REVIEW

Biocompatible micromotors for biosensing

Roberto Maria-Hormigos[1,2] · Beatriz Jurado-Sánchez[1,3] · Alberto Escarpa[1,3]

Abstract

Micro/nanomotors are nanoscale devices that have been explored in various fields, such as drug delivery, environmental remediation, or biosensing and diagnosis. The use of micro/nanomotors has grown considerably over the past few years, partially because of the advantages that they offer in the development of new conceptual avenues in biosensing. This is due to their propulsion and intermixing in solution compared with their respective static forms, which enables motion-based detection methods and/or decreases bioassay time. This review focuses on the impacts of micro/nanomotors on biosensing research in the last 2 years. An overview of designs for bioreceptor attachment to micro/nanomotors is given. Recent developments have focused on chemically propelled micromotors using external fuels, commonly hydrogen peroxide. However, the associated fuel toxicity and inconvenience of use in relevant biological samples such as blood have prompted researchers to explore new micro/nanomotor biosensing approaches based on biocompatible propulsion sources such as magnetic or ultrasound fields. The main advances in biocompatible propulsion sources for micro/nanomotors as novel biosensing platforms are discussed and grouped by their propulsion-driven forces. The relevant analytical applications are discussed and representatively illustrated. Moreover, envisioning future biosensing applications, the principal advantages of micro/nanomotor synthesis using biocompatible and biodegradable materials are given. The review concludes with a realistic drawing on the present and future perspectives.

Keywords Micromotors · Biosensing · Propulsion · Biofluid · Biomedical analysis

Introduction

Self-propelled micro/nanomotors (MNMs) are micro- or nanoscale particles that represent an innovative tool for various fields such as drug delivery, environmental remediation

✉ Roberto Maria-Hormigos
  roberto.maria@uah.es

✉ Alberto Escarpa
  alberto.escarpa@uah.es

1   Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering, University of Alcala, Alcala de Henares, 28871 Madrid, Spain

2   Future Energy and Innovation Laboratory, Central European Institute of Technology, Brno University of Technology (CEITEC-BUT), Purkyňova 123, 61200 Brno, Czech Republic

3   Chemical Research Institute "Andres M. Del Rio", Alcala de Henares, 28871 Madrid, Spain

and monitoring, and diagnosis [1–4]. The use of MNMs has grown considerably over the past few years due to their intrinsic properties of propulsion and consequent intermixing in solution compared with their respective static forms [nanoparticles (NPs) or other static substrates] [5–10]. Autonomous propulsion of MNMs allows the design of new motion-based bioassays and/or decreases analysis time and volume samples of existing procedures. Such propulsion of these microscale objects can be achieved by a chemical reaction occurring at the interface of the device and the liquid environment (i.e., bubble, Marangoni propulsion, and self-electrophoresis) [5, 11–13] or by providing an external energy input from a power source (i.e., ultrasound, magnetic and electromagnetic radiation) [14].

Indeed, the fuel toxicity and the inconvenience of use in relevant samples such as blood have prompted researchers to explore biocompatible means of propulsion. Recent progress in fuel-free and biocompatible catalytic (enzymatic and biohybrid) MNMs has led to novel biosensing applications. Some aspects have already been reviewed, such as the use of Janus particles (static and dynamic) for analytical applications and the use of light-driven micromotors for

bioanalytical applications, with a deep focus on propulsion mechanisms and as in our previous review that described the progress in micromotors for analytical applications [15–17]. However, these advances in biocompatible means of propulsion have not been reviewed to date from this point of view.

The aim of this review is to discuss recent developments (last 2 years) on MNMs in biosensing, with special emphasis on fuel-free propulsion schemes. First, an overview of bioreceptor attachment to catalytic MNMs for biosensing applications is provided as an example of biofunctionalization possibilities. Secondly, we briefly describe the synthesis and biocompatible propulsion mechanisms of the main MNMs used for biosensing, along with key analytical applications. We conclude with the limitations and advantages of such novel biosensing approaches and the future perspectives and directions in the field.

Catalytic MNMs for biosensing

MNMs can be smartly functionalized with bioreceptors such as antibodies [18–20], peptides [21], aptamers [22], lectins [23, 24], and ligands [25, 26], as well as through the use of molecularly imprinted polymer technology [27].

Antibodies for MNM-based immunoassays have generally been the main strategy for micromotor development in past years due to their selectivity, sensitivity, and ability to tune the sensing steps, thereby avoiding matrix effects [28–33]. One example of antibody-functionalized MNMs is the development of a sandwich immunoassay for C-reactive protein (CRP) determination carried out by our research group. Catalytically reduced graphene oxide (rGO)/Ni/PtNP micromotors were functionalized with the capture antibody (anti-CRP) by covalent bonding. In the first step, these anti-CRP micromotors were incubated with the plasma sample, the secondary antibody (tagged with horseradish peroxidase, HRP), surfactant, and fuel solution (H_2O_2) to perform the immunosandwich in one step. Then, micromotor propulsion was stopped, and thanks to their magnetic characteristics (intermediate magnetic layer of nickel), micromotors were separated from the sample. Finally, micromotors were added to an electrochemical transducer for signal readout from benzoquinone reduction mediated by the HRP-tagged secondary antibody (Fig. 1a) [28]. As can be seen in this example, the combination of propulsion, magnetic, and biorecognition properties of antibody-functionalized MNMs allows the development of rapid immunoassays in few-microliter samples that can be exploited by biocompatible means of propulsion.

Aptamers are an interesting alternative to antibodies for genetic biomarker detection, as the use of complementary chains for mutation detection is a highly reliable biosensing or diagnostic approach [34–39]. Aptamers have been explored in connection with MNM technology for motion-based and fluorescence (bio)sensing schemes. For example, Zhang et al. developed jellyfish-like (Au/Ag/Ni/Ag convex-concave structure) micromotors for DNA sensing. The concave side of the micromotor was modified with a thiolate aptamer-catalase complex. Under H_2O_2 addition, micromotors were propelled by the catalase enzymatic reaction, and as a result of target DNA hybridization, micromotor propulsion was hampered (Fig. 1b) [34]. This is an example of how MNMs can benefit from aptamer functionalization for DNA sensing applications.

Affinity peptides have also been explored as an alternative to antibodies or aptamers for biofunctionalized MNM biosensor development [40–42]. For example, our group developed catalytic Janus micromotors wrapped with two-dimensional (2D) nanomaterials including graphdiyne, black phosphorus, and graphene oxide for cholera toxin B determination. In the first step, micromotors were incubated with the peptide probe, and the adsorption of the peptide in the 2D nanomaterials produced the quenching of fluorescence-labeled peptide probe (OFF). Then, in the presence of the target molecule (cholera toxin B), biorecognition between the target and the peptide was achieved, the complex was detached from the micromotors, and the fluorescence of the peptide was recovered (ON) (Fig. 1c) [40].

Table 1 summarizes the catalytic MNMs designed and developed for biosensing applications during the last 2 years. As can be seen, myriad biosensing strategies using MNMs can be identified. However, they are all based on the use of hydrogen peroxide as fuel for micromotor propulsion. The high fuel concentration needed for MNM propulsion made their use impossible in whole blood samples, where hydrogen peroxide produces a foam and interference from its reaction with the catalase present in red blood cells. This encourage the development of MNM-based biosensing based on biocompatible propulsion mechanisms, specially for in vivo applications.

Biocompatible MNM propulsion in biosensing applications

Biocatalytic MNMs

Enzymatic micromotors rely on propulsion by biocompatible substances and even metabolites present in the sample to generate catalytic reactions that induce motion, overcoming the need for toxic fuels in specific applications [43–47]. For example, urease can be used for urea decomposition for MNM propulsion by an ionic self-diffusiophoretic mechanism [48]. Luo et al. developed a urease-powered Janus urease-Au/magnetic micromotor as self-sensor for urea determination. As propulsion was clearly dependent on urea concentration, motion-based urea determination was possible using this micromotor [44]. Patiño et al. functionalized urease-SiO_2 hollow micromotors with DNA pH-switches. These micromotors can navigate urea solution for

Fig. 1 MNM functionalization strategies for biosensing applications. (**A**) Tubular MNMs functionalized with antibodies for electrochemical detection of CRP sepsis biomarker detection. Reprinted with permission from [28]. (**B**) Janus MNMs modified with aptamers for motion-based sensing of DNA. Reprinted with permission from [34]. (**C**) Janus MNMs modified with peptides for the affinity fluorescence sensing of cholera toxin B. Reprinted with permission from [40]
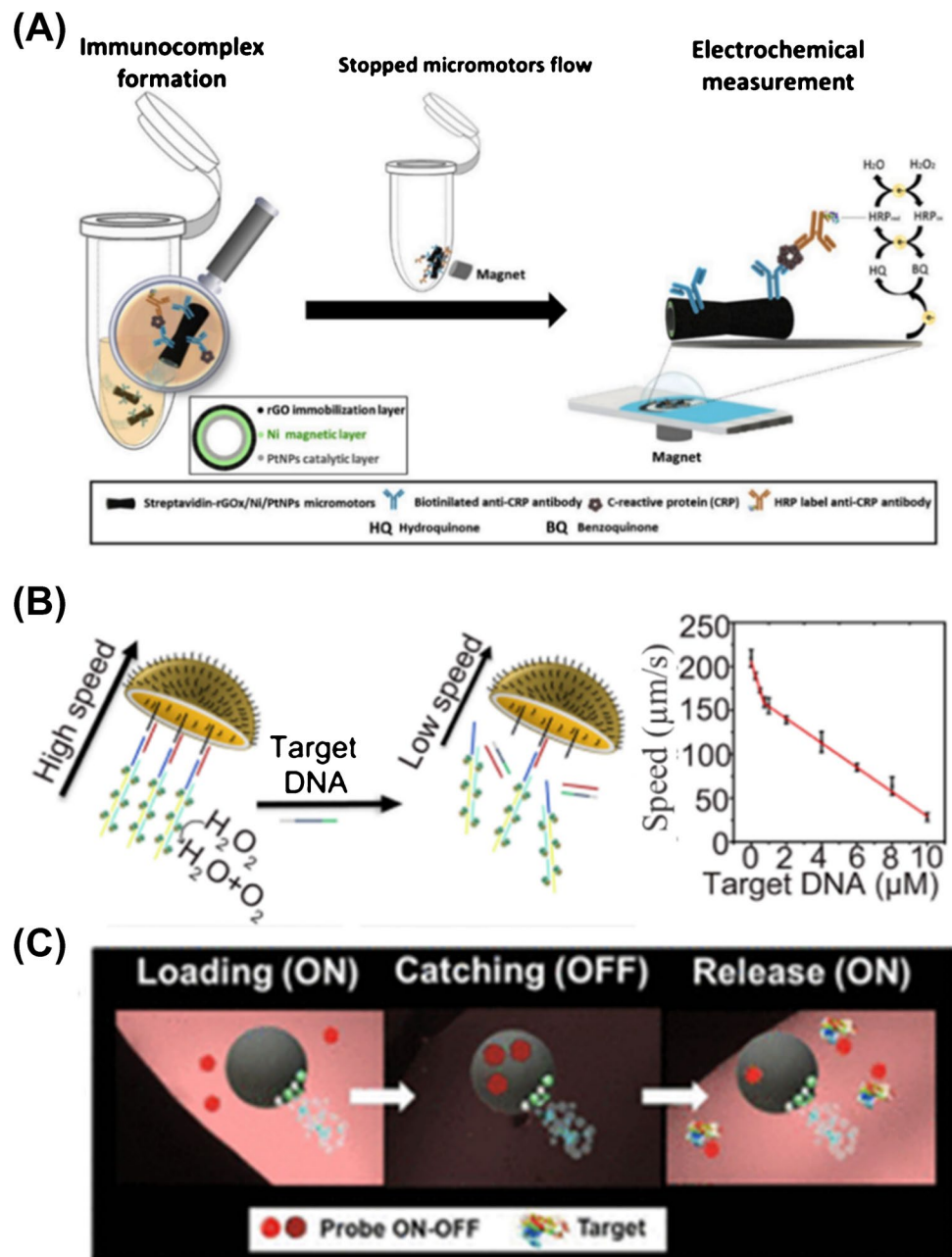
pH sensing and enzyme activity determination by fluorescence detection. At acidic pH (< 6), fluorescence resonance energy transfer (FRET) was the main signal obtained, but at pH > 6, DNA switches opened and FRET was hampered relative to the normal fluorophore emission [45]. Yuan et al. designed a $SiO_2/NaYF_4$:Yb/Tm Janus nanomotor functionalized on a SiO_2 face with urease and HRP enzymes. As a result of uric acid oxidation, H_2O_2 was generated and used by the HRP enzyme for ortho-phenylenediamine (OPD)-mediated oxidation (oxOPD). oxOPD near-infrared (NIR) light adsorption hampered Janus nanomotor luminescence under the same NIR irradiation, which enabled uric acid biosensing (Fig. 2A) [46].

Enzymatic MNMs have great potential for enantiomeric biosensing due to their intrinsic enzymatic enantiomeric selectivity. Arnaboldi et al. developed enzymatic bilirubin oxidase millimeter boats functionalized with enantiomeric oligothiophene for selective enantiomeric 3,4-dihydroxyphenylalanine detection/oxidation on the boat tail. Because of asymmetric oxidation of 3,4-dihydroxyphenylalanine on both sides of the boat tail, electron flux on the boat was asymmetric as well. Consequently, the swimmer trajectory is clockwise or counterclockwise, as a function of the enantiomer present in solution, and the curvature of the track can be used as a direct readout of enantiomeric excess (Fig. 2B) [47].

Table 1 Catalytic MNMs for biosensing applications*

Fuel (H_2O_2) concentration	(Bio) receptor/analyte	Detection (tag)	Approach/application	Assay time (min)	Ref.
1%	Anti-CRP/CRP	Electrochemical (HRP)	*On-the-fly* immunoassay/ sepsis diagnosis**	35	[28]
1%	Anti-CRP/CRP	Electrochemical microfluidic (HRP)	*On-the-fly* immunoassay and *on-flow* detection/sepsis diagnosis**	8	[29]
2%	Anti-PCT/PCT	Fluorescence (FITC)	*On-the-fly* immunoassay, "OFF-ON"/ sepsis diagnosis**	30	[30]
10%	Anti-PCT/PCT	Colorimetry (particle density)	Motion trigger detection-based immunoassay/sepsis diagnosis**	13	[31]
0.5%	Anti-IgG/IgG	Electrochemical (AgNPs)	*On-the-fly* immunoassay/ inflammation	10	[32]
0.8%	Anti-IgG/IgG	Electrochemical (IrO_2/Pt HER)	*On-the-fly* immunoassay/ inflammation	45	[33]
5%	Aptamer HIV-1	Motion-based	DNA hybridization/ HIV diagnosis	7	[35]
10%	Aptamer miRNA-21	Fluorescence (FAM)	DNA tag "ON-OFF"/ cancer diagnosis	30	[37]
3%	Aptamer miRNA-21	Motion-based/fluorescence (FAM)	DNA tag "OFF-ON"/ cancer diagnosis	45	[38]
1% (Ultrasound-assisted)	Aptamer miRNA-21	Fluorescence (FAM)	DNA tag "OFF-ON"/ cancer diagnosis	31	[39]
3%	Peptide anti-*cholera* toxin B	Fluorescence (rhodamine)	Peptide affinity "ON-OFF-ON"/ bacteria diagnosis	10	[40]
3.75%	Peptide anti-*E. coli* endotoxin	Fluorescence (rhodamine)	Peptide affinity "OFF-ON"/ bacteria diagnosis	1	[41]

*HER: hydrogen evolution reaction; FITC: fluorescein isothiocyanate; FAM: 6-carboxyfluorescein

**Reported results below disease cutoff.

Another biocompatible MNM propulsion method relies on the natural movement of organisms such as bacteria or sperm. The combination of these biological systems with inorganic or other organic sensing materials to create biohybrids has been used for biomedical applications, such as drug delivery or imaging, and offers great potential for biosensing applications [49]. However, only Sun et al. have developed a hybrid micromotor based on flagella bacteria for sensing purposes. They used *Bacillus subtilis* bacteria genetic engineering to create biosensing platforms based on fluorescence readout of biological pathways in the presence of the target molecule. Moreover, they demonstrated the capacity of inorganic particle attachment to bacteria to create hybrid MNMs (Fig. 2C) [50].

Magnetic-driven MNMs

Magnetic fields have been used in traditional biosensing for many years because of the possibility for simplified separation and preconcentration steps. Moreover, they are a harmless source of energy that can be used to propel MNMs even in vivo [51, 52]. Zhang et al. modified *Ganoderma lucidum* spores with Fe_3O_4 magnetic NPs and fluorescent carbon dots (CDs) to produce magnetic fluorescent hybrid micromotors. As result of CD modification in the hydrothermal process, the spore hybrid micromotor surface is mainly composed of oligosaccharides. Therefore, spore hybrid micromotors have high affinity for amino acid residues (repetitive oligopeptides) on the *Clostridium difficile* (*C. diff*) toxin that quenches the fluorescence of propelled micromotors to create a motion-based fluorescence biosensor (Fig. 3A) [53]. Yu et al. also used motion-based detection for glucose and cholesterol biosensing. They synthesized a Janus SiO_2/Ni microdimer whose amplitude, speed, and angle of rotation were shown to correlate with glucose and cholesterol concentrations [54]. Uygun et al. fabricated chitosan/Ag/Ni magnetic nanomotors. They explored double-stranded DNA motion-based detection due to the absorption and electrostatic properties of chitosan against negatively charged DNA. The attachment of DNA to the nanomotors caused a decrease in their motion speed [55].

Fig. 2 Enzymatic and biohybrid MNMs in biosensing applications. (**A**) Schematic illustration of enzymatic propulsion and uric acid detection by SiO2/NaYF4:Yb/Tm Janus nanomotors (**a**) and luminescence emission spectra of micromotors after the addition of different concentrations of uric acid (**b**). Reprinted with permission from [46]. (**B**) Design of the enantio-sensitive boat and propulsion mechanism (**a**) clockwise and counterclockwise trajectory, as a function of the DOPA enantiomer present in solution (**b**) and curvature direct readout of enantiomeric excess (**c**). Reprinted with permission from [47]. (**C**) Design and mechanism of two-component system-based signal transduction in bacteria connects specific inputs with a measurable output response for a hybrid biosensor platform design (**a**) and *B. subtilis* hybrid biosensor microswimmer time-lapse images with fluorescence response in the presence of target bacitracin and inorganic cargo towing (**b**). Reprinted with permission from [50]
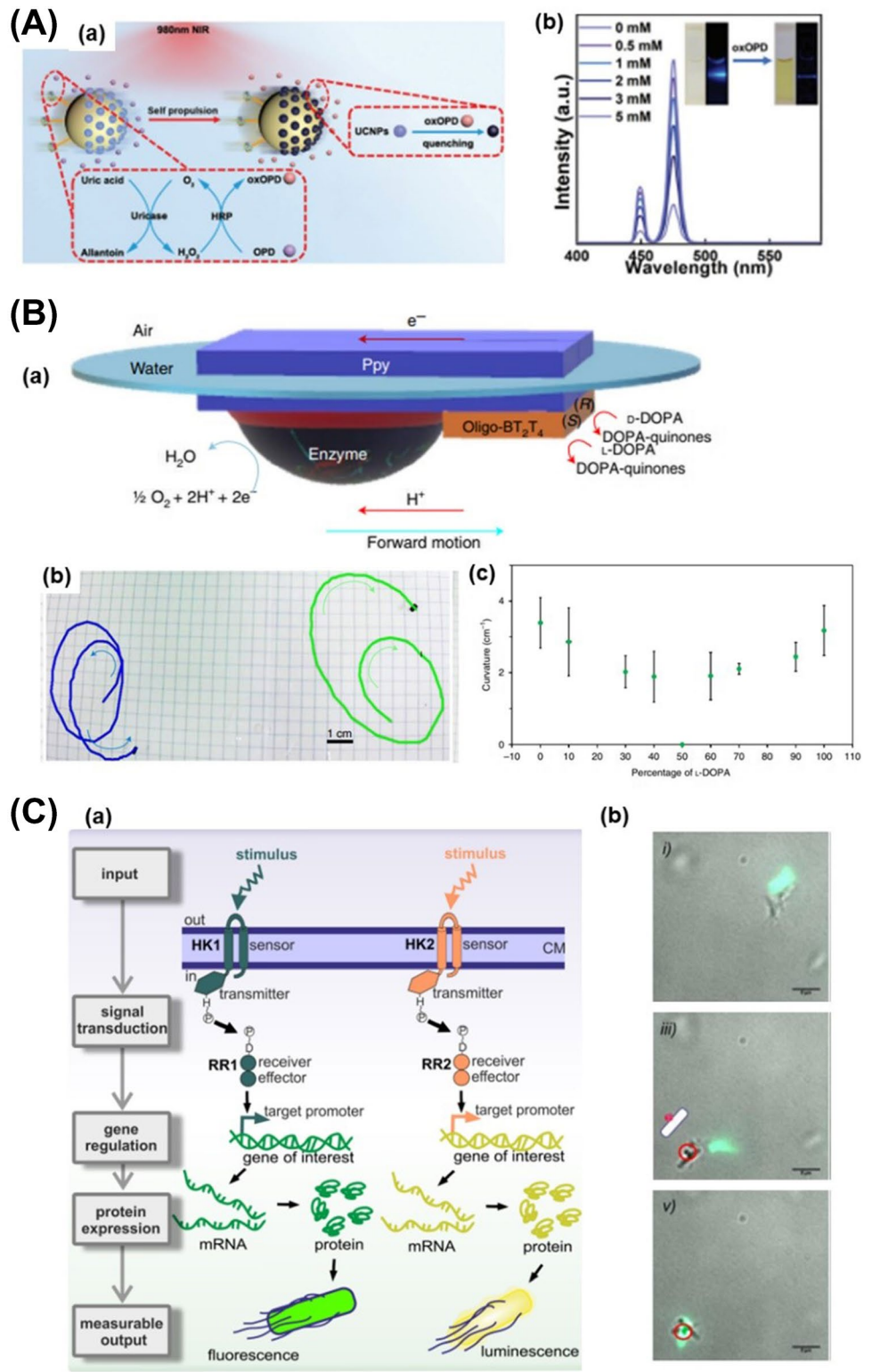
Surface-enhanced Raman spectroscopy (SERS) is a powerful analytical technique that provides fingerprint molecular information by analyzing the Raman shift in the optical scattering of molecular bonds. In this sense, Ma et al. prepared Fe$_3$O$_4$ magnetic particles covered with SiO$_2$ and

AgNPs to create magnetic nanorods that were propelled under a magnetic field. These nanomotors act as a SERS substrate that can navigate and be taken up by eukaryotic cells for SERS biosensing of different intracellular molecules (Fig. 3B) [56]. Ma et al. also used these magnetic

Fig. 3 Magnetic MNMs in biosensing applications. (**A**) Schematic illustration of the preparation of fluorescence and magnetic spore micromotors and the detection strategy for *C. diff* bacterial toxin from diluted hospital samples. Reprinted with permission from [53]. (**B**) SERS magnetic nanomotor probes for in vivo biosensing. (**a**) Schematic illustration of the fabrication and SERS sensing of the magnetic nanomotors. (**b**) Video snapshots of a magnetic nanomotor rotating inside a living cell at different time intervals. (**c**) Typical SERS spectra from the site of the magnetic nanomotor within an intracellular environment after rotation. Reprinted with modifications with permission from [56]. (**C**) Schematic representation of (**i**) MagRobots modified with antibody against SARS-CoV-2 spike protein (SP) that is (**iii**) driven using a transverse rotating magnetic field in the presence of SARS-CoV-2 SP and (**ii**) secondary antibody against SARS-CoV-2 SP labeled with Ag–AuNRs. (**iv**) MagRobots show collective self-assembly through the immunosandwich assay of SARS-CoV-2. (**v**) The detection was performed through hydrogen evolution reaction (HER) of Ag–AuNRs. Reprinted with permission from [58]
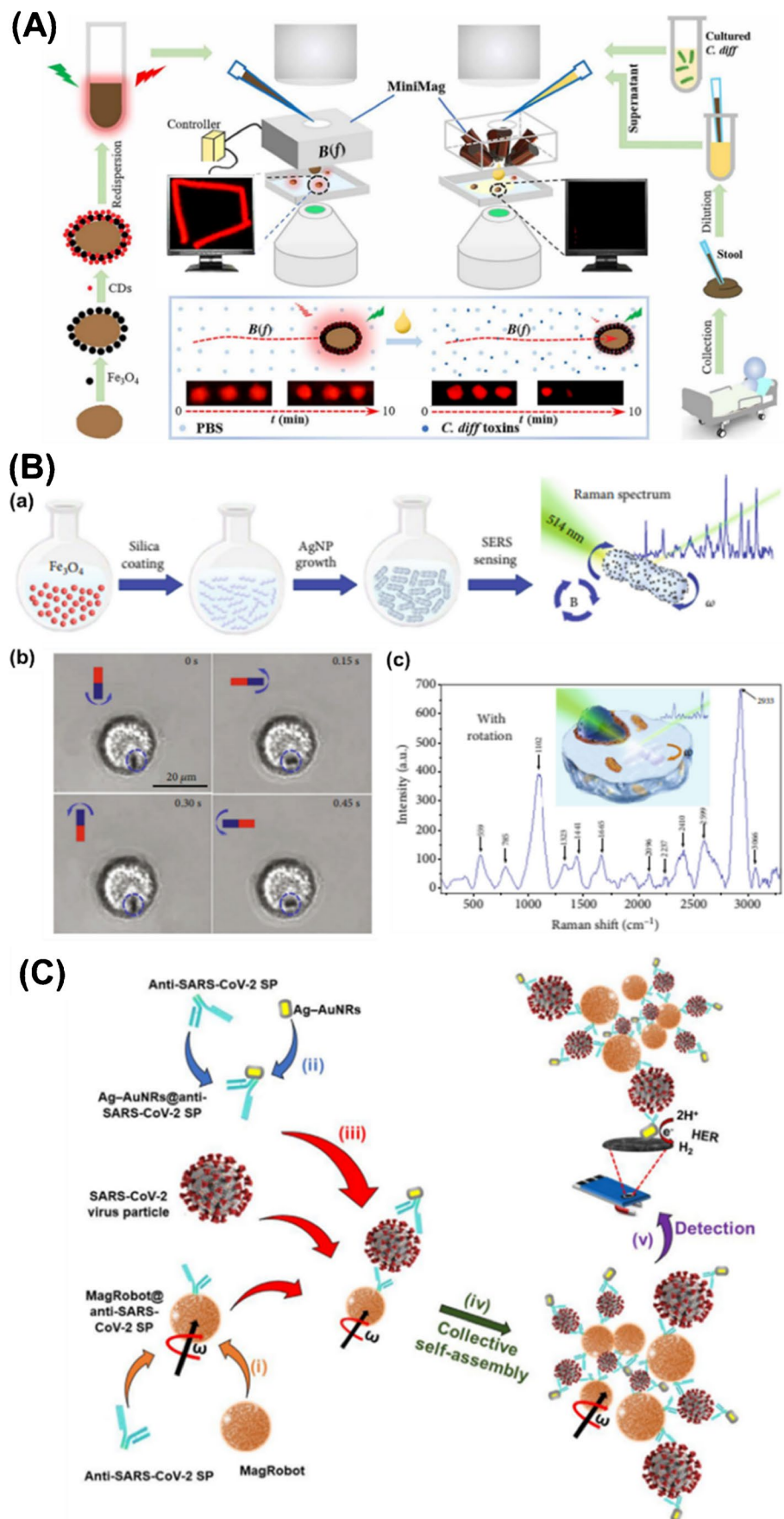
nanorods for antibody functionalization. They developed an automatic magnetic-assisted immunoassay in a microfluidic platform for procalcitonin (PCT) biomarker colorimetric determination [57].

The SARS-CoV-2 pandemic situation over the past 2 years has had a great impact on biosensors, with the development of fast, accurate, and affordable methods for SARS-CoV-2 screening. Pumera et al. developed a magnetic nanomotor for the electrochemical determination of the virus spike protein (VSP) as a biomarker. Commercial magnetic nanoparticles were functionalized with anti-VSP antibodies. These particles were able to be propelled under a magnetic rotating magnetic field to perform VSP on-the-fly biorecognition. A secondary antibody tagged with a Ag-Au nanorod was used for the electrochemical detection of the VSP determination due to HER on a commercial electrochemical platform (Fig. 3C) [58].

Light-driven MNMs

Light is a suitable external stimulus to trigger micromotor propulsion, avoiding the use of sophisticated setups. Indeed, nanomotors and micromotors can be viewed as solar cells with a suitable (asymmetric) design integrating photocatalytic or photoactive materials [59–62]. Even though the main efforts in light-driven MNMs are still focused on achieving efficient propulsion under less powerful and more biocompatible light sources, some MNM biosensing approaches have been developed in the last 2 years exploiting light as a source of energy for their propulsion [63, 64]. Ma et al. developed matchlike nanomotors with a body of Ag nanowire covered with SiO_2 (Ag@SiO_2 NWs) with a AgCl tip to achieve self-diffusiophoresis propulsion due to photocatalytic decomposition of the AgCl tail. A spontaneous aggregation of the nanomotors at the center of the light beam was observed when they were exposed to a focused UV light. Moreover, Ag@SiO_2 NWs acted as SERS substrate for crystal violet and breast cancer cell detection (Fig. 4A) [63]. Xu et al. fabricated a magnetic NIR light-driven fluorescent nanomotor with efficient navigation in human blood samples for circulating tumor cell (CTC) determination (MHS/Pt/F/aE nanomotors). The MHS/Pt/F/aE nanomotors were obtained from a Fe_3O_4 NP covered with a SiO_2 and Pt NPs and functionalized with a FITC fluorescent probe and antibodies against epithelial cell adhesion molecule (anti-EpCAM). PtNPs exhibited absorption and scattering in the NIR light bands due to the surface plasmon resonance effect. Then, the absorbed energy can be further converted into heat energy, causing nanomotor propulsion in the thermophoretic mechanism. Finally, the nanomotors are attached to CTCs, enabling their isolation from the blood sample and fluorescence determination (Fig. 4B) [64].

Acoustically driven MNMs

Ultrasound-driven propulsion mechanisms greatly enhance the prospects for biomedical applications of MNMs due to their compatibility with ultrasound sources of biomedical instrumentation, enabling biosensing even inside living cells [65–69]. Xu et al. developed gold nanorods functionalized with aptamers against microRNA (miRNA)-1246. Self-propulsion of gold nanorods was achieved at 1.8 MHz for *on-the-fly* biorecognition. Then, aggregation was induced at low frequency (620 kHz) to form a cluster. DNA detection was performed using the gold nanorods as SERS substrate (Fig. 5A) [68]. Wang et al. designed ultrasound-propelled graphene-oxide-coated gold nanowire motors, functionalized with fluorescein-labeled DNA aptamers (FAM-AIB1-apt), for the qualitative detection of overexpressed AIB1 oncoproteins in MCF-7 breast cancer cells. The fluorescence of the labeled aptamer was quenched by the graphene oxide nanomotor surface. FAM-AIB1-aptamer-modified nanowires propelled by ultrasound penetrated the cells. In the presence of the target molecule (overexpressed in breast cancer cells), FAM-AIB1-aptamer was released from the nanowire surfaces, and the fluorescence of the labeled aptamers was recovered inside the cells for the OFF-ON detection of MCF-7 breast cancer cells (Fig. 5B) [69].

MNMs propelled by biocompatible sources of propulsion are promising platforms for biosensing due to the enhanced mixing (especially in low-volume samples) and easy isolation from the sample for attachment and simplified methodologies. To provide an overview of the field in the last 2 years, MNMs for biosensing applications using biocompatible means of propulsion sources are summarized in Table 2. As can be seen, huge progress in biosensing has been achieved using MNMs propelled by a biocompatible source of energy. However, further efforts are needed to implement these methodologies in real clinical samples, given the lack of real sample analysis, as the studies described herein only used simulated samples or media in most cases and comparison with hospital reference values.

Moreover, while it is not the focus of this review, we want to highlight the importance of considering how media composition (pH, ions, ionic force, viscosity, immune system, etc.), biofunctionalization, biorecognition, and propulsion mechanisms affect one another [70, 71]. In order to obtain MNMs with suitable bioanalytical performance for their intended application, all these interactions should be addressed. For example, propulsion mechanisms such as enzymatic propulsion can produce a solution pH change when protons are implicated that can interfere with the biorecognition event and vice versa. The same occurs with thermal changes produced in light-driven micromotor or high-powered ultrasound applications that produce

Fig. 4 Light-driven MNMs in biosensing applications. (**A**) Fabrication and self-propulsion of the matchlike nanomotor (**a**). Illustration (**b**) and video snapshots showing the phototactic behavior of the nanomotors (**c**). Light-guided biochemical SERS sensing by the phototactic nanomotor. Schematic illustrations of SERS sensing of crystal violet (**d**) and cancer cells (**f**). Raman spectra and intensity change of characteristic peaks of crystal violet (**e**) and cancer cells (**g**) before and after nanomotor light irradiation for 10 s. Reprinted with permission from [63]. (**B**) Schematic illustration of NIR-driven fluorescent magnetic nanomotor application for the isolation and detection of CTCs in blood samples. Reprinted with permission from [64]
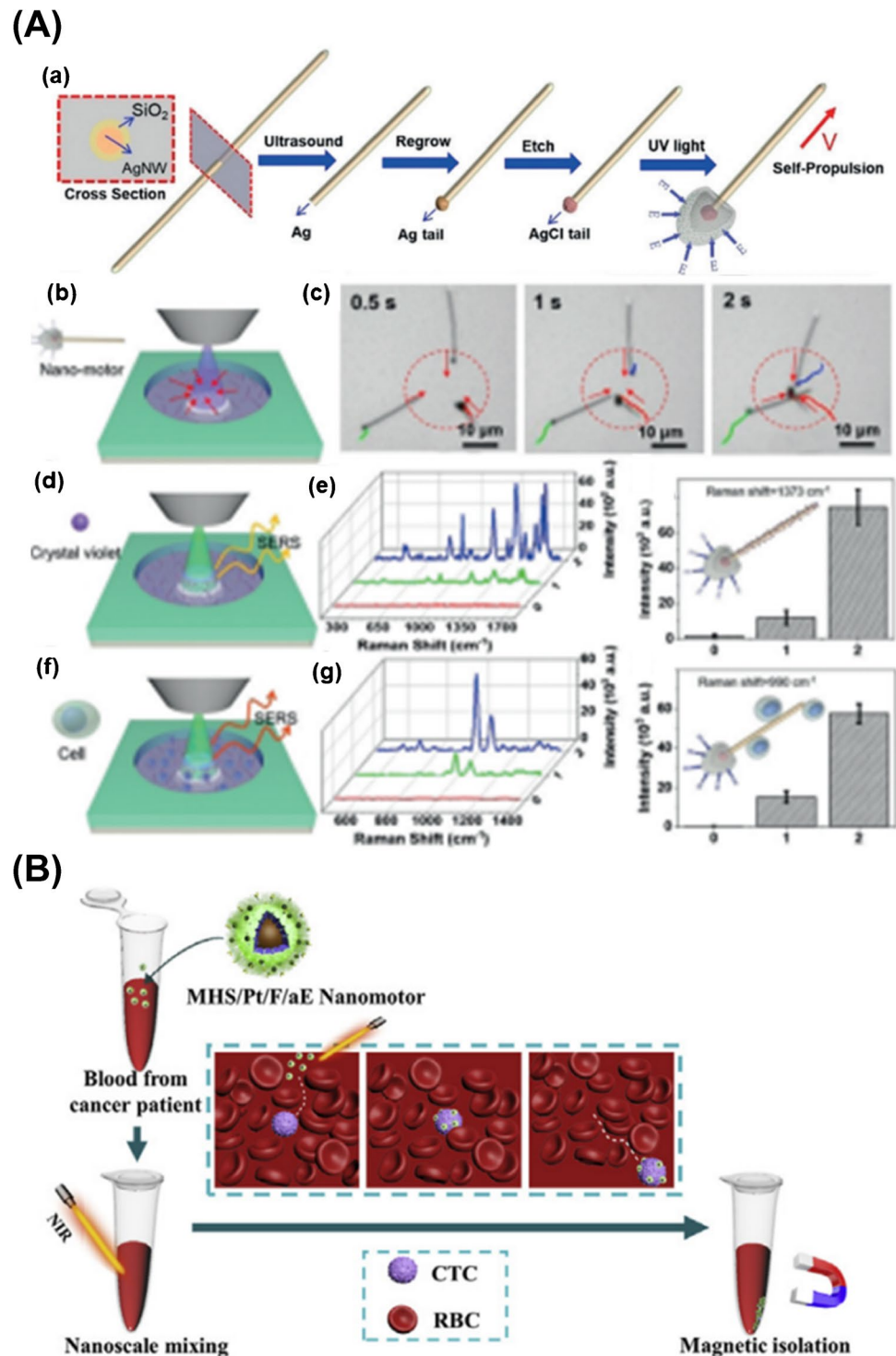
changes in aptamers, affinity peptides, or protein conformations, which can hamper their biorecognition or destroy MNM functionalization. For in vivo biosensing, it is also important to study the immune response against MNMs. Therefore, all these interactions should be taken into consideration in the development of MNMs for biosensing applications.

Biocompatible and biodegradable materials in MNMs for future biosensing applications

Another important factor to consider in MNM technology for future biosensing applications is the materials employed in MNM fabrication. Biocompatible and biodegradable materials are desirable for developing
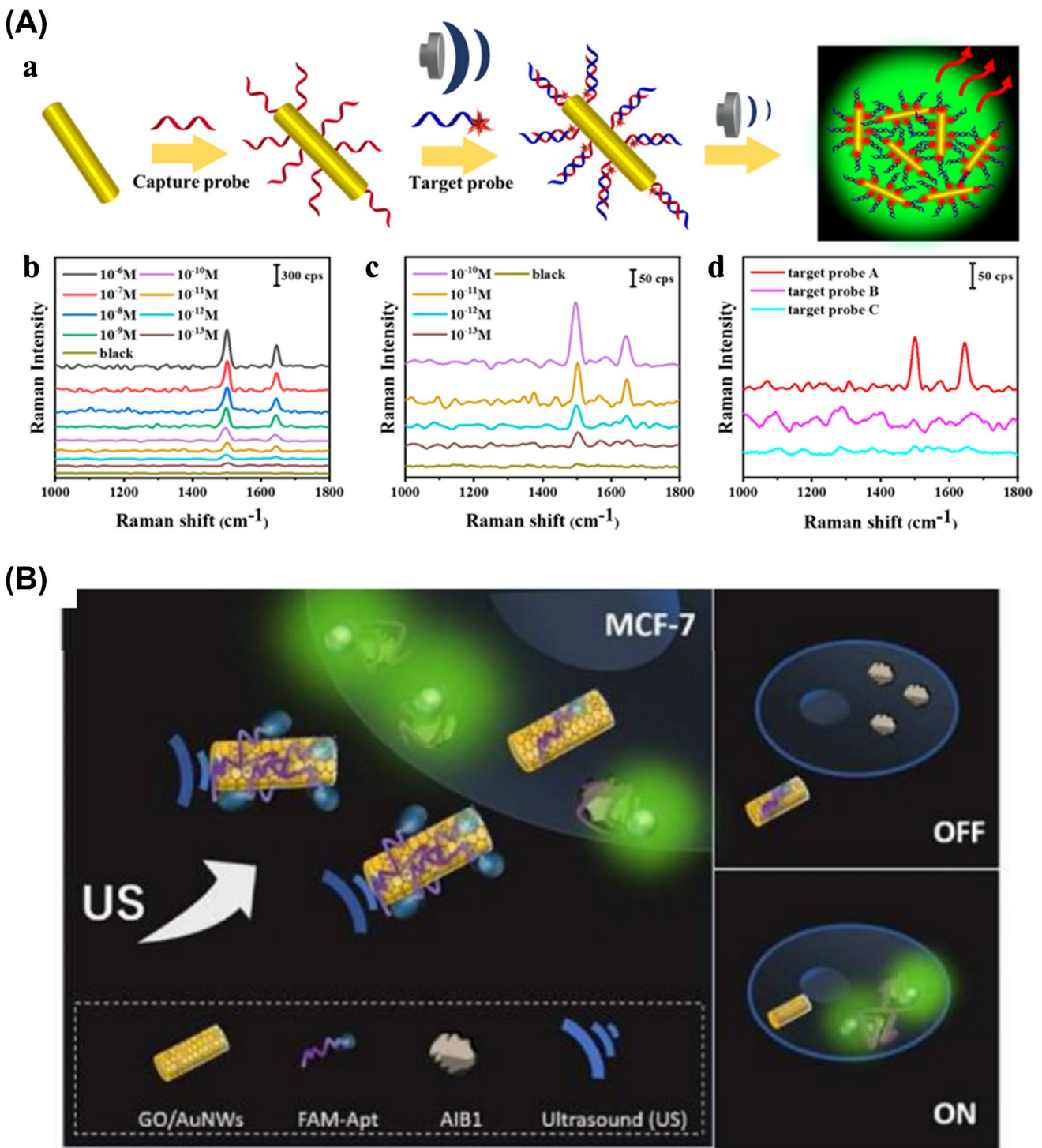
Fig. 5 Ultrasound-driven MNMs in biosensing applications. (**A**) Schematic diagram of the ultrasound Au nanorod modification, biorecognition, and SERS sensing (**a**). SERS spectra of target DNA detection at different concentrations (**b**, **c**). Selectivity of the SERS signal between different target probes (**d**). Reprinted with permission from [68] (**B**) Schematic illustration of AIB1 detection in living cancer cells using ultrasound-propelled FAM-AIB1-apt-GO/AuNW motors based on OFF-ON fluorescence switching. Reprinted with permission from [69]

in vivo biosensing applications. Several advances have been achieved in this direction, principally motivated by MNM use in drug delivery and environmental remediation [72–75]. The use of biocompatible materials which interact with biological sample components such as cells is needed to avoid any side effects during

Table 2 MNMs powered by biocompatible means for biosensing applications*

Propulsion force (fuel/source)	(Bio)receptor/analyte	Detection (tag)	Approach/application	Assay time	Ref.
Biocatalytically driven					
Urease (urea)	Enzyme urease/urea	Motion-based	Enzyme activity/urea concentration	20 s	[44]
Urease (100 mM urea)	DNA switch	FRET-fluorescence (Cy5/Cy3)	FRET/Cy3 ratio/pH assessment	Real-time	[45]
Uricase (uric acid)	Enzyme uricase-HRP/uric acid	Luminescence (NaYF$_4$:Yb/Tm)	"On-off" particles/uric acid concentration	4 min	[46]
Artificial enzyme (DOPA)	Ligands (BT$_2$T$_4$ oligomers)	Motion-based	Propulsion angle/DOPA enantiomeric excess	**	[47]
Bacteria flagella	Bacteria genetic modification	Fluorescence (cell activity)	Overexpression of protein fluorescence/bacitracin detection	1.25 h	[50]
Magnetic field-driven					
Rotatory (10 mT, 4 Hz)	Carbon dots/*C. diff* toxins	Fluorescence (carbon dots)	Fluorescence "on-off" over time/*C. diff* diagnosis	10 min	[53]
Rotatory (5 mT)	—/Glucose	Motion-based	Motor rotation frequency/glucose determination	Real-time	[54]
Drag (22 mT)	Chitosan/DNA	Motion-based	DNA electrostatic attachment/not reported	30 min	[55]
Drag (20 mT)	AgNPs/cytoplasm molecules	SERS	Motor-based SERS substrate/cytoplasm molecules determination	30 min	[56]
Rotatory (10 mT, 4Hz)	Anti-PCT/PCT	Colorimetry (particle density)	Enzyme-linked immunosorbent assay (ELISA)/sepsis diagnosis**	1 h	[57]
Rotatory (5 mT, 5 Hz)	Anti-spike protein/spike protein	Electrochemical (Au HER)	*On-the-fly* immunoassay/SARS-CoV-2 diagnosis	30 min	[58]
Light-driven					
UV (0.27 W/cm^2)	AgCl tail	SERS	Motor-based SERS substrate/cells and crystal violet determination	10 s	[63]
NIR (2.68 W/cm^2)	Anti-EpCAM/EpCAM	Fluorescence (FITC)	Cell membrane biorecognition and magnetic cells isolation/tumor cells	5 min	[64]
Ultrasonic-driven					
1.8 MHz	Aptamer miRNA-1246	SERS	DNA hybridization and motor-based SERS substrate/prostate cancer diagnosis	**	[68]
2.66 MHz	Aptamer AIB1	Fluorescence (FAM)	Selective DNA tag "OFF-ON" inside the tumor cells/breast cancer diagnosis	15 min	[69]

*HER: hydrogen evolution reaction; FITC: fluorescein; FAM: 6-carboxyfluorescein; FRET: fluorescence resonance energy transfer; Cy: cyanine; BT$_2$T$_4$: 2,2′-bis(2,2′-bithiophene-5-yl)-3,3′-dibenzothiophene; *C. diff*: *Clostridium difficile*.

**Data not reported.

in vivo biosensing and drug delivery. Moreover, the biodegradability of MNMs by metabolic routes or biological fluid enzymes into excretable compounds is highly recommended to avoid the bioaccumulation of MNMs in the biological system when they are employed for in vivo applications.

Transient inorganic MNMs

Transient inorganic materials that are oxidized during MNM propulsion and biosensing into non-harmful ions are normally biocompatible. MNMs composed of transient inorganic metals are based on Zn and Mg metals used as catalyst.

These metals dissolve in saline water and acid media by their oxidation into Zn^{2+} and Mg^{2+}. During this process, H_2 gas bubbles are generated and propel MNMs until catalyst depletion. This approach is still used for developing applications in gastric acid, especially for drug delivery [76–78]. Even though these materials are clearly limited to specific applications, they offer a smart solution for developing biocompatible and biodegradable catalytic MNMs for biosensing applications in the gastrointestinal tract.

Polymeric MNMs

Also, biocompatible polymers that are metabolized by the body into excretable compounds are the main approaches used for MNM design. Natural polymers such as chitosan and alginate, and synthetic biodegradable polymers such as poly(amino acid)s (PAAs) and polydopamine (PDA), are the more common polymers used in MNM design.

Chitosan and alginate are natural polymers used extensively in the food industry and pharmacological formulations due to their biocompatibility and biodegradability. Thus, MNMs synthesized with these materials have emerged in recent years as a trend in biomedical, environmental, and analytical applications [79–85]. Even though these are promising materials, however, few biosensing applications have been developed [79, 80]. Our group developed chitosan (CHI)/Prussian blue micromotors functionalized with acetylcholinesterase enzyme for nerve agent determination. These micromotors were employed for the screening of acetylcholinesterase inhibitors in forensic samples related to possible poisoning sources in murder attempts. The detection approach consisted of a colorimetric fast assay of only 20 min in myriad beverage samples [79]. Karaca et al. developed CHI/Pt catalytic micromotors for DNA determination by their adsorption on the external CHI layer. The assay consisted of the motion-based detection of DNA attachment by electrostatic interaction with the chitosan external layer of the micromotors. This results in real-time monitoring of DNA present in biological samples for future biosensing applications [80]. However, both of these applications lack biocompatibility because they are both based on catalytic propulsion using H_2O_2 as fuel. This highlights the importance of combining a biocompatible source of propulsion with biocompatible materials in MNM design for future biosensing assays, especially for in vivo applications.

PPAs and PDA monomers can be easily electropolymerized to obtain biodegradable polymers by enzymes present in biological fluids and animal stomachs. For this reason, these polymers have attracted much attention in the last 2 years for MNM development [86–93]. However, all these developments have focused on drug delivery and environmental remediation. Nevertheless, the biodegradability of MNMs (using these synthetic polymers) makes them an excellent alternative for developing new biosensing strategies while avoiding possible bioaccumulation problems of other inorganic materials commonly used in MNM design such as gold, silver, carbon nanomaterials, or other petrol-based polymers that are difficult to biodegrade.

Conclusions and future perspectives

MNMs are promising platforms for biosensing due to the enhanced mixing and easy isolation from the sample for attachment and simplified methodologies. Overall, the field is still dominated by catalytic MNMs due to the low-cost technological facilities required and the high chemical compatibility with sophisticated bio-architectonics available from a rich body of biofunctionalization knowledge. Moreover, catalytic MNMs fueled by hydrogen peroxide have high towing force and generate bubbles, which greatly enhances the analytical performance without limitations from media constituents. Therefore, all kinds of bioreceptors and sensing techniques have been coupled with catalytic MNMs. Yet, the requirements for peroxide fuel, which is toxic for living cells, and the interference in some biological samples, such as blood, hampered future in vivo and several in vitro biosensing applications. Inspired by this challenge, several advances have been made in biocompatible propulsion mechanisms for the development of MNM biosensing approaches.

Enzymatic MNMs are a great alternative to H_2O_2 catalytically driven MNMs due to the employment of natural fuels present in the sample which can even act as analytes themselves. Hybrid MNMs that employ natural organisms such as bacteria can propel samples to perform myriad applications. Indeed, the genetic bioengineering of bacteria has demonstrated great potential for biosensing applications. Enzymatic and biohybrid MNMs are promising biocompatible means of propulsion, but they are strictly limited to optimal biological conditions, such as pH, ionic force, or temperature, which can hamper their coupling to specific detection techniques such as SERS or fluorescence that use focused light, which can lead to additional undesired local heating. We believe that future efforts should aim to protect enzymatic components from media conditions or substitute them with artificial enzymatic materials. This will expand the range of applications for enzymatic MNMs for biosensing applications. Magnetic MNMs for biosensing have great potential due to the already developed magnetic particle bioassays that are largely compatible with magnetic field sources employed for MNM propulsion. However, they suffer from lack of design and propulsion improvement such as the limited availability of suitable magnetic materials based on only three elements (Ni, Co, and Fe). Nevertheless, although the lack of designs can hamper the development of magnetic MNMs, several alternatives in terms of

magnetic setup designs have been developed (Helmholtz coils, rotating magnets, and other homemade setups). Future efforts should be aimed at combining these magnetic setups for different bioassay steps to improve the performance of magnetic MNMs for biosensing applications. Light-driven MNMs still face problems with biocompatible and less energetic light sources for MNM propulsion in vivo. However, light-driven MNMs have the potential to be employed for in vitro biosensing, especially coupled with detection techniques that already use focused light sources such as SERS and fluorescence. Indeed, it is expected that advances in photosensitive materials, such as MXenes or upconversion nanoparticles, will reduce the need for energy sources to propel light-driven MNMs for future biosensing applications. Meanwhile, ultrasound-driven MNMs have special potential for in vivo biosensing due to the compatibility of MNM propulsion with ultrasound medical devices and the straightforward cell uptake of nanosized motors which enables the selective detection of the cells by biorecognition in the cytoplasmic media, as has been described in this review. However, ultrasound-driven MNMs are difficult to develop to obtain different acoustic behavior around the MNMs needed for this propulsion mechanism. But, at the same time, ultrasound can be modulated to perform different tasks associated with biosensing steps, such as DNA strand separation or aptamer detachment from the MNM surface that is impossible or more difficult to trigger in other propulsion mechanisms.

Even though MNMs driven by biocompatible means of propulsion show exceptional results in specific applications, future efforts should be aimed at improving these approaches by taking advantage of the huge development in catalytic MNMs, functionalization, and detection techniques. This can lead to novel biosensing applications where the use of hydrogen peroxide is toxic or interferes with the analytical signal. We envision novel in vitro applications in such directions using light- and magnetic-driven MNMs and in vivo biosensing applications using ultrasound, enzymatic, and biohybrid MNMs. Given the impressive results reported from SERS-based sensing using MNMs, future advances are expected using this detection technique in micromotor technology, especially coupled to ultrasound- and light-driven micromotors due to the compatibility between materials and source of energy with the detection technique. Moreover, new and more efficient photocatalytic materials are needed for energy conversion and wavelengths employed for MNM propulsion (as the light will need to penetrate the dermis and other parts of the human body). Thus, future in vivo biosensing applications using light-driven MNMs can be expected. The same prognostics are applied to magnetic MNMs coupled with electrochemical detection techniques due to the actual development of magnetic NPs in electrochemical biosensing. Moreover, automatic magnetic platforms for point-of-care testing are also envisioned due to the possibility of magnetic field informatics programming.

Finally, biocompatible and biodegradable materials should be included in MNM design and synthesis in order to avoid bioaccumulation side effects of their use in in vivo applications. In this sense, a long road lies ahead, as the combination of biocompatible and biodegradable materials with biocompatible means of propulsion for biosensing applications remains largely unexplored.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

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