locations due to physical confinement, microscale operational spaces, complex geometries, topographies and anatomical structures provide major treatment challenges. The combination of unique microrobotics functionalities offers a compelling advantage for dealing with the complex nature of biofilm infections in biomedicine. Image reproduced, with permission, from [33,42,56,60,61,66,69,85,86].



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Figure 2. Motion actuation methods. Intrinsically powered through (A) electrochemical reactions. Image reproduced, with permission, from [28]. (B) Bubble generation. Image reproduced, with permission, from [30]. (C) Enzyme-powered catalysis. Image reproduced, with permission, from [36]. (D) Microorganism-induced mobility. Image reproduced, with permission, from [69]. (E) Schematic of different extrinsically powered methods. Image reproduced, with permission, from [38]. (F) Schematic of magnetic torque and magnetic force. Image reproduced from [87] under a Creative Commons license. (G) Individual helicoidal robot and its motion principle. Image reproduced, with permission, from [42]. (H) Formation and manipulation of robotic swarms based on magnetic nanoparticles. Image reproduced from [52] under a Creative Commons license. (I) Ferrofluid-based macrostructures. Image reproduced, with permission, from [56].

switchable adhesion and friction liquid pumping on a centimeter scale that interferes with biofilm formation (Figure 2I) [56]. However, the high deformability of ferrofluids might hinder their ability to physically scrub preformed biofilms. This could be addressed by including additional functionalized nanomaterials to improve the ferrofluid properties to enhance biofilm removal.

The versatility and design flexibility of microrobot-based approaches provides extensive control options for targeting biofilms. Intrinsic and extrinsic powering can be combined to address the limitations of each other [57–60]. For example, the lack of directionality of self-propulsion, catalysis-driven microrobots can be ameliorated by including additional control elements, such as light, acoustic wave, or magnetic source to improve antibiofilm efficacy.

Targeting biofilm infections

The human body presents many niches where biofilms grow on hard-to-reach anatomical and implanted surfaces (Figure 1A) providing unique challenges that require access to the site of infection, adjustment to different surface topographies, physical disruption, and chemical treatment (Figure 3A–D). Upon reaching the target, the EPS matrix (i.e. protective material of the biofilm) must be breached to expose the bacteria, including dormant cells. Once exposed, the microbes can be killed by on-site chemical reactions or drug delivery (Figure 3C,D). Finally, biofilm sampling can be integrated to obtain microbiome data or detect pathogens to diagnose early infections. These robotic functionalities can be tailored to address specific clinical challenges across the body to effectively detect and treat biofilm infection, prevent recolonization due to residual biofilm components and increase susceptibility to antimicrobial therapy through physical disruption of the biofilm [61–63].

Accessibility and precision targeting

Magnetically directed assemblies of IONPs can reconfigure and adapt to confined/complex surfaces via tetherless, tunable magnetic fields to treat biofilms on teeth, in the root canal and on oral mucosal surfaces. IONPs can assemble into a bristle-like shape that can be controlled with microscale precision to conform to varying topographies and anatomical features while adapting to curved geometries of teeth (Figures 3B and 4A) [43]. These assemblies can also access complex and narrow anatomical sites such as **isthmuses** inside the tooth that are inaccessible using current clinical instrumentation [49]. Using programmable algorithms, IONP-derived assemblies performed precise spatial targeting of mucosal fungal biofilms without damaging surrounding host tissue avoiding off-target effects (Figure 4B). Magnetic fields have been also used to control the movement of IONP-decorated microrobots over craniofacial meshes [64] and plate implants [65], in addition to guiding individual robots and swarms to remote locations such as the inner ear [66] and the biliary tree [61].

Intrinsically powered microrobots containing photoactive nanoparticles made from black-TiO₂ can self-propel through catalytic reactions activated by a broad range of light wavelengths from UV to the near infrared allowing movement over the surface of biofilm covered titanium miniplates to perform localized antibiofilm action (Figure 4C) [67]. Similarly, hollow rod-shaped diatom particles filled with manganese oxide nanocatalysts or tubular TiO₂ microrobots decorated with platinum nanoparticles can generate oxygen bubbles when exposed to H₂O₂, which has been exploited for self-propulsion to facilitate cleaning motion over biofilm infected skin wound or dental surfaces [32,68]. TiO₂/CdS-microrobots coated with ureases self-propel in urine due to the enzymatic reactions for targeting urinary catheter biofilms [33]. The natural motion of *Chlamydomonas reinhardtii* has been used to create an intrinsically powered hybrid microrobot, capable of moving through biological fluids and achieving diffusion throughout the deepest lung tissues [69].

For administration in the human body, microrobots can be deployed endoscopically, bronchoscopically, via a catheter or orally in a safe manner during standard medical or dental procedures. For example, helical IONP-microrobots introduced following endodontic surgery can be guided via remote magnetic control toward the apical end of the root canal to perform antibiofilm functions (Figure 3E) [42,49]. The complex anatomy of the ear or the gastrointestinal system can be bypassed to deliver microrobots to their target using endoscopes (Figure 4D,G) [61,66]. The bacterially contaminated upper airway can be avoided by bronchoscopic delivery of hybrid microrobots directly into the trachea [69]. The possibility of using current medical equipment for delivery of microrobots near target areas (macroscale accessibility) combined with their ability to adapt different surface topographies and navigate tight spaces (microscale accessibility) provide feasible and precise biofilm targeting methods.

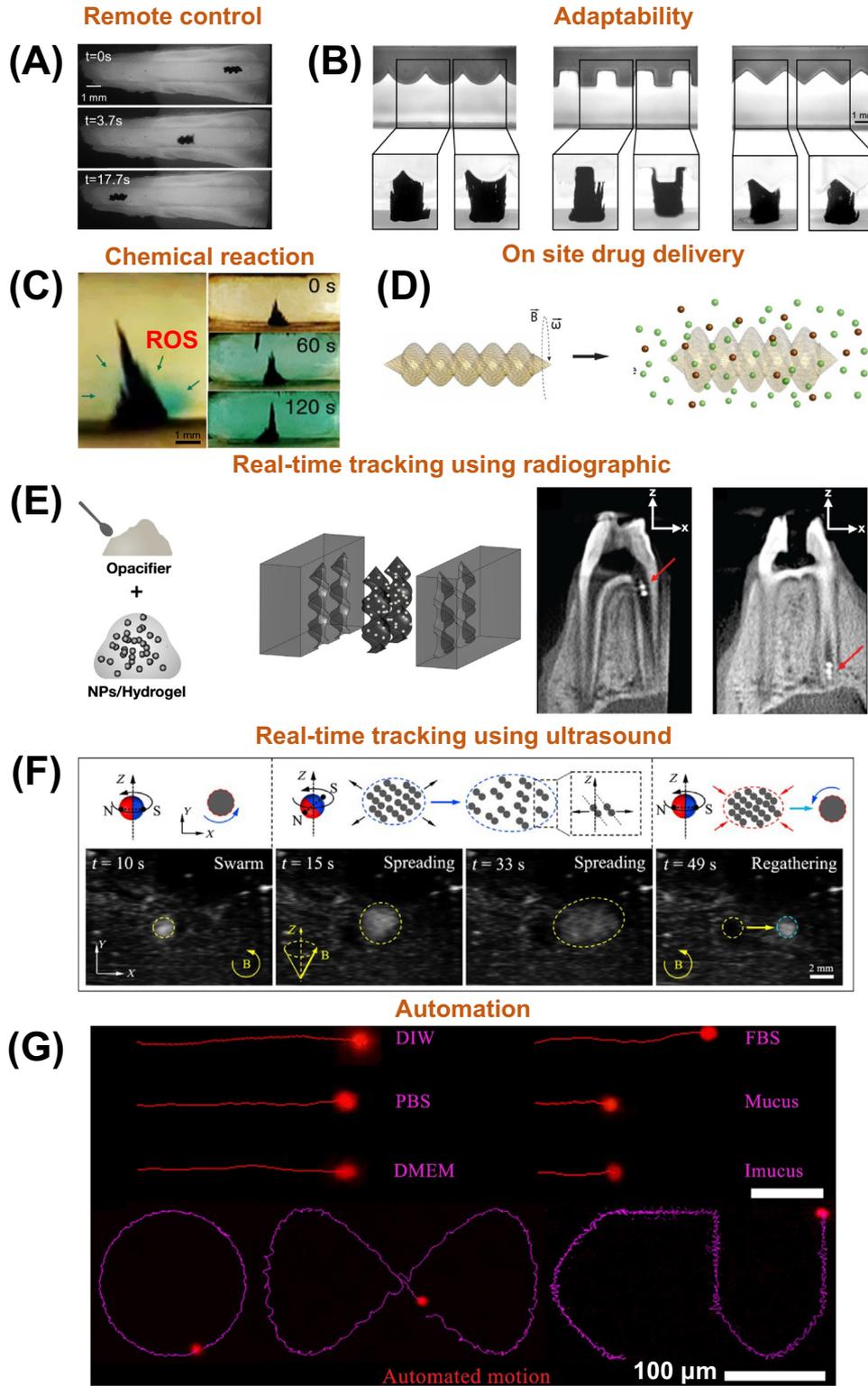
Physical biofilm degradation

Microrobots capable of physically penetrating and degrading the protective EPS matrix allow direct microbial damage, boosting the effectiveness of antimicrobials delivered on site. Physical abrasion can be achieved using microrobots via diverse mechanisms allowing for site and microbe specific treatment protocols. Bristle-like IONP superstructures that are capable of generating 10–230 N/m surface shear stress can be used to mechanically remove plaque from the dental surface [43], or IONPs can form collectives that generate a vortex motion sufficient to penetrate the EPS matrix [64]. 3D molded IONP-based helicoid microrobots can physically disrupt biofilms in enclosed spaces such as the apical region of a root canal where biofilms are commonly found as a result of chronic odontogenic infections [42,49,66]. Magnetic hyperthermia created by alternating magnetic fields can be combined with physical disruption to enhance biofilm degradation as shown in a mouse model of skin infection [70]. Apart from friction-based techniques, the fracture of EPS can be achieved by using the force of bursting bubbles to penetrate *Pseudomonas aeruginosa* biofilms [68] or through spiked architecture that tears the EPS apart when the microrobots come into contact with the target area as demonstrated in the biofilm clearance of biliary stents [61].

Combining physical breakdown with chemical degradation can weaken the biofilm structure and increase drug access to the embedded microbes. Chemical degradation can be achieved intrinsically through reactive oxygen species (ROS)-generation by nanocatalysts inherent to the microrobot material [42,70] or by including EPS-degrading enzymes like glucanohydrolases, dispersin, or DNAses [71] to the treatment protocol, thereby enhancing biofilm dispersion and mechanical removal [42]. Photocatalytic magnetic microrobots made of BiVO₄ (for ROS generation) and Fe₃O₄ (magnetic actuator) use the combination of these two materials to disrupt *Staphylococcus aureus* biofilms formed on titanium mesh implants [65]. Another example includes the integration of catalytically produced oxygen bubbles with an antimicrobial solution containing gentamicin for biofilm degradation, resulting in 99% of microbial cell removal in a skin infection model [68].

Drug delivery and microbial killing

Concurrently, with physical disruption, the antimicrobial activity can be enhanced through on-site drug delivery. The most common mechanism is ROS generation, either through inherent catalytic activity of nanomaterials in the presence of substrate or via photocatalysis after exposure to specific light wavelengths [33]. The *in situ* ROS generation by microrobots has been used to kill biofilm microbes in the oral cavity, inside the small diameter of the tympanostomy tube [66], on the skin surface [70], and within the urinary bladder. Likewise, a tubular black-TiO₂ microrobot decorated with Ag nanoparticles can release antimicrobial Ag ions upon irradiation to kill biofilms from facial implants [67], while microrobots releasing magnetic liquid metal droplets that morph into



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spine shaped structures can penetrate and destroy bacteria to kill biofilms clogging biliary stents [61].

To improve efficacy, it is crucial to increase drug localization and accumulation at the infection site through *in situ* delivery (Figure 4E,F) or selective pathogen binding while avoiding off-target effects. For example, IONP assemblies have been shown to preferentially bind to *Candida albicans* (fungal pathogen) over human gingival cells/tissue, enabling localized accumulation and targeted ROS generation *in situ*, potentiating antifungal effects [72]. Conversely, microrobots can be loaded with chemotactic agents like l-aspartic acid that attracts and captures *Escherichia coli*, enhancing antimicrobial specificity [61].

Drug loading and controlled release can be integrated into biofilm-targeting microrobots for transport and localized delivery. For example, click-chemistry can graft ciprofloxacin-loaded nanoparticles with neutrophil coatings to microalgae, protecting against macrophage clearance while achieving pathogen killing by lung-targeted microrobots (Figure 2D) [69]. Conversely, polyethyl-eneimine has been used to load ampicillin onto microrobots and transport the drug to the implant surface. This compound is a β -lactam potentiator, which counters the β -lactamase-based antimicrobial resistance mechanisms, thereby increasing antimicrobial efficacy. Cargo can be also released in response to pathological environment (pH or toxins) or by magnetic actuation allowing on-site drug delivery.

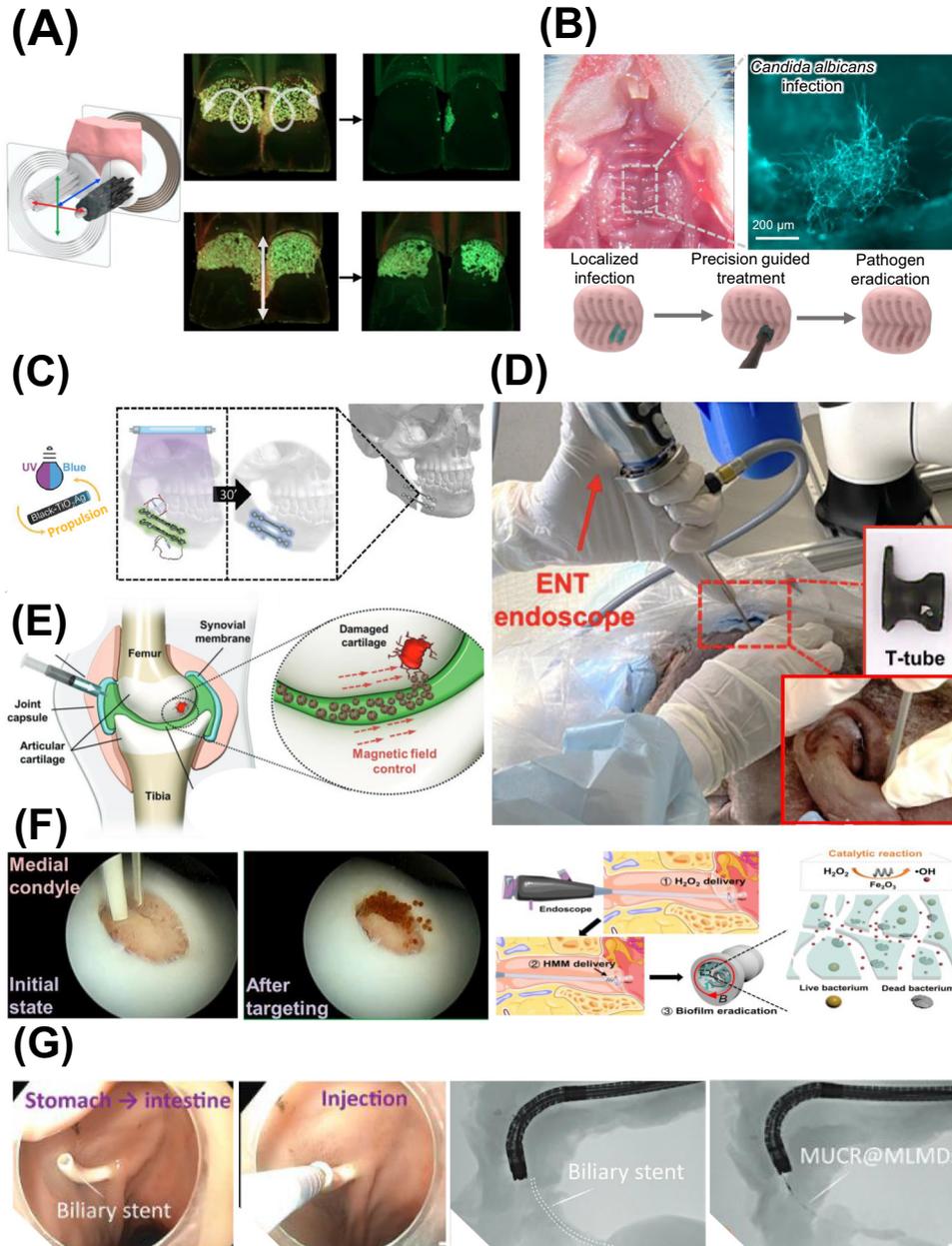
Diagnostic sampling and pathogen detection

Microrobots offer alternatives for diagnostic sampling by navigating complex pathways to retrieve samples that require surgically invasive procedures. For example, IONPs–bristles can capture and retrieve microbes (bacteria, fungi, and viruses) and biomolecules (enzymes) in sufficient quantities for biofilm composition analyses [49,73,74]. In addition, sensors can be integrated into microrobots that can bind and detect pathogens *in situ* through real-time feedback. For instance, fluorescent magnetic spore-based microrobots can detect *Clostridium difficile* toxins and be tracked in real time [75]. Antimicrobial-peptide-coated microrobots have been used for *in vivo* biofilm infection [76], opening possibilities for using peptide chemistry for selective pathogen binding and detection. Robotic systems integrating physical sampling with sensor technology can enhance diagnostic accuracy, which combined with real-time feedback and *in situ* detection capabilities could lead to early disease intervention [77]. However, this area of microrobotics remains underexplored.

Visualization and automation

Research in microrobotics has largely been conducted in artificial settings. Moving to the human body introduces significant hurdles like tracking and visualization in obscured environments, navigating intricate tissue structures, and working with dynamic biological fluids. A variety of

Figure 3. Functionality of microrobots for targeting biofilm purposes. (A) Navigation to hard-to-reach areas through remote control. Image reproduced, with permission, from [42]. (B) Surface topography adaptability. Image reproduced from [43] under a Creative Commons license. (C) On-site chemical reaction through catalytic property as shown by blue color generation due to reactive oxygen species (ROS) production from H_2O_2 catalysis by microrobotic bristle-like superstructures made of iron oxide nanoparticles with peroxidase-like activity. Image reproduced from [43] under a Creative Commons license. (D) Drug-loading and releasing by helicoid microrobot. Image reproduced from [88] under a Creative Commons license. (E) A microrobot loaded with opacifiers inside the mini-pig root canal, showing possibilities for both (i) visualization using a simple inclusion of opacifiers and currently available equipment and (ii) tracking to the intended target area (apical end of the root canal). Image reproduced, with permission, from [49]. (F) Microrobots with real-time tracking ability using ultrasound. Image reproduced from [89] under a Creative Commons license. (G) Automation of microrobots using real-time feedback through fluorescence emission. Image reproduced from [75] under a Creative Commons license.



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Figure 4. *In vivo* and clinical applications for microrobotics. (A) Use of iron oxide nanoparticle (IONP) bristles to remove biofilm, kill bacteria, and retrieve samples from the interproximal space. Image reproduced from [43] under a Creative Commons license. (B) Precision-guided spatial targeting of fungal infection using IONP-made microrobotic assemblies. Image reproduced, with permission, from [72]. (C) Intrinsically powered nanorobots remove multispecies biofilm from facial titanium miniplates [67]. (D) Endoscopic delivery of helical microrobots to target site in ear for clearance of biofilm from tympanostomy tube. Image reproduced from [66] under a Creative Commons license. (E) Magnetically actuated microsccaffold with ability for targeted drug/cell delivery to damaged articular cartilage. Image reproduced from [90] under a Creative Commons license. (F) Time lapse sequence of microrobots during and after targeting a site of cartilage damage on the medial condyle of the femur and the patella in an ex vivo porcine model. Image reproduced, with permission, from [85]. (G) Endoscopic delivery and fluoroscopic visualization of magnetic urchin-like capsule robots to target biofilm within biliary stents. Image reproduced, with permission, from [61].

visualization methods based on magnetism, acoustics and radiation have been investigated using standard medical imaging equipment (Figure 3E,F) [78]. Endoscopes with magnetic deploying devices have been used not only to place the microrobots in proximity to their target but also for direct visualization of the robotic movement at biofilm infection sites in the ears [66] and gastrointestinal tract [61]. Tracking and control functions are usually external, so fully automated demonstrations mostly involve extrinsically powered microrobots, where imaging directly informs actuation [79]. To facilitate visualization, robots can be optimized for enhanced contrast. For example, inclusion of opacifiers in helicoidal microrobots magnetically controlled inside the mini-pig root canal shows possibilities for both visualizing movement of the robot and guiding it towards the infected area (i.e. apical end of the root canal) (Figure 3E), unobstructed by the surrounding hard tissue [49].

For automated navigation in complex geometries, embedding physical intelligence in the robot's design can enhance its functionality. For example, swarms of nanoparticles excel due to their shape adaptability while conforming to surface topography [43]. Swarms can navigate in dynamic biological flow environments [80]. For instance, a wall-roller strategy inspired by immune cells aids movement by avoiding the highest velocity of blood flow in central region of the vasculature [81], which would be applicable to biofilms formed in the inner walls of catheter-based infections. The possibility of combining physical intelligence (or sensor-based real-time feedback, Figure 3G) with path-planning algorithms could potentially allow self-adjustment as the robot navigates across intricate anatomical areas and flow dynamics to target hard-to-reach biofilm infection sites. However, current microrobots are unable to make autonomous decisions, a factor that may be improved with deep learning techniques [80]. As methods for fabrication, actuation, sensing and control are improved and miniaturized in conjunction with AI-optimized navigation [80], automation may be within reach in the near future.

Concluding remarks

Advances in microrobotics are creating previously unimagined opportunities to diagnose, treat and prevent biofilm-mediated infections while controlling the spread of antimicrobial resistance. Significant progress has been made in the design and fabrication of multifunctional microrobots. Their unique capabilities can be summarized into five main categories.

- (1) Tether-free control and high-precision targeting. Diverse shape and control modalities (particularly magnetic) enable unparalleled flexibility to design robots (as individual or collective) to navigate different physiological environments and anatomical complexities to achieve localized therapeutic effects in difficult-to-reach locations.
- (2) On-site drug delivery and chemical reactions. Chemical agents, including antimicrobials and biologics, can be loaded into robotic structures, while free radicals can be generated *in situ* by catalytic robots. Furthermore, selective pathogen binding-and-killing can be achieved. These properties can maximize killing potency and minimize off-target effects on healthy tissues and host microbiota while hindering antimicrobial resistance development.
- (3) Physical intelligence. Reconfigurable robots can adapt to different surface topographies and geometries, access confined spaces, and exert mechanical forces by adjusting their shape, stiffness and rheology. These properties enhance physical biofilm disruption and enable sample retrieval.
- (4) Pathogen detection and diagnostics. Selective pathogen binding and physical biofilm sampling can be achieved using biomolecules, such as bioadhesive proteins, antibodies and lectins, or physical entrapment. Conversely, pathogens, biomarkers or toxins can be detected in real-time via imaging, fluorescence or electrochemical signals.

Outstanding questions

How can hardware designs be improved for portability, affordability and accessibility to overcome barriers to widespread implementation of robotics approaches for biofilm treatment and diagnostics?

How can automation of robots *in vivo* be improved, particularly in deep tissue, especially considering advancements in machine learning and artificial intelligence?

Considering the current technological capabilities and limitations, which specific biofilm related infections are most suited for advancement to *in vivo* and human trials?

What considerations should be taken into account regarding the biosafety of microrobots including removal or biodegradation, and what strategies should be implemented to address these concerns in future research?

How can sensors or on-demand activation be implemented to provide real-time diagnostics or controlled drug release for biofilm detection and infection treatment?

- (5) Multitasking and automation. Simultaneous diagnostics and mechanochemical treatment can be achieved for effective biofilm eradication in a single platform. For example, magnetic IONP superstructures can retrieve biofilm samples for pathogen detection while performing physical disruption and ROS-mediated bacterial killing *in situ*. Imaging and tracking with AI integration could realize automation for spatial targeting and biofilm treatment.

A key challenge to accelerate clinical translation is how to improve locomotion, real-time tracking, and biocompatibility with mechano-chemical functionalities. Feasibility must be assessed in complex physiological environments and different anatomical niches without causing tissue damage, immunogenic effects or changes in the host microbiota. Furthermore, most *in vivo* studies have not fully demonstrated disease resolution and lack standard protocols from microrobot delivery procedures to post-operative management. Some areas for improvement include:

- (1) Multitasking microrobots. Physical parameters (including shape, texture, structure) combined with surface modification, coating or functionalization can enhance mobility in biological fluids and tissues while executing specific tasks (pathogen sensing or on-site drug delivery). Self-assembly and fabrication techniques with advances in 3D printing and click chemistry can create robots with arbitrary shape and structure with different coatings or loads. Physical capture can be integrated to enable diagnostic sampling.
- (2) Practical actuation systems. One challenge of external control systems is complexity and size, often requiring large components typically found in medical facilities. Specifically, magnetic manipulation systems must improve efficiency and precision of microrobots. The magnetic field generated by non-superconducting electromagnetic actuation systems are relatively weak, limiting applied forces. However, application-specific, portable control systems can be developed, such as using mobile permanent magnets in place of electromagnets to minimize costs and heating concerns. The complexity of the physiological environment requires a stronger propulsion ability and fine spatial control, and more *in vivo* tests are urgently needed (see [Outstanding questions](#)).
- (3) Harnessing imaging technology. Enhancing precise maneuvering with real-time localization and monitoring through visualization techniques is imperative. While conventional medical imaging methods like X-ray, MRI, and ultrasound face limitations in real-time tracking, combining these techniques can minimize latency and navigation issues. Inclusion of opacifiers as demonstrated for endodontic microrobots can greatly improve visualization and tracking. However, a key challenge for real-time tracking is the need for fast imaging at high resolution. Incorporating machine learning algorithms could improve tracking and control for closed-loop operation of microrobots *in vivo*. Furthermore, integrating imaging diagnostics for pathogen or toxin detection would improve targeting.
- (4) Addressing biosafety. Ensuring safety and biocompatibility is critical for clinical implementation ([Box 2](#)). Existing FDA-approved nanomaterials (such as IONPs) can be used as building blocks, or biocompatible coatings/capsules can be employed for cloaking to avoid inflammatory response by the immunogenic effects while biodegradable or retrievable robots can alleviate safety concerns. Microrobots can be retrieved using magnets in catheter-like devices while biodegradable hydrogels allow microrobots to be decomposed by surrounding enzymes, local physiological conditions or in a time-controlled manner. Therefore, designing microrobots with biocompatible

Box 2. Biosafety, regulatory challenges, and translational opportunities

The use of small-scale robotics in biomedicine is a new field and as such microrobot-based modalities will need to overcome several technical, regulatory, and market challenges along with demonstrating safety and efficacy before they can reach human trials. There are multiple regulatory agencies, such as the US Food and Drug Administration, European Medicines Agency, and the Japan Pharmaceutical Manufacturers Association, that will need to evaluate and approve the use of microrobotic platforms in the clinic, which is costly and time-consuming. Whether these treatment systems are classified as a medication, or, more likely, as a **medical device** and its various classes will guide the regulatory frameworks for clinical trials and the safety/biocompatibility testing requirements. Ideally, the fate of the medical microrobot after it has performed its desired function would be reabsorption by the body, retrieval by a medical device or by excretion from the host system.

Existing knowledge about the preclinical safety and toxicity of small-scale robotic systems to target biofilm infections is primarily based on evaluation of blood parameters and histology of tissues and major organs after a single dose of therapy [69,82]. While this is an important first step, in-depth studies are required to determine the toxicity and immunogenic effects of repeated dosing, longer treatment periods and resident time or accumulation at the target site [20]. Alternatively, these potential issues can likely be ameliorated by creating biodegradable microrobots, cloaking with cell membranes/biocoatings or by retrieving the robots [83]. Retrieval has the added benefit of allowing for sampling of the treated area, such as obtaining a localized biofilm sample for microbiome sequencing or pathogen detection [43]. Artificial intelligence could also help to increase safety; local path planning algorithms could help train the robots to navigate in the unknown and dynamically changing biological environments for targeting and retrieval.

Finally, the reproducibility and cost need to be considered when scaling up the production of microrobots for commercial use and appropriate methods of sterilization and storage [84]. Securing funding for early development, prototyping, and patent applications remains a major barrier on top of manufacturing and regulatory costs. Concurrently, technological developments without proper protection are unlikely to receive investment from the private sector. Hence, kickstarting new robotics technologies could benefit from academic-industry partnerships and academic-entrepreneurship. In sum, researchers must design efficient and safe robots that meet the requirements of low-cost, large-scale/high volume preparation in GMP facilities using environmentally friendly fabrication technologies, while cost-effective and portable actuation devices must be developed.

coatings with tunable surface charge, stretchability, hydrophilicity, morphology, and degradability could limit adverse results and improve safety. Although existing microrobots have shown low toxicity, more *in vivo* experiments are required for verification, paving the way for clinical trials.

Many groundbreaking approaches are rapidly emerging to allow high spatial maneuverability, on-demand reconfigurability, and precise programmability. Cooperation between material scientists, engineers and roboticists with biomedical experts and clinicians is essential to achieve translation of microrobots into clinical applications. Long-term safety assessment remains crucial before large-scale clinical trials. Despite challenges, solutions are increasingly within reach due to a dynamic scientific community, rapid advances in manufacturing and lowering component costs. The design and control flexibility combined with multifunctionality and multitasking capability of microrobotics provide unparalleled opportunities to advance the standard of care against biofilm infections that are becoming obsolete in the face of increasing antimicrobial resistance.

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Declaration of interests

HK and ES have an interest in patent application Small-scale Robots for Biofilm Eradication 17/291,326. HK, ES and AB have an interest in patent application Automated and Precise Device for Dental Plaque Detection, Monitoring and Removal 17/764,587. HK, ES, AB and MJK have an interest in patent application Wireless Retrieval of Biological Samples for Diagnostics 63/339,132.

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