Micro- and nano-motors: the new generation of drug carriers

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Micro- and nano-motors are emerging as novel drug delivery platforms, offering advantages such as rapid drug transport, high tissue penetration and motion controllability. They can be propelled and/or guided by endogenous (i.e., chemotaxis) or exogenous stimuli (e.g., ultrasound, magnetic fields, light) toward the area of interest. Moreover, such stimuli can be used to trigger the release of a therapeutic payload when the motor reaches certain location in order to improve the drug targeting. In this review article, we highlight medically oriented micro-/nano-motors, in particular the ones created for targeted drug delivery, and discuss their current limitations and possibilities toward *in vivo* applications.

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Drug-delivery systems have greatly evolved since 1952, when the first sustained release systems were introduced (see the graphical abstract) [1]. In the 1980s, self-regulated drug release carriers as well as the first drug delivery nanostructures were developed. From then on, research in this field has been focused on improving the targeting and systemic circulation of those carriers as well as to study different nanoparticle-based drug formulations, topics which have been widely reviewed in the literature [2–7]. Several nanocarriers (between 5 and 200 nm diameter) have been explored for therapy (e.g. inorganic, polymeric, liposomes, nanocrystals, nanotubes and dendrimers) [8], many of them already approved by the Food and Drug Administration (FDA) to treat, for example, neurovascular diseases, neurological cancers and neurodegenerative maladies [9]. The study of their pharmacokinetics has led to the creation of new carriers with reduced drug dose and protection from efflux or degradation [10]. Most of these nanocarriers include sophisticated surface biofunctionalization and coatings like polyethylene glycol (PEG) [11] or biomimetic modifications, [12] to increase their specificity and circulation time [13]. However, challenges still remain for those carriers such as the improvement of their targeting (currently only about 0.7% – median – of the administrated nanoparticle dose is found to be delivered to a solid tumor [14]), their penetration ability, drug loading capacity and delivery on the subcellular level. Other type of drug carriers are the cellular ones, which have many advantages such

as the ability to encapsulate higher amounts of drugs within their membrane, to interact with other cells/tissue and are more biocompatible compared to the mentioned nanocarriers, but they do not possess controlled motion to penetrate tissues or to perform actuation tasks [15–17]. To overcome these hurdles, micro- and nano-motors appear as a new generation of targeted drug-delivery systems, as they combine the advantages of previous drug carriers (e.g., drug protection, selectivity, biocompatibility) with the ability to swim and penetrate tumor tissue, and can be provided with specific actuation mechanisms to trigger the drug release in the right time/space [18,19]. They can also be easily functionalized for *in vivo* imaging, to detect molecules in their surrounding environment, and/or to capture single cells. Moreover, they can be controlled precisely using external sources such as magnetic fields, ultrasound or light [20]. For example, they have been used to isolate cells [21], perform microbiopsies [22], sense proteins [23], ligands or other molecules of interest, deliver drugs or even help sperms with motion deficiencies to reach the oocyte, all *in vitro* [24–27]. Nonetheless, it is worth noting that the use of these new systems entails additional difficulties, such as their control and visualization in deep tissue in real time [28]. In this review article, we describe synthetic and bio-hybrid micromotors that have been conceived for targeted drug delivery as well as the current achievements and challenges toward their *in vivo* application.

Synthetic micromotors

The most common synthetic micromotors are the catalytic ones, whose biomedical applications have been mainly limited to on-chip proof of concept studies. As they rely on toxic fuels, it is not possible to use them in *in vivo* scenarios, unless the catalyst material and fuel are replaced by biocompatible ones [29,30]. Some attempts have been made to move tiny motors in biologically available fuels such as glucose or urea. However, the required concentration of these fuels was much higher than physiological levels in most of the reported works. Besides, the velocities of these motors have been very low, in the order of a few micrometers per second on average. So far, numerous efforts have been made to improve speed [31], directionality [32], and to enhance the enzymatic motor activity by using, for example, electroactive polymers to encapsulate higher amounts of enzyme [33], or hollow mesoporous structures to increase the reactive surface area [34]. Alternatively, materials that decompose in a slightly acidic environment, such as zinc, manganese or calcium carbonate, have also been employed to fabricate micro-/nano-motors that can be operated in environments like the stomach or the vicinity of cancer cells, both exhibiting a pH below 7 [35,36]. External field-driven synthetic micro-/nano-motors which can be propelled toward the area of interest by using external magnetic fields, ultrasound or light also fall into the category of synthetic micromotors. These micromotors can also be fabricated from different materials and with various functionalities. They can be functionalized with biomolecules or chemical substances to perform the same tasks as the catalytic ones. They have better controllability but, as mentioned above, they require an external control to be maneuvered in three dimensions. In consequence, a proper imaging technique is essential to track them, since they cannot reach the area of interest autonomously. In the following sections we will describe specific works in which cargo release of drugs or other therapeutic substances have been demonstrated by using chemically or physically actuated micro- and nano-motors.

Chemical actuation (catalytic reactions)

The first demonstration of catalytic nanomotors to capture and transport cargo was done by Sen's group in 2008 – self-propelled Au-Pt bimetallic nanorods which moved in presence of hydrogen peroxide solution. The decomposition of hydrogen peroxide to oxygen on the Pt side and the water reduction on the Au part of the rod resulted in a forward motion due to the generated proton gradient along the rod. This nanomotor was able to transport polystyrene particles which were captured via electrostatic interaction or covalent binding between the biotin-functionalized rod and the streptavidin-functionalized particle. They also contained a Ni segment which allowed the direction control via external magnetic field actuation [37].

Later, in 2010, Kagan *et al.* showed for the first time the capture, transport and release of drug containers, specifically biodegradable polymeric particles (poly-D, L-lactic-co-glycolic acid) and liposomes containing doxorubicin (DOX) drug, using a similar bimetallic nanomotor (see Table 1). It was shown that the release of such cargo was possible by a fast reversal of the nanomotors' motion whereby the drag force imposed on the particle overcame the magnetic attraction between the particle and the nanomotor. The micromotor velocity was also affected by the cargo size, as expected [38]. In the same year, Schmidt's group reported the controlled manipulation and release of multiple micro objects [39], including cells, [21] using rolled-up catalytic microengines. This work opened a way to transport cells on demand to build or repair tissues, as now has been shown by several groups [25,40–41]. The release of such cargo was also achieved by a fast change of the micromotor direction. To achieve a more controlled release

Table 1. Synthetic micro- and nano-motors.

Carrier material	Type of cargo (drug)	Loading mechanism	Target	Propulsion	Guidance	Release	Ref.
Chemically actuated micro-/nano-motors							
Au/Pt bimetallic nanorods	Biodegradable PLGA particles and liposomes containing DOX	Electrostatic interaction	–	Catalytic (hydrogen peroxide)	Magnetic	Drag forces	[38]
Hollow Janus particles with Au nanoparticles and catalase enzyme	DOX	Physical adsorption	HeLa cells	Catalytic (hydrogen peroxide)	Magnetic	NIR light	[43]
Biodegradable BSA/poly-L-lysine/gelatin and enzyme rocket	DOX	Physical adsorption	HeLa cells	Catalytic (hydrogen peroxide)	Magnetic	NIR light	[44]
Hybrid stomatocyte nanomotors made of block copolymers	DOX	Physical entrapment	HeLa cells	Catalytic (hydrogen peroxide)	–	pH-reducing agent	[45,46]
PEDOT/MnO ₂ Janus micromotors	CPT	Electrostatic interaction	–	Catalytic (hydrogen peroxide)	–	–	[47]
Mg/pH responsive polymers Janus micromotors	Gastric acid neutralization payload and fluorescent dyes	Physical adsorption	Mouse stomach	Catalytic (acid)	pH-taxis	pH	[48]
Mg/TiO ₂ /PLGA-chitosan Janus micromotor	Gastric acid neutralization payload and clarithromycin (an antibiotic drug)	Physical adsorption	Mouse stomach	Catalytic (acid)	pH-taxis	pH	[49]
Calcium carbonate-micromotors	Coagulation factors	Physical adsorption	Bleeding sites in mouse models	Catalytic (acid)	–	pH	[50]
Mesoporous nanomotor	DOX	Physical adsorption	HeLa cells	Catalytic (urea)	–	Diffusion	[51]
Physically actuated micro-/nano-motors							
Ni flexible nanoswimmer	Fe ₃ O ₄ /DOX encapsulated PLGA particles	Mechanical	HeLa cells	Magnetic	Magnetic	Drag forces	[52]
Au/Ni/Au nanowire motor	DOX	Electrostatic interaction	HeLa cells	Ultrasound	Magnetic	NIR	[53]
Ni/Ti helical microswimmers	Lipoplexes containing pDNA	Surface functionalization	Single HEK 293 cells	Magnetic	Magnetic	Physical contact	[54]
Nanowire motor	siRNA	Surface functionalization	HEK-293 MCF-7 cells	Ultrasound	Magnetic	Ultrasound internalization	[55]

BSA: Bovine serum albumin; CPT: Camptothecin; DOX: Doxorubicin; HEK: Human embryonic kidney; NIR: Near IR; PEDOT: Poly(3,4-ethylenedioxythiophene); PLGA: Poly-D, L-lactico-glycolic acid.

of cargo, Sundararajan *et al.* proposed the use of photochemical stimuli (UV light) to trigger cargo delivery of polystyrene (PS)-streptavidin and amidine particles (drug particle model) via silver-dissolution or photocleavable bifunctional linkers from Pt/Au/polypyrrole (PPy) nanorod motors (Table 1) [42].

Hollow Janus particles (particles with two faces) with embedded Au nanoparticles were used to transport and release DOX (Figure 1A). These magnetically guided carriers were propelled by the reaction of catalase with hydrogen peroxide at a very low concentration. Upon reaching the area of interest, the drug was released via near infrared (NIR) light activation, inducing breakage of the polymeric shell upon local heating of the embedded Au nanoparticles through the photothermal effect (Table 1) [43]. The same group reported a biodegradable self-propelled bovine serum albumin (BSA)/poly-L-lysine/gelatin multilayer rocket to transport and deliver anticancer drugs such as DOX. The release of drugs and other substances (Au nanoparticles and catalase) was triggered by NIR light that dissolved the heat-sensitive gelatin layer (Table 1) [44].

In 2015, Ma *et al.* reported the use of mesoporous silica nanomotors as potential drug carriers. These nanomotors moved also in low concentrations of hydrogen peroxide. They were produced via sol-gel synthesis and coated with Pt on one side. Fluorescein isothiocyanate and methylene blue were used as a model drugs. The presence of H₂O₂ did not affect the sustained release kinetics which are beneficial for small cargo loading and targeted delivery [56].

Hybrid stomatocyte nanomotors have also been reported as drug carriers. They were made of soft self-assembled block copolymers which contain platinum nanoparticles and DOX anticancer drug. These nanomotors moved in presence of hydrogen peroxide reaching velocities of up to 39 µm/s. Drug release took place at the subcellular level once the nanomotors degraded upon a slight pH change. Moreover these nanomotors were capable of loading

Figure 1. Chemical micro- and nano-motors for drug release. (A) Schematic of an enzymatic Janus micromotor. This micromotor can load doxorubicin and release it via external NIR activation onto cancer cells and its motion relies on the reaction of Pt with hydrogen peroxide. (B) Mg-based micromotor coated with a thin Au layer and a payload embedded in a pH-sensitive polymer layer for the neutralization of gastric acid. (C) CaCO_3 self-propelled micromotors that move due to the reaction of an organic acid (tranexamic acid) in aqueous solutions, forming CO_2 bubbles. These micromotors can deliver hemostatic drugs (i.e., thrombin) to aid wound healing. (D) Enhanced drug release by self-propelled urease enzymatic nanomotor in presence of urea (left). Doxorubicin fluorescence emission quantification in nanomotors-treated HeLa cells when they were in presence and absence of urease.

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hydrophilic as well as hydrophobic drugs due to their bilayer structure (Table 1) [45]. They were also able to sense their local environment and trigger motion and drug release upon the presence of a reducing agent (glutathione) which cleaved their external PEG shell (Table 1) [46].

The introduced systems paved the way to develop smart carriers but were not ideal for medical applications as they relied on the use of toxic hydrogen peroxide as fuel for their motion, making it necessary to identify more suitable materials and fuels. An alternative material is Mg, which is stable at room temperature and can react with water-forming bubbles in an efficient manner. The metallic form of Mg is biocompatible and its ions are among the most abundant in the human body, being essential for the function of many organs. With this in mind, Mou *et al.* developed a biocompatible micromotor based on Mg and Pt. The Mg(OH)_2 passivation layer was quickly dissolved in NaHCO_3 , then the reaction between water and Mg enhanced hydrogen bubble production, which created the main driving force of the micromotors. However, their lifespan was only of the order of 80 s, and no drug release application was shown [57].

In 2014, the same group reported temperature-controlled drug delivery by Mg/Pt-poly(N-isopropylacrylamide) Janus micromotors in simulated body fluid and blood plasma. These motors were prepared similarly to the previous work, but with an added layer of poly(N-isopropylacrylamide) (PNIPAM) which is a temperature-responsive polymer. This new material served to encapsulate and release the drug by using external temperature stimulation. This system was able to take up the drug molecules in its swollen state (at 4°C) and, when the temperature increased to 37°C, shrink and release the drug in the process. For proof of concept, fluorescein isothiocyanate was used as drug model, achieving complete release after 30 min operating at physiological temperature. It was also shown that those micromotors moved in body fluids and blood plasma quite efficiently [58]. In 2015, Wang *et al.* reported the use of similar micromotors made of poly(3,4-ethylenedioxythiophene)/MnO₂ for camptothecin drug delivery. These motors were able to move in BSA solution and pure bovine serum, showing a potential use in complex physiological environments. Here, the drug was captured by the micromotors by electrostatic interactions as the micromotors were positively charged after modification with poly(sodium 4-styrenesulfonate) and poly(diallyldimethylammonium chloride), while camptothecin was negatively charged (Table 1) [47].

Sattayasamitsathit *et al.* constructed self-propelled engines based on zinc, fabricated by electrodeposition. These motors moved in acidic media, forming hydrogen bubbles which were ejected from the large opening of the tubes. Such mechanisms by dissolving zinc allowed not only the propulsion of the micromotors but also the release of cargo while moving. The encapsulation process was done during the Zn electrodeposition process. They also demonstrated the possibility to have multicargo within the microtube. As a proof of concept, these motors were evaluated in 0.7 M HCl solution. Although their lifespan was only about 15 s, this system opened a way for a new generation of acid-propelled micromotors to perform tasks such as therapy in the stomach or cancerous tissue as both exhibit a slightly acidic environment [59]. Later, the same group reported similar micromotors based on zinc capable of moving inside a mouse's stomach, being one of the first *in vivo* demonstrations. Their distribution, retention, cargo delivery and toxicity properties were evaluated, showing high biocompatibility and potential application to treat stomach diseases [35]. Recently, Zhang and Wang reported the use of acid-driven micromotors to neutralize gastric acid in a mouse model. They were based on Mg coated with a pH-responsive polymer. They demonstrated an effect within 24 h post administration without any noticeable toxicity. The Mg engine converted the acid fuel into a propulsive force, depleting the protons and neutralizing the pH within the stomach of the mouse. The pH alteration itself acted as release mechanism of the model payload from the pH-sensitive coating for post-treatment fluorescence imaging (DiD (1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine)) (Table 1) [48]. As follow-up project, similar Mg-based micromotors were reported to treat gastric bacterial infection (*Helicobacter pylori*) in a mouse's stomach. This time the micromotors were loaded with clarithromycin antibiotic. Their performance was shown to be superior to regular drug administration as the Mg present in the micromotor neutralized the gastric acid locally while releasing the drug, thereby increasing the drug effectiveness (Table 1) [49].

Calcium carbonate is another promising material. Guix *et al.* reported calcium carbonate-based Janus particles, which moved in slightly acidic environments generated by cancer cells through the lactic acidosis phenomena. The slight extracellular pH decrease facilitated the dissolution of calcium carbonate, resulting in an enhanced diffusive motion. Although this system was not used to deliver any drugs, it was an interesting approach to use effervescent systems to move and deliver substances while moving in slightly acidic media [36].

Similar calcium-based particle micromotors were employed to serve as effective hemostatic agents. Such systems were conceived to regulate blood coagulation through the release of coagulation factors, or to remove factors for thrombosis prevention (Figure 1C). Coagulation factors should be delivered on bleeding sites to increase safety and efficacy, and avoid side effects such as induced hypovolemic shock, even preventing death. The evaluation was done in two mouse models showing high efficiency in halting hemorrhage compared to nonpropelling particles and to a solution of recombinant thrombin that is commonly used in clinical interventions (Table 1) [50].

Al-Ga/Ti is another material combination that allows the motion of micromotors in water without the need of toxic fuels [60]. This motion principle is based on the continuous hydrogen generation via liquid metal embrittlement in the Al-Ga system, a reaction that happens between the Al alloy and the water. Such micromotors were also able to move in biological media but they exhibited a very short lifespan (few minutes). To date, no cargo release has been demonstrated with this system.

Enzymatic micro- and nano-motors can also move with biocompatible fuels such as glucose and urease. Here the enzymes convert the chemical energy into kinetic motion. In 2005, Feringa *et al.* reported the first nanomotor driven by enzymatic reactions. Carbon nanotubes were used as a platform to immobilize the glucose oxidase enzyme which catalyzes the oxidation of glucose to gluconolactone and hydrogen peroxide. Then, the resulting hydrogen

Figure 2. Physical micro- and nano-motors for drug delivery. (A) Flexible magnetic nanoswimmers to transport drug-loaded magnetic polymeric particles and deliver them onto HeLa cells. (B) Ultrasound-driven drug-loaded nanoporous Au nanomotors. Here, the drug release is triggered by near IR light once the motor reaches the cancer tumor after magnetic guidance. (C) Magnetic artificial bacteria flagella, functionalized with lipoplexes-containing pDNA (marked in green fluorescence) for gene transfection. (D) Schematic of a nanomotor-based gene silencing approach based on an ultrasound-driven nanomotor which is internalized inside an HEK293-green fluorescent protein (GFP) cell to deliver pRNA as a promising method to treat various types of diseases.

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peroxide was decomposed by catalase enzyme into oxygen and water. The oxygen bubbles were expelled from one side of the carbon nanotube, inducing its forward motion [61]. From then on, different synthetic structures such as porous Janus nanomotors, [62] rolled-up microtubes [31] and stomatocytes [63,64] have been used to immobilize or carry enzymes which can react with the surrounding environment, leading to self-propulsion of the micro- and nano-motors via enhanced diffusion or bubble formation [65]. Recently, Sanchez's group reported the use of enzymatic mesoporous nanomotors which move in urease-based media to carry and deliver DOX onto cancer cells *in vitro*, showing that the drug-loaded nanomotors were more effective in killing cancer cells when they were in presence of urea, compared to passive carriers, due to the motor kinetics and the ammonia production by the catalytic decomposition of urea (Figure 1D & Table 1) [51].

Physical actuation

Physically driven micro- and nano-motors are of great importance due to their high controllability. They do not need fuel for their motion as they can be actuated by external means such as magnetic fields, ultrasound or light, making them very attractive for several biomedical applications [66]. Gao *et al.* reported the first example of drug release of a magnetically driven, flexible nanoswimmer. This system was fabricated as a flexible magnetic nickel structure which was actuated by externally rotating magnetic fields through a predefined route (Figure 2A). The authors suggested the possibility to use cleavage linkers to attach the drug to the nanomotor surface to enable controlled release by proper tumor microenvironment conditions (Table 1) [52]. Ultrasound can also be used to propel synthetic micro- and nano-motors like the ones reported by Wang's group. The authors fabricated three segment nanowire motors with payload release capability by using a pH-sensitive polymeric segment, with magnetic property added by a nickel segment and with an additional Au segment for biofunctionalization (Figure 2B). Their speed was analyzed in different media (water, seawater, serum and saliva) [67]. As a follow-up project, the same

Table 2. Bio-hybrid micro- and nano-motors.

Cell/organism/molecule	Type of cargo (drug)	Loading mechanism	Target	Propulsion	Guidance	Ref.
Red blood cells	DOX, QDs, MB	Hypotonic dilution encapsulation	Human umbilical vein endothelial cells	Ultrasound	Magnetic	[77,78]
Neutrophil	DOX-loaded mesoporous silica nanoparticles	Phagocytosis	<i>Escherichia coli</i>	Cell migration	Chemotaxis	[79]
Raphides biotubes coated with iron	CPT	Physical adsorption	HeLa cells	Magnetic	Magnetic	[80]
Virus	Zinc phthalocyanine	–	Macrophage cells	Virus migration	–	[81]
Sperms	DOX-HCl	Nucleus binding	HeLa cell spheroids	Flagella beating	Magnetic	[82]
<i>E. coli</i> bacteria	Polyelectrolyte multilayer microparticles with embedded magnetic beads and DOX	Physical adsorption	Breast cancer cells	Flagella beating	Magnetic/chemotaxis	[83]
Magnetotactic bacteria (<i>Magnetococcus marinus</i>)	SN-38 drug-loaded liposomes	Surface functionalization	MC-1 cell	Flagella beating	Hypoxic regions of the tumor/magnetic	[84]

CPT: Camptothecin; DOX: Doxorubicin; MB: Methylene blue; QD: Quantum dot.

group demonstrated the release of DOX onto cancer cells with the same kind of motor. Once more, ultrasound was used for propulsion and magnetic fields for guidance. Drug release was achieved by the photothermal effect, induced by applying an external NIR light stimulus that interacted with the Au segment (Table 1) [53]. Bioinspired magnetically propelled helical microswimmers were functionalized with calcein-loaded liposomes [68] and with lipoplexes-containing pDNA [54] for *in vitro* targeted drug delivery and gene transfection to single cells (Figure 2C & Table 1), respectively. Selective delivery of siRNA was also demonstrated by using an acoustically propelled nanowire motor (Figure 2D & Table 1) [55]. This motor was loaded with a hybrid siRNA-DNA nanotechnology platform to facilitate efficient intracellular uptake and gene knockdown, showing superior performance compared to existing gene-silencing methods. A 94% silencing was achieved by these micromotors after 5 min of treatment due to the ultrasound-enhanced siRNA-DNA internalization. Micro- and nano-motor-assisted gene therapies are promising for different applications, such as treatment of various types of diseases, development and screening of new drugs, and for functional genomics and diagnosis.

Bio-hybrid motors for drug delivery

Despite the success of synthetic micro-/nano-motors for drug delivery, many challenges remain regarding the use of artificial materials inside the human body. Biological hybrid systems have recently emerged as a promising alternative with advantageous biocompatibility, biodegradability and their functional interactions with body tissue [69]. Biological organisms or substances have shown great abilities to protect the payload or the whole micro-/nano-motors by encapsulating drugs inside them or by covering all the synthetic parts [70], owing to their high physiological adaptability [71] and their functional immunosuppression [72]. Being of biological origin, these encapsulating or covering agents can also significantly decrease the toxicity of the drug and the carrier [73]. Besides, compared with synthetic materials which hardly assisted in drug release and cellular uptake, bio-hybrid systems have shown an improvement of bioavailability and may allow to further decrease the dose amount as a result of the high cell affinity of biological molecules [30,74] and the enhanced drug uptake mediated by biological organisms [75].

Cells/microorganisms as drug carriers

Cell-based structures such as red blood cells, bacteria and stem cells have been shown to be promising alternatives to carry drugs in an effective and biocompatible way [15]. Their membrane systems can protect therapeutic payload from rapid clearance by phagocytosis and hepatic filtration, decrease drug toxicity in healthy tissue and enhance intracellular delivery due to their efficient endosomal escape mechanism [16]. Since the 1970s, red blood cells (RBCs) have been widely studied as drug carriers to prolong the circulation time of encapsulated drugs, [76] which is possible due to their immunosuppressive properties and versatile drug-loading ability (Table 2) [77]. Combined with an external propulsion system, RBCs were successfully controlled even against blood stream current, making them very interesting for diagnosis and therapy in vascular networks, especially when precise localization is required. Wu *et al.* applied ultrasound fields to propel Fe₃O₄ nanoparticles-loaded RBCs, also called RBC motors (Figure 3A) [78]. Asymmetric distribution of the magnetic particles enabled magnetic alignment and guidance. These RBC motors

Figure 3. Bio-hybrid drug-delivery micromotors. (A) Schematic of multicargo-loaded, ultrasound-driven red blood cells micromotors designed for theranostic applications. Red blood cells are loaded with quantum dot nanocrystals for imaging, the anticancer drug doxorubicin and magnetic Fe_3O_4 nanoparticles for guidance by a hypotonic dilution-based encapsulation method. (B) Schematic of *Escherichia coli* membrane-camouflaging mesoporous silica nanoparticles neutrophil micromotors. (C) Camptothecin-loaded porous biotubes. These biotubes were coated with iron for magnetic steering and control. HeLa cells were treated with these biotubes to show their dual effect of cell drilling and induced cell apoptosis due to the delivered drug within 72 h. (D) Drug-loaded sperm-hybrid micromotor for targeted drug delivery: schematic of magnetic microstructure (tetrapod) for the capture, guidance and release of drug-loaded sperm cells, SEM image of polymeric tetrapods fabricated by direct laser writing. A micrograph of a motile sperm coupled to a tetrapod, a microscopic image sequence of sperm release after hitting a HeLa cell cluster and overlaid z-stack images of sperm fusion with HeLa cells, showing local drug release, are shown. (E) Magneto-aerotactic bacteria to transport drug-loaded liposomes to the hypoxic regions of a cancer tumor in a mouse model. They were first guided magnetically and then by aerotaxis. MSN: Mesoporous silica nanoparticle; SEM: Scanning electron microscopy.

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were successfully transported in a controlled way in diluted whole blood without apparent biofouling. After applying ultrasound waves, the cells remained stable and functional, conserving the hemoglobin inside. As a follow-up study, Wu *et al.* incorporated quantum dots as imaging agents, DOX as an anticancer drug and magnetic nanoparticles to enable magnetic guidance, into RBCs (Table 2) [77]. A cell viability test showed that these multicargo-loaded RBCs exhibited a toxicity three-times lower than freely diluted DOX over a 24 h incubation time.

White blood cells are also interesting candidates to transport drugs. As immune cells, they can autonomously target pathogens and eliminate them via phagocytosis [85]. Munerati *et al.* first reported the drug-encapsulating ability of macrophages [86]. The loading mechanism was described as ATP-mediated permeabilization, which explained their high loading capacity (2 pg DOX per cell). As stated in their cell-killing results, DOX-loaded cells significantly inhibited the proliferation of tumor cells compared with unloaded cells. Shao *et al.* developed a hybrid neutrophil micromotor system which was guided along the chemoattractant gradients secreted by *Escherichia coli* for targeted drug transport (Figure 3B & Table 2) [79]. In their research, DOX was embedded within mesoporous silica nanoparticles and then taken up by the neutrophils. Cell viability results showed that 80% of the loaded neutrophils remained active after drug loading. The prepared drug-loaded neutrophils exhibited an oriented movement at a maximum velocity of 0.165 $\mu\text{m/s}$. The directed motion of the hybrid motors was decided by the concentration of *E. coli*. Apart from cells with complete membrane systems, researchers are exploiting many more natural derivatives. For example, Srivastava *et al.* extracted raphides from plants as anticancer drug carriers (Table 2) [80]. After coating with a magnetic layer, drug-loaded raphides played a dual-action role as drug carriers and as microdaggers to kill cancer cells deterministically in a magnetic field (Figure 3C). Sporopollenin exine capsule-based micromotors proved to be also a good candidate to deliver drugs [87]. Coated with a layer of Pt, these plant-derived motors could transport preloaded BSA and release it. Viruses also show a potential on drug packaging due to their structural uniformity and versatile hierarchical assembly of protein subunits, despite the difficulty to be guided (see Table 2) [81].

Cells/microorganisms as propellers

The research of micro-/nano-motors faces the big challenge to propel the motors *in vivo* [28]. Self-propelled biological structures are very intriguing due to their ability to adapt and swim in complex environments by their independent propelling systems [27]. Sperms, as a type of motile cells whose main function is the fertilization of an oocyte, are one of the most powerful natural microswimmers. Their compact membrane system can encapsulate drugs inside the cytoplasm and thus reduce unwanted toxicity. Apart from several substances on the membrane which can protect loaded drugs from clearance by immunosuppression, [88] their unique acrosome structure was confirmed to be able to induce cell fusion between sperms and somatic cells [89]. Magdanz *et al.* [26] and Medina-Sanchez *et al.* [25] reported the use of micromotors to guide or transport motile and immotile sperms to eventually overcome two of the main male infertility problems: oligozoospermia (low sperm count) and asthenozoospermia (poor sperm motility), respectively. In our group, a sperm-hybrid micromotor system was also developed as a new drug-delivery system to potentially treat diseases in the female reproductive tract (Figure 3D & Table 2) [24,82]. In this work, DOX was loaded into bovine sperms by a co-incubation method. The loading amount was up to 15 pg per sperm. DOX showed no significant negative influence on the sperm motility. HeLa cells, derived from female cervix cancer cells, were used as a model disease target. Cell viability experiments indicated that DOX-loaded sperms exhibited higher tumor cell-killing efficacy (87%) than a DOX solution with the same dose (55%). To achieve the goal of precise guidance and local release, a synthetic, tetrapod-like structure with a tubular body and four flexible arms was designed. A drug-loaded sperm was coupled into the tubular body of the tetrapod-like structure, and released when the tetrapod arms hit a barrier or a cell spheroid. This drug-loaded sperm-hybrid micromotor was able to swim 1.8 cm through a narrow constriction and finally was discharged from the tetrapod structure and swam into a tumor spheroid. After that, the preloaded DOX was released into the tumor spheroid. According to SEM observations, the DOX-loaded sperm was able to fuse with a HeLa cell and induce its apoptosis, while unloaded sperms only fused with HeLa cells without any observable effect. Such hybrid micromotors open up great possibilities in using sperms to address *in vivo* biomedical issues.

Some flagellated bacteria have also been shown as efficient swimmers at the microscale [90]. A study of bacteria-driven microswimmers demonstrated the transport of fluorescent beads. These bacteria exhibited a strong preference of swimming towards a L-serine gradient without significant speed change [91]. Singh *et al.* developed a type of microemulsion-embedded bacteria-based micromotors [92]. Such hybrid motors could squeeze across narrow hollow structures, such as Transwell® membranes, and then transport imaging agents into cells by diffusion. Bacteria-driven motors can be also applied for the delivery of anticancer drugs. Taking the advantage of the chemotaxis of *E. coli*, Park *et al.* refitted bacteria to be an active delivery system (Table 2) [83]. By coupling with polyelectrolyte multilayer microparticles that contained embedded magnetic nanoparticles, these motile bacteria could be efficiently guided to target breast cancer cells under a chemoattractant gradient or a magnetic field, and release preloaded DOX into them. Based on fluorescence images, the cellular uptake of DOX released by the hybrid swimmers was found to be remarkably higher compared to the case of drug-loaded passive (immotile) microparticles. Some bacteria are

endowed with specific guidance features which enable them to swim in complicated environments. For example, *Magnetococcus marinus* strain MC-1 can efficiently migrate to an area with low oxygen concentration, relying on magneto-aerotaxis. Felfoul *et al.* (Table 2) [84] attached anticancer drug-loaded liposomes onto MC-1 and guided these complexes to the target tumor tissue *in vivo* under a magnetic gradient field (Figure 3E). As a consequence up to 55% of the hybrid cells swam into the tumor region.

Nanoscale biological motors have recently been explored with some new functions to serve as drug carriers. Autonomous DNA nanorobots have been demonstrated as promising transporters for nanoparticles or molecular payloads. With the help of a design tool for DNA origami, scientists created a hexagonal barrel-like DNA nanorobot. This nanorobot featured two domains which can be regarded as DNA locks. This sensitive system could successfully couple payloads with a modification linker and release them in response to different cell types as specific keys [93]. In a recent work, Thubagere *et al.* developed a DNA-based robotic walker for cargo delivery at the molecular level [94]. This robot was able to pick up two types of cargo and deliver them to separate destinations simultaneously by random walking. However, there are no reports yet about their therapeutic application.

Conclusion & future perspective

Despite great development of technologies to fabricate numerous kinds of micro- and nano-motors over the last years, there are still various difficulties that limit the application of these systems *in vivo* [28,95–96]. In a few cases the micromotors were shown to accomplish the task of drug delivery inside a mouse to treat stomach infection or even cancer, offering promising results. However, we are still far from being able to track them in real time to control their positioning and other functions inside the body. There is still a lot to be done toward this objective such as studying microbots in more complex environments, not only regarding the composition of biological media but also the intricate cavities and channels that can be found inside the body by mimicking them *in vitro*. Additionally, different functions like propulsion, manipulation, substance delivery and sensing need to be further explored. Another necessity is to analyze the toxicity of the materials employed for fabrication and, if possible, make them biodegradable as it has been already reported by several groups for some systems [44,45]. The immunoresponse to those materials should also be studied further in more detail. Considering targeted drug delivery, more progress is needed to demonstrate the killing efficiency compared to traditional treatments. Strategies and protocols should be optimized regarding the necessary doses to load the appropriate amount of drug per micromotor, toward personalized micromotor-assisted therapies. Also, very robust and reproducible protocols that can be extrapolated to *in vivo* scenarios are needed. On-demand cargo release based on exogenous (light, ultrasound, magnetic fields) or endogenous stimuli (chemical gradients), or a combination of both, would be desirable to avoid drug dilution in body fluids and undesired accumulation in healthy tissue. An interesting direction to follow is soft microrobotics, in which soft and smart synthetic materials are used to perform more complex tasks in the body, as well as to adapt to different environments, working as individuals or a swarm.

Executive summary

- Micro- and nano-motors are conceived as a new generation of drug-delivery systems with superior performance compared to their passive (immotile) counterparts, as they are designed for targeted and personalized therapies. They can move in complex environments and penetrate into damaged tissue to deliver drugs at the subcellular level.
- One of the most challenging problems is the directional and tactic motion of micro- and nano-motors when moving in more realistic scenarios (physiological environments). This calls for smart design and proper material selection.
- Cells and microorganisms feature fascinating advantages toward *in vivo* therapies, such as self propulsion, biocompatibility and a compact membrane system which helps to protect therapeutic cargo during its transport. In combination with synthetic materials, such bio-hybrid systems are great candidates to perform accurate and effective drug release on the affected tissue. They can be guided by external fields or by their own tactic capabilities.
- Although scientists have made great demonstrations of micro- and nano-motor performance *in vitro*, more work toward *in vivo* applications is required and many challenges need to be overcome, such as precise control in complex environments, *in vivo* imaging in deep tissue and in real time, reduction of undesired immunological reactions and toxicity, and subcellular delivery of drugs with controlled dosage.

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