Advanced Drug Delivery Reviews 138 (2019) 41–55

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Micromotors for drug delivery *in vivo*: The road ahead

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ARTICLE INFO

Article history:
Received 20 April 2018
Received in revised form 27 August 2018
Accepted 11 September 2018
Available online 17 September 2018

Keywords:
Micromotors
Drug delivery *in vivo*
GI tract
Solid tumor
External trigger actuation

ABSTRACT

Autonomously propelled/externally guided micromotors overcome current drug delivery challenges by providing (a) higher drug loading capacity, (b) localized delivery (less toxicity), (c) enhanced tissue penetration and (d) active maneuvering *in vivo*. These microscale drug delivery systems can exploit biological fluids, as well as exogenous stimuli, like light-NIR, ultrasound and magnetic fields (or a combination of these), towards propulsion/drug release. Ability of these wireless drug carriers towards localized targeting and controlled drug release, makes them a lucrative candidate for drug administration in complex microenvironments (like solid tumors or gastrointestinal tract). In this report, we discuss these microscale drug delivery systems for their therapeutic benefits under *in vivo* setting and provide a design-application rationale towards greater clinical significance. Also, a proof-of-concept depicting 'microbots-in-a-capsule' towards oral drug delivery has been discussed.

Contents

1. Factors governing design-application relationship . . . 42
   1.1. Active vs passive targeting: how can externally actuated DDS make a difference?. . . . 42
   1.2. Micromotors: mode of guidance/actuation *vs.* mode of release/activation . . . 43
2. Exogenous control: effects of light – NIR under *in vivo* conditions . . . 43
3. Exogenous control: effects of ultrasound (US) under *in vivo* conditions. . . . 45
4. Exogenous control: effects of magnetism under *in vivo* conditions . . . 47
5. Chemical activation: utilizing body fluids as a fuel for propulsion *in vivo* . . . 48
6. Micromotors in GI tract: exploring clinical relevance and future opportunities . . . 48
7. Can size and shape of micromotors affect their drug efficacy *in vivo*?. . . . 51
8. Conclusion . . . 51
. . . 52
References . . . 52

Drug delivery systems (DDS) have not only promoted a novel generation of therapeutics, like proteins/peptides (pharmaceuticals with short half-lives), or nucleic acids offering greater site-specificity [1], but also better predictability of pharmacokinetic & pharmacodynamic profiles of conventional drugs [2]. This has resulted in their improved biodistribution, decreased side-effects and enhanced therapeutic efficacy. Clearly, choice of a DDS is critical as (a) pharmacologically active agents are not inherently effective by themselves; (b) the efficacy of a drug is greatly influenced by the route and methodology of administration and; (c) improved drug pharmacokinetics, especially for routes, which have traditionally demonstrated poor impact [3]. For instance, oral administration of several drugs is hindered due to poor bioavailability in harsh gastrointestinal (GI) microenvironment [4], and destruction by liver enzymes (also known as *first pass* effect) [5]. In contrast, parenteral (intravenous, subcutaneous and intramuscular) forms of administration yield rapid effects, with very high drug bioavailability, but low patient compliance [6].

Another advantage of using a DDS, over direct administration of active pharmaceutical ingredient (API)/drug, is to reduce the undesired toxic side-effects — higher therapeutic dosage and therapeutic index. This has particular relevance for cytotoxic drugs, like chemotherapeutics, which are very potent against cancer, but also exhibit high toxicity

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https://doi.org/10.1016/j.addr.2018.09.005

towards healthy tissues (thereby, impeding their usage at clinically relevant doses/concentrations) [7,8]. This important medical need, coupled with effective dosage requirement, has created the need for site-specific drug delivery systems. Ideally, such a dynamic DDS should achieve therapeutic drug levels at the target site, limit exposure to healthy tissues, and consequently, minimize the occurrence of side-effects. This can be achieved by incorporating a "trigger switch" or "control mechanism", that facilitates drug release in the target tissue, only under specific local or external stimuli. Based upon their stimuli-responsive behavior, DDSs can be divided into two broad categories: (a) endogenously triggered DDS (using pH, redox microenvironment, ATP/glucose, enzymes and hypoxia, among others); and (b) exogenously triggered DDS, or externally actuated DDS (*via* temperature, light, magnetic fields and ultrasound). At this point, it is important to highlight that such synthetic, physically actuated or chemically propelled DDS, have been referred as nano/micromotors (depending upon their size scale), in certain scientific communities [9,10]. While several reviews exist in the area of nano/micromotors for drug delivery, each discussing their *in vitro* and *in vivo* significance, the quintessential pharmacological perspective remains elusive from these reports.

Herein, we have made an effort to expand the discussion of microscale DDS *in vivo*, by establishing a design-application relationship and associated pharmacological significance. Focus has been kept towards *in vivo* applications (see Fig. 1A), to create a common ground between chemical engineers, material scientists, fabrication experts, pharmacologists and clinicians, towards designing of clinically relevant solutions (*i.e.* clinical significance). Owing to limited number of such studies, this review draws inspiration from the fundamentals in clinical drug delivery, discussing current challenges and exploring new opportunities – All with an aim to provide a clear blueprint towards translational drug delivery research.

1. Factors governing design-application relationship

1.1. Active vs passive targeting: how can externally actuated DDS make a difference?

Passive targeting refers to drug transport *via* blood plasma, at the site of interest, and is directly dependent on blood circulation parameters (like circulation time and activity of macrophages, among others). Therefore, all oral and systemic DDS, are essentially passive targeting systems. On the other hand, active targeting has been referred to approaches that include target-specific moieties, to enhance drug accumulation in the target tissue *via* blood circulation (*i.e.* Paul Ehrlich idea of a "magic bullet") [13]. However, such an active targeting should not be confused with site-directed targeting, achieved *via* autonomous propulsion or external actuation [14]. For instance, inflammatory tissues and solid tumors, both exhibit increased drug retention owing to their leaky vasculature system; a phenomenon known as 'Enhanced Permeability and Retention' (EPR effect) [15] – which is more prominent in murine models than human beings [16]. Despite 'homing groups' and EPR effect, internalization of such a DDS into a solid tumor (extravasatation), is still dependent on blood circulation and associated flow gradients. This becomes a serious challange when we correlate blood flow rate (>5 L/min), which is approximately 1.5–33 cm/s (at capillaries and venules) [17], with the total length of the human blood vessels *i.e.* ~100,000 km [18] (diameter of planet Earth is 12,742 km). Consequently, following scenario unfolds: (i) a nanomedicine/drug carrier circulating the blood stream, faces an arduously long journey, at extremely high speeds (>several cm/s); (ii) Nano-DDS bypasses a tumor, at most, a few centimeters in size, in sub seconds and; (iii) ligand-receptor interaction (even if it exists at endothelial surface), must happen in this extremely narrow 'window of opportunity', without being accumulated in the normal tissues (where such receptors are also expressed). Clearly, cancer nanomedicine has come under strong scrutiny [19,20] and requires a radically new approach which can: (a) influence the motion/direction of a DDS, to direct it at the target site, (b) without being entirely dependent on the local fluid gradients (like blood flow) and, (c) followed by site-specific drug release. Such a dynamic DDS with its ability to propel/navigate against the flow, can provide new insight for drug delivery against solid tumors. A systematic assessment of cancer nanomedicine has revealed that less than 1% of the injected drug actually gets internalized into a solid tumor [21]. While, one may argue that nanoparticle dosage (inferred from injected dosage), is not a true representation of pharmacokinetic activity [22], there exists little information to corroborate any further [23]. Clearly, recognizing the scope for improvement (Fig. 1B), efforts have been focused on physically actuated nano/micro DDSs, to provide higher degree of control, and improved drug efficacy [24,25]. This becomes important when taking into account the chemical nature of a drug. For instance, drugs with low solubility in aqueous environment, have limited uptake in diseased tissues (which includes most of the chemotherapeutics).

In this regard, micromotors have shown significant progress, ranging from chemotherapeutic delivery (Medibots) [26,27], nucleic acid delivery [28], performing microbiopsies [29] and even assisted fertilization [30] — all under *in vitro* setting. Being a 'motor' (*i.e.* characteristic motion properties), may offer a significant advantage as they can be

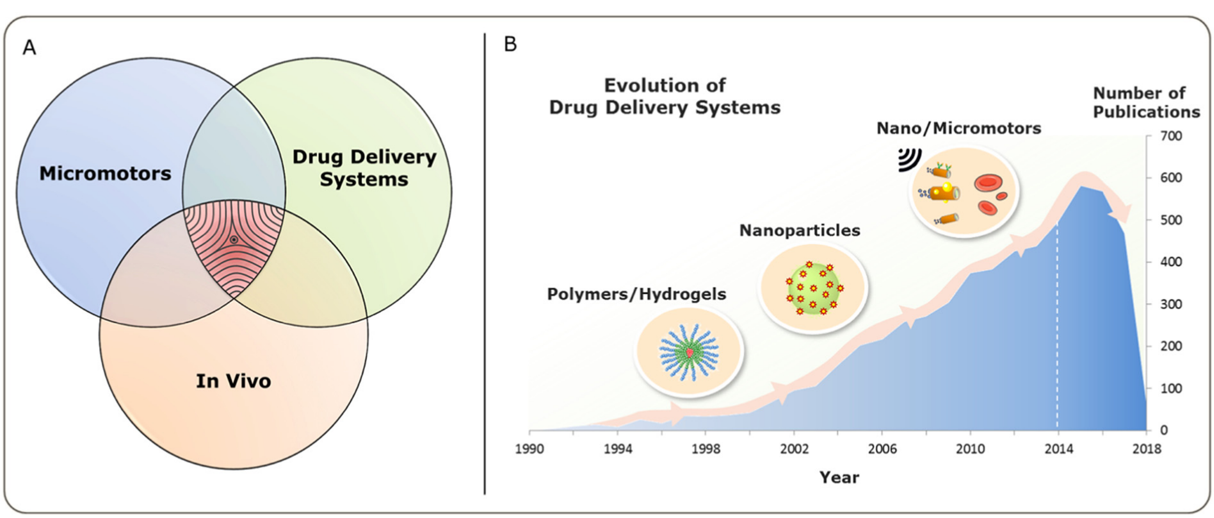

Fig. 1. (A) Diagram depicting the main focus of this review as *in vivo* applications of micromotor for drug delivery. (B) Timeline showing the evolution of the number of publications per year on exogenous-triggered drug delivery systems [PubMed search: (ultrasound OR magnetism OR light OR NIR) AND "drug delivery systems"].

externally actuated (along with activated drug release), or autonomously propelled for local drug delivery. Therefore, nano/micromotors can help bridge the gaps for site-specific drug delivery, by having directed motion, which is independent or less dependent on local fluid gradients.

1.2. Micromotors: mode of guidance/actuation vs. mode of release/activation

Conventionally, the term 'actuate' has been reserved for mechanical processes, and is often used to describe motion properties. The term 'activate' on the other hand, has been used to elucidate chemical conversion of a compound (reactant), or its state. For externally-triggered DDSs, the trigger or control mechanism should be applied locally and non-invasively. Therefore, it is important to briefly discuss these parameters, with respect to their clinical significance, towards micromotors development. These DDSs are able to respond to an external or exogenous trigger i.e. ultrasound, magnetism, light, heat, electricity, by incorporation of specific stimuli-responsive materials. For example, iron oxide particles will respond to magnetic fields, or UV/Vis light can execute photoreactions [31]. While one can recognize their relevance towards local drug delivery, it is equally important to understand how the choice of an external trigger, affects the design of DDS, and associated drug delivery parameters.

In this section, we have briefly discussed chemical and physical triggers/control mechanisms, followed by another section on their clinical relevance.

Micromotors for drug delivery are mostly fabricated *via* a multi-stage process, such that the drug is loaded into a hollow microcontainer, drop-casted or adsorbed onto a surface. It comprises of distinct assemblies such as a therapeutic agent, a support layer/carrier matrix, polymeric chassis, stimuli-responsive layer/particles – U solubilitynlike a conventional 'liquefied drug polymer' production line [32]. Therefore, if dissolution process is drug-controlled, overall bioavailability is largely governed by the chemical properties of the drug itself (as noted in case of low-water solubility like most chemotherapeutics, BCS II & III). To this end, propulsion-induced micromixing effects, for increased solubility, is commonly observed in stationary/less turbulent fluid environment [33–35]. Clearly, such micromixing effects might be limited under physiological conditions, like blood circulation (owing to high intrinsic flow gradient), but more valuable elsewhere (like GI tract).

Exogenous stimuli under clinical setting includes ultrasound, magnetic fields and light. For a successful therapeutic delivery/effect, radiation beam should be strong enough to penetrate deep into the tissue, interact locally with the DDS for actuation/drug release, and avoid any detrimental effects on the target tissue. From a biomedical perspective, only three windows of complete spectrum, facilitate deep-tissue penetration and absorption: X-ray, radio frequency and acoustic radiation [36,37]. Since, the ionizing nature of X-ray poses significant health hazards, it is not considered as an ideal candidate for external stimuli [38]. Likewise, radio frequency waves cannot be focused on smaller tissues/regions, owing to their large wavelength of 1 m or above. This leaves us with electromagnetic and ultrasound (US) waves, which can be focused into a focal point, using principles of wave interference [39]. By reviewing the progress made in the area of exogenously controlled drug delivery *in vivo*, and drawing parallels with micromotor technology, we provide a benchmark for developing combinatorial approach for advanced biomedical applications (see Fig. 2).

At this stage, it is important to understand that there are three components in any active micromotor system: (i) mode of propulsion; (ii) mode of guidance; (iii) mode of release/activation. Externally actuated DDSs, either incorporate all of the above elements, or any combination thereof (refer to Table 1). Better understanding of these parameters, where each has a distinct advantage over the other (see Fig. 2), depending upon the route/mode of application, is critical for improving drug delivery efficacy.

2. Exogenous control: effects of light – NIR under *in vivo* conditions

One of the fundamental requirements of a drug carrier, is its ability to release drugs 'on demand' (to reduce toxicity elsewhere), and enhance efficacy by controlling time and duration of exposure. This is where photoactivation has found extensive usage *in vivo* [51,52]. These photo-triggered DDSs are externally activated by light/NIR, which causes site-directed drug release, with photointensity-dependent release rate. Unlike US or magnetic fields, light-NIR triggers are mostly employed for controlled drug release, rather than actuation *in vivo*. However, photoactivation has been demonstrated towards propulsion/docking of micromotors *via* a photo-catalytic reaction [53,54]. Limited *in vivo* studies can be attributed to the fact that, UV and visible light (< 650 nm) cannot penetrate deep into tissue as: (i) radiation below 650 nm cannot penetrate deeper than 1 cm due to high scattering and; (ii) absorption by hemoglobin, oxy-hemoglobin, and water present in the tissue. Nonetheless, it has found clinical relevance towards topical drug delivery applications. In this regard, Near-infrared (NIR) mediated nano/microsystems garnered special attention, as they can be focused onto a small specific area, and penetrate deeply into the tissue with insignificant damage. NIR light of 650–900 nm (water absorbs wavelengths longer than 900 nm), can penetrate up to 10 cm into living tissue, with minimal tissue damage at the site of application [55]. Therefore, NIR-responsive microcarriers [56], with local payload release, has provided significant advances in drug delivery applications (including photodynamic therapy (PDT), photothermic therapy (PTT), laser mediated tissue sealing). Readers can find a detailed review on photochemical mechanisms for drug delivery applications elsewhere [31].

As for NIR-triggered micromotors *in vivo*, He et al. demonstrated Janus micromotor particles (3 μm), towards photothermal tissue welding, by magnetic/thermal influences (Fig. 3A) [40]. The particle composition comprised of sputtered Au on one side and polyelectrolyte multilayers-SPIONs on the other side of the Si microparticles. The system exploited collagen denaturation (50–70 °C), followed by tissue softening and melting; whereby, a temperature decrease allows for condensation and a hard wound closure. Presence of Au particles in such a system has been well recorded for enhancing photothermal effects [57,58]. While laser tissue welding is a well-studied phenomenon [59], authors claimed that the ability to magnetically guide the "dyes" into a wound, facilitated sealing of actively bleeding wounds; which in a normal case, would flush the passive dye and nanoparticles out. Study performed in a mouse model demonstrated comparable results between motors, nanoparticle gluing and medical suturing (healing completed ~9 days) as shown in Fig. 3A. Further, mechanical suturing introduced additional injuries and tissue deformation, which was avoided in case of laser tissue welding (although, thermal damage was observed for the latter). Also, animal tissue study performed *ex vivo* (beef liver), suggested that liver's mechanical properties can be restored with laser tissue welding, and may provide an alternative for hemostatic treatment for targets with limited suturing potential (like liver and lungs).

While plethora of studies exist, discussing light-activation of nano/microscale DDSs, we have made an effort to discuss some interesting microscale systems, that can provide valuable insight for micromotor technology. In this regard, Zha et al. reported hollow polylyyrole microspheres (2–3 μm) for dual-action ultrasound imaging with photo-trigger drug release *in vivo*. [41] Coupled with ultrasound imaging, a NIR laser completely ablated the tumor in a U87-MG mouse model within two weeks. An easy yet intelligent design scheme, incorporating oil-in-water emulsion method (Fig. 3B), has been applied for synthesizing NIR absorbing hollow microspheres from polypyrrole. Resulting polypyrrole hollow microspheres (PPyHMs), can act as an efficient theranostic agents, by greatly enhancing the US imaging, as well as excellent photohyperthermic effects (earlier demonstrated with incorporation of Au nanoparticless) [60]. What makes this study interesting, is the fact that Polypyrrole (PPy) materials have been tested previously

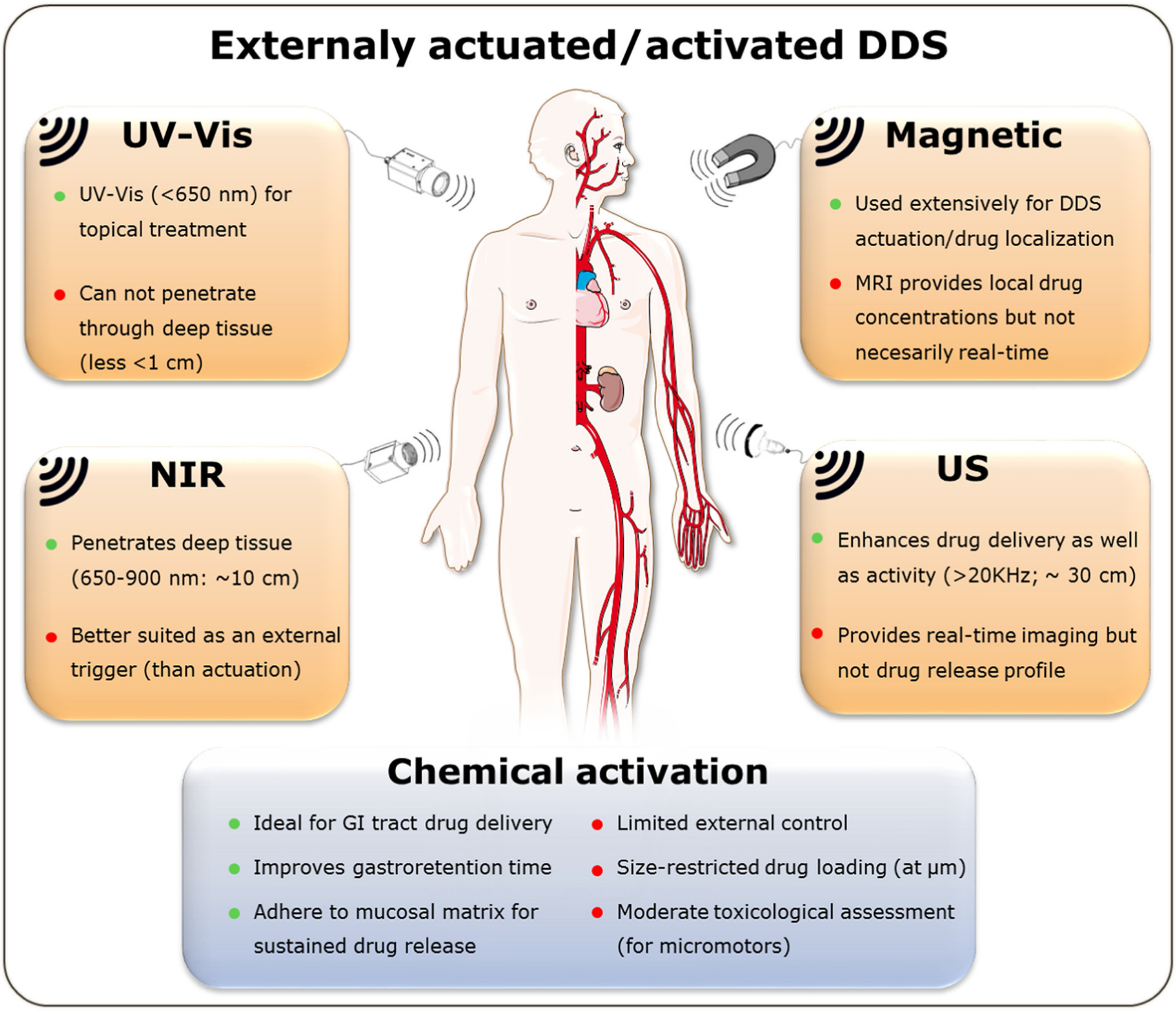

Fig. 2. Schematic summary of external triggers used to actuate/activate drug delivery systems.

Table 1
Synthetic micro-DDS with various physical and chemical actuation/activation schemes.

Carrier and active material	Proposed action/delivery	*In vivo* animal model	Mode of propulsion/guidance	Mode of release/activation	Ref.
Janus microparticles (Au-SPIONs; 3 μm)	Tissue welding	BALB/C mice model	Magnetically guided	Photothermal activation	[40]
Hollow Ppy microspheres (2–3 μm)	Imaging with trigger release	U87-MG tumor mouse model	Blood circulation (passive targeting)	US-NIR	[41]
Fluorescence labelled dextran particles	GI tract delivery	Yorkshire pigs (*ex vivo*); C57BL/6 mice (*in vivo*)	US propelled	Mucosal layer adhesion	[42]
Octafluoropropane trapped magnetic microspheres (3–4 μm)	Pulmonary tract; Real-time imaging and drug delivery (Cy5.5)	Murine LL/2 Lewis lung carcinoma in SCID mice	Magnetically guided	US activation	[43]
PLGA microparticles (10 μm) with SPIONs (magnetic nanoparticles)	Inflamation in joints, (Dexamethasone acetate)	C57Bl/6 mice	Magnetically guided	Diffusion (*via* local delivery)	[44]
Magnetic microhelices (20 μm)	Local delivery in intra-peritoneal cavity	BALB/C mice	Magnetic actuation	NIR fluorophore mediated imaging	[45]
PLGA-FeCo microparticles (50 μm)	Anti-tumor drug delivery, Doxorubicin (DOX)	New Zealand rabbits	Magnetically guided	Diffusion (*via* local delivery)	[46]
PEDOT/Zn tubular micromotors (20 × 5 μm)	*In vivo* propulsion in stomach	ICR mice	Chemical propulsion (Gas evolution reaction)	n/a	[47]
Mg based Janus micromotors (20–30 μm); Eudragit L100 coating	*In vivo* propulsion in stomach	ICR mice	Chemical propulsion (Gas evolution reaction)	n/a	[48]
Mg-loaded PEDOT/Au microtubes (20 × 5 μm); Eudragit L100 coated	*In vivo* propulsion through GI tract	ICR mice	Chemical propulsion (Gas evolution reaction)	n/a	[49]
Mg-microspheres (20–30 μm); Chitosan coated	Drug-PLGA matrix (clarithromycin)	C57BL/6 mice	Chemical propulsion (Gas evolution reaction)	Diffusion (*via* oral delivery)	[50]

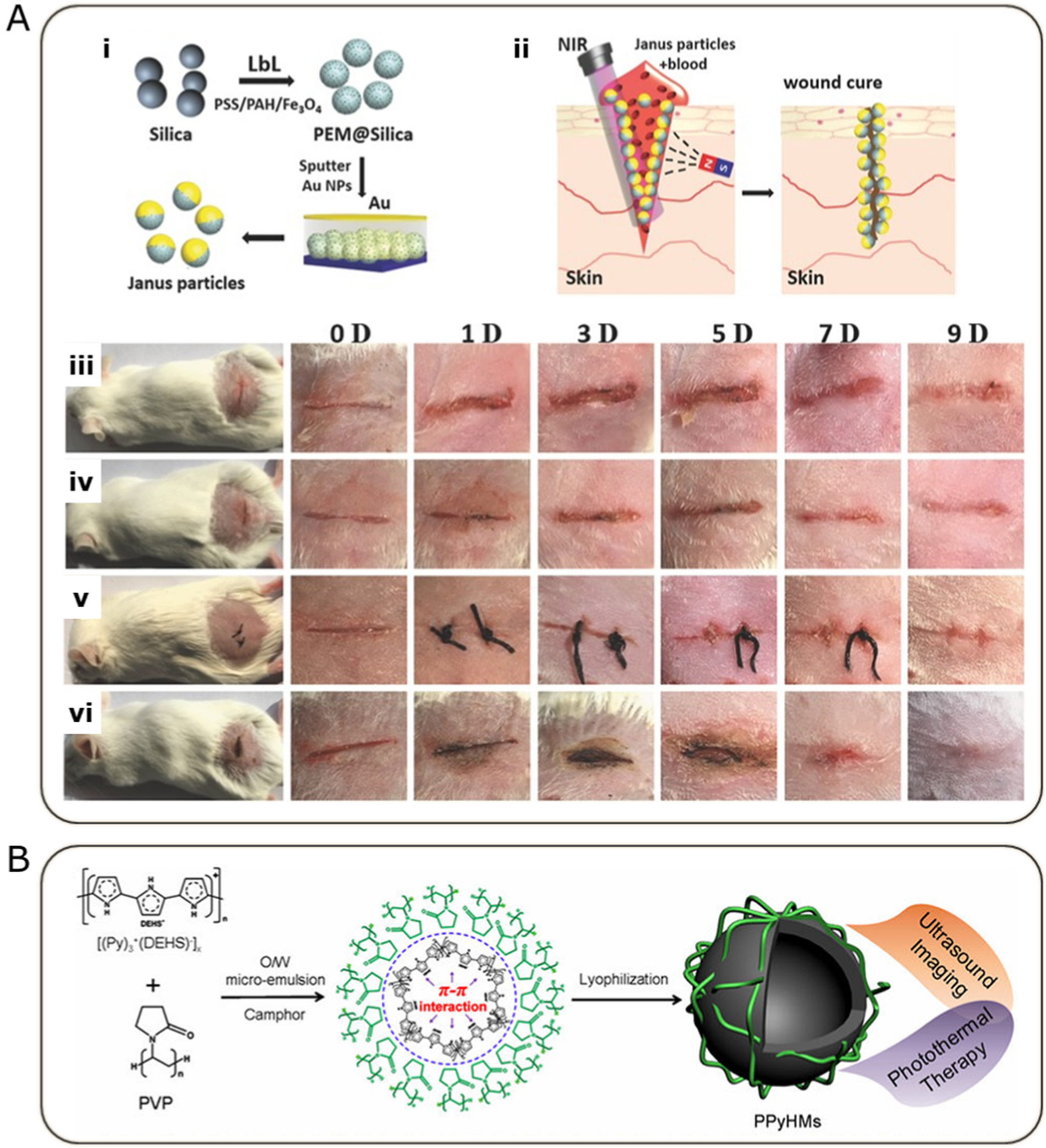

Fig. 3. Exogenous control: effects of light – NIR under *in vivo* conditions (3A) Guidable Thermophoretic Janus Micromotors Containing Gold Nanocolorifiers for Infrared Laser Assisted Tissue Welding. (i) Production of Janus composite particles by LbL self-assembly of PEM and magnetite nanoparticles followed by sputter coating with gold and resuspension in water. (ii) Laser tissue welding with magnetic assistance, due to magnetite particles being homogeneously distributed in the particles the particle orientation is random during welding. Examples of (iii) control sample (no treatment), (iv) laser tissue welding, (v) medical suturing, (vi) nanoparticle glue. Only the non-treated wound heals worse than the treated wounds. Laser tissue welding based on autonomous movable dyes heals comparable to medical suturing or nanoparticle gluing. (3B) Polypyrrole Hollow Microspheres as Echogenic Photothermal Agent for Ultrasound Imaging Guided Tumor Ablation. Schematic illustration of the formation of echogenic PPyHMs for combined US imaging and PTT *via* a facile O/W microemulsion method. Image reproduced with permission from [40,41].

for micromotor fabrication, including cargo delivery under *in vitro* setting. [61,62] Further, PPy offers both stability [63] and biocompatibility [64] towards designing such a photoactive DDSs for *in vivo* applications.

3. Exogenous control: effects of ultrasound (US) under *in vivo* conditions

US (frequency above the audible range of humans; >20 kHz) can be focused on very small areas due to its wavelength in the order of millimeters. It has been employed for both, enhancing the delivery, as well as improved drug activity, for over two decades [65,66]. Enhancement in drug uptake by US, relies on a phenomenon known as *acoustic cavitation*, typically, seen as a cloud of bubbles forming in the vicinity of the ultrasonic source. [67] Cavitation is the formation of low-pressure voids (vacuum bubbles or cavities) in the liquid, which grows, briefly oscillate, and then asymmetrically implode with great intensity. The resulting microjets can physically propel drug/particles into tissue, as well as reversibly permeabilize tissue for enhanced drug uptake. Therefore, we can corraborate that US mediated cavitation appears to play two key-roles: (i) it propel/disrupt the structure of carrier vesicle (and releases the drug) and; (ii) makes cell membranes and capillaries more permeable to the target drug.

While US has found significant clinical application towards imaging, lithotripsy and even transdermal drug delivery, its direct application with conventional systemic and local drug delivery has been rather limited. Nonetheless, US could also serve as an external trigger for drug delivery *in vivo*. A key-feature that makes US interesting is its dual-capacity for imaging as well as actuation/drug release. This is important as imaging has been a key-challenge towards *in vivo* deployment of micromotors [68]. Recent research suggests that US can also be used for drug delivery across GI tract [69] (in addition to the skin [70]), thereby facilitating successful delivery of small molecules, biologics, and nucleic acids [42,71].

Schoellhammer et al. [42] demonstrated US-mediated targeted drug delivery in a mice model. As a proof-of-concept study, they delivered fluorescence-labelled dextran particles (2 μm), into the colon of mice via local US exposure. It was observed that the dextrans could be delivered very fast, deep into the epithelial tissue (Fig. 4A). This was also confirmed by SEM images as: (i) untreated tissue showed thick mucus layer without crypts; (ii) US-treated tissue clearly showed evenly distributed crypts, suggesting that, US dissipates the mucus layer for enhanced delivery and; (iii) US-mediated delivery demonstrated uniform distribution of microparticles with no clustering. Nonetheless, as they observed removal of mucus layer, there could have been detrimental consequences for the underlying intestinal tissues [72]. This 'gap' can be covered by chemically-propelled micromotors (as discussed later), which tends to 'propel and penetrate' the mucosal lining in stomach (mucopenetration), opening novel options for per oral (P.O.) drug delivery.

Another important area, where US may find a slight edge over magnetic systems, is its ability to get focussed in a smaller area. Magnetically controlled systems *in vivo* are highly dependent on magnetic field strength. Technically, it is difficult to build-up sufficient field strength, focussing on a small area, in order to counteract linear blood flow rates in tissues (>10 cm/s) and arteries (0.05 cm/s). While one may argue that sufficiently strong magnets are now available (MRI operates at 1–3 T, or using rare earth magnets for localization), avoiding normal tissue clearance, is still a major challenge. Under clinical setting, it is important not only to achieve local drug delivery, but also to monitor real-time pharmacokinetics. For example, while US imaging allows rapid visualization using contrast agents, it does not provide much information about drug release profile and local action. However, Magnetic Resonance Imaging (MRI) can provide local drug concentrations with limilted real-time capabilities. Therefore, upon combining them together, one may achieve both, local drug administration and pharmacokinetic assessment.

Recently, Langer et al. demonstrated such a combinatorial theranostic system by integrating both, magnetic and US theranostic systems, towards fabrication of magnetic microbubbles (MagMB) [43]. These MagMB are essentially hollow (gas-trapped) lipid microparticles (3–4 μm; Fig. 4B), that are responsive to both magnetic (owing to the presence of iron oxide nanoparticles) and acoustic modulation, imaged

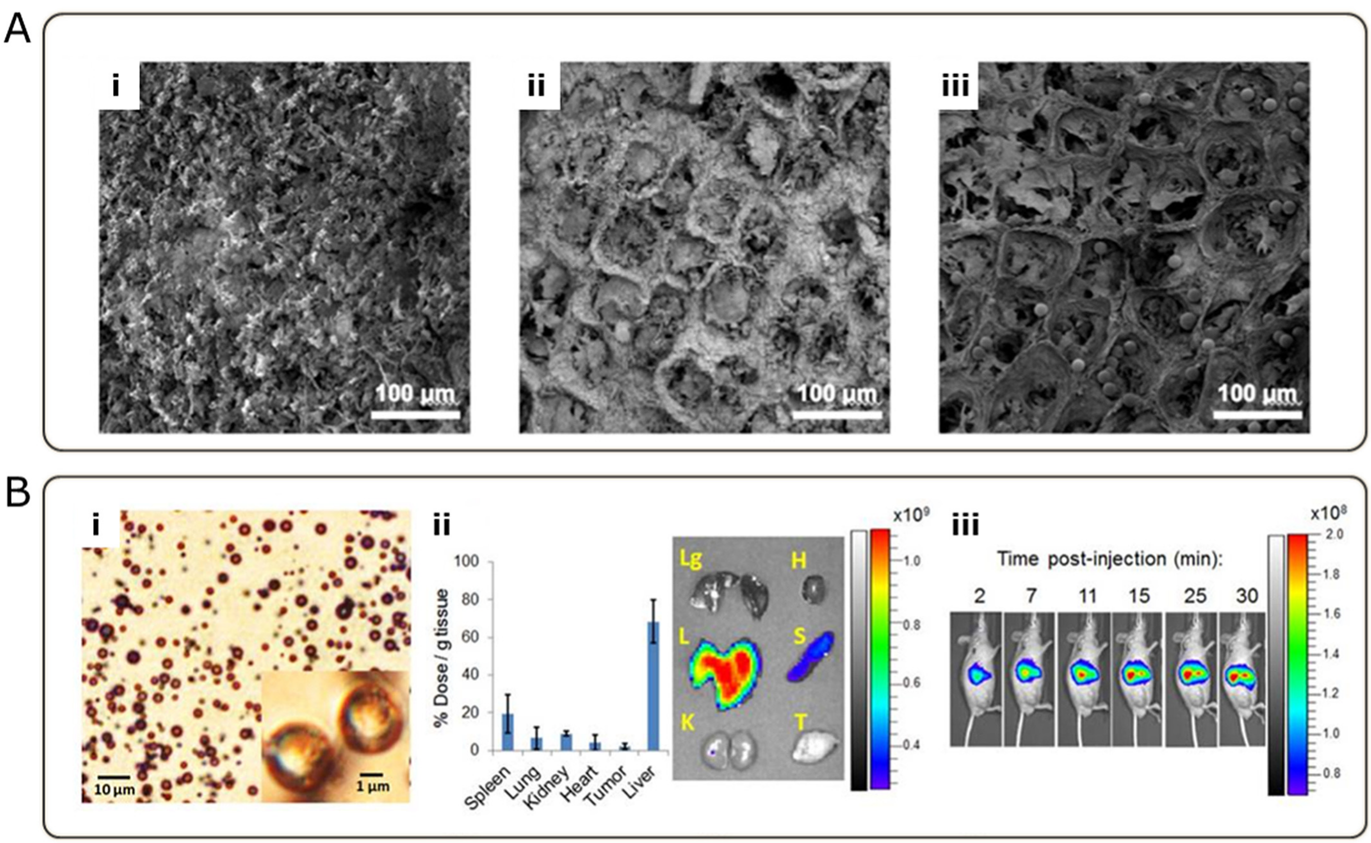

Fig. 4. Exogenous control: effects of ultrasound (US) under *in vivo* conditions. (4A) Defining optimal permeant characteristics for ultrasound mediated gastrointestinal delivery. SEM micrographs of porcine colonic tissue not treated with ultrasound (i) and after treatment with ultrasound (ii). (iii) Porcine colonic tissue after simultaneous treatment with ultrasound and 15 μm diameter latex beads. (4B) Circulating magnetic microbubbles for localized real-time control of drug delivery by ultrasonography-guided magnetic targeting and ultrasound. (i) Representative bright-field phase contrast micrographs of MagMB (Inset: high magnification (100×) phase-contrast micrograph of MagMB). (ii) Organ biodistribution of MagMB-Cy5.5 quantified *ex vivo* by fluorescence spectrophotometry 30 min post administration. Data represent mean ± SD, $n = 4$. Representative qualitative biodistribution profiles of MagMB-Cy5.5 analyzed by fluorescence imaging 30 min post-administration (Lg: lungs; L: liver; K: kidneys; H: heart; S: spleen; T: tumor). Color bar represents fluorescence radiant efficiency expressed in [photon/s/cm^2/steradian]/[μW/cm^2]. (iii) Representative kinetic sequence of the MagMB-Cy5.5 organ distribution analyzed by *in vivo* whole-body fluorescence imaging over a 30 min time interval post administration. The color bar represents fluorescence radiance expressed in [photon/s/cm^2/steradian]. Image reproduced with permission from [42,43].

via ultrasonography towards real-time control of drug delivery. As shown in Fig. 4B, following intravenous injection in mice, MagMB exhibited a 17–90 fold lower pulmonary entrapment, as compared to previously reported magnetic microbubbles (circulation time > 10 min). It accumulated in tumor vasculature ('L' symbolizes lungs) by magnetic targeting, monitored by ultrasonography, and collapsed by focused US to activate drug deposition at the target. US guided burst delivery of microbubbles, carrying nucleic acids as payload, can be a dual-action solution, promoting both imaging and biologics delivery [73].

4. Exogenous control: effects of magnetism under *in vivo* conditions

Early studies of magnetic guidance/drug localization, towards selective cancer chemotherapy, can be traced back to a series of reports by Kato et al. [74]. Starting from albumin-magnetic microbeads, to ferromagnetic mitomycin microcapsules (about 300 μm), these DDSs were magnetically localized *in vivo* at the site of a tumor. This suggests that the idea of overcoming side-effects of systemic chemotherapeutic administration has been a key-driver for magnetically activated DDSs. It has been achieved by incorporating a layer/assembly of magnetically responsive nano/microparticles, which respond to an external magnetic field. Generally, Iron, Cobalt, Nickel and superparamagnetic Iron particles (SPIONs) are used for drug delivery applications.

However, one key-difference between previous studies, and current generation of micromotors, is the fact that external fields are directed towards active mobilization of DDS, and not merely limited to accumulation of DDS (*i.e.* tissue localization). This can be understood as, a magnetic object under a homogeneous magnetic field, will not experience gradient forces. Therefore, the DDS cannot be moved (actuated), but rather localized at a specific site, to prevent random diffusion. On the contrary, in case of magnetically actuated DDS (like micromotors), it is important that the dipole is not aligned with the direction of the applied magnetic field, to generate a net torque for guided motion [75]. In either case, it promises to improve the targeting efficiency of magnetic carriers, as well as enhance the drug availability at the molecular site of action.

While, it is known that magnetic localization of a therapeutic agent results in achieving effective dosage concentration, some studies have also demonstrated it as a prolong release trigger. A study by Butoescu et al. demonstrated that, magnetically retainable DDS (1 μm and 10 μm), may overcome current limitations of intra-articular corticosteroid injections in joints (*i.e.* drug-crystal induces arthritis and repeated injections in joint). They demonstrated slow release of dexamethasone, over a period of three months, by applying an external magnetic field to overcome issues with drug re-crystalization in joints [44,76].

Initially, there was no significant difference regarding presence or absence of a magnet near the joint (two weeks post-administration). This was speculated due to the action of macrophages, clearing out these magnetic microparticles, thereby, minimizing the influence of a magnet. However, over a longer period of observation (Fig. 5A), histological images confirmed that microparticles are internalized into the joint (three-months post-injection), with no inflammatory response, or damage to the synovial lining. This also indicates that magnetic particles pose no significant health risk and may provide pharmacologically relevant dosage over a long period of time.

While, a few years ago, lack of better controls and imaging modalities would have hampered the growth of such magnetic microswimmers *in vivo*, a recent study by Nelson et al. offers a breakthrough solution for the same [45]. They demonstrated that magnetically-actuated synthetic microswimmers, loaded with NIR-fluorophores, could navigate in the peritoneal cavity of mice, providing real-time tracking *via* live fluorescent imaging (Fig. 5B). These

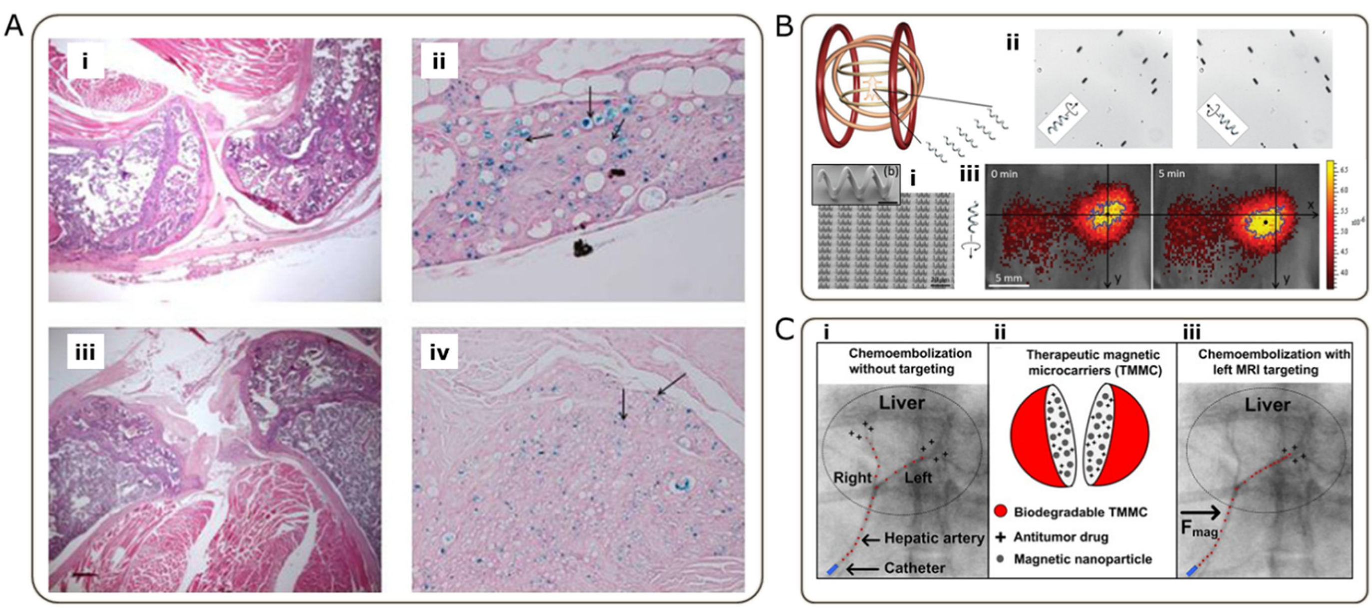

Fig. 5. Exogenous control: effects of magnetism under *in vivo* conditions. (5A) Magnetically retainable microparticles for drug delivery to the joint: efficacy studies in an antigen-induced arthritis model in mice. Histology of mouse knee joints 3 months after intra-articular injection of either 10 μm (i and ii) or 1 μm (iii and iv) microparticles. Of note, even after 3 months, both types of microparticles are present in the tissue surrounding the joint cavity. Prussian blue (PB) staining provides evidence of iron within the microparticles; see arrows in (ii and iv). No major signs of inflammation are evident. Original magnifications: x20 (i and iii), x400 (ii) and x100 (iv). Stains: haematoxylin and eosin (i and iii) and PB (ii and iv). (5B) Controlled *in vivo* swimming of a swarm of bacteria-like microrobotic flagella. (i) Image of a single or an array of 16 μm artificial bacterial flagella (ABF) fabricated by Direct Laser Writing (DLW). (ii) Controlled swimming of f-ABF swarm *in vitro* under 9 mT and 90 Hz on a polished Si wafer in distilled water tracked by optical microscopy. (iii) Swimming of a swarm of f -ABFs in the intra peritoneal cavity of a Balb-C mouse under 9 mT and 90 Hz (total movement of the swarm center of mass: 1.3 mm). The contour of the yellow cloud was determined using a homemade Matlab code and was used to determine the movement of the swarm of f -ABFs. (5C) *Co*-encapsulation of magnetic nanoparticles and doxorubicin into biodegradable microcarriers for deep tissue targeting by vascular MRI navigation. Representation of MRI targeting with TMMC for liver chemoembolization by fluoroscopy images of the rabbit hepatic artery with superposed images of the TMMC distribution without (i) and with (iii) the MRI targeting. On image (i), the microparticles are released from the catheter in the artery and distributed to both lobes. (ii) Schematic representation of a cut of the TMMC loaded with an antitumor drug and magnetic nanoparticles embedded into a biodegradable matrix. (iii) displays the MRI targeting of the left bifurcation using the magnetic force (Fmag) to preserve the right lobe from the chemoembolization. Image reproduced with permission from [44,45]. Fig. 5C Reprinted from Biomaterials, 32, P. Pouponneau, J.C. Leroux, G. Soulez, L. Gaboury, S. Martel, *Co*-encapsulation of magnetic nanoparticles and doxorubicin into biodegradable microcarriers for deep tissue targeting by vascular MRI navigation, 3481–3486, Copyright (2011), with permission from Elsevier.

microhelices respond to a magnetic field produced by AC currents, and in turn, exhibit micromotor motion, by modulating direction and magnitude of the resulting magnetic field (note that DC magnetic field is constant).

This is a significant step towards site-directed 'motion & tracking'. In this regard, Magnetic resonance navigation (MRN) provides a significant opportunity for nano/micromotor development. In another study, Martel et al. demonstrated PLGA-microparticles (50 μm), loaded with the antitumor drug doxorubicin, and iron-cobalt nanoparticles for optimal steering properties (Fig. 5C) [46]. MRN was demonstrated for tumor targeting of microparticles and systemic concentration of doxorubicin was minimized due to localized drug release. Microparticles were steered in the hepatic artery with a depth of 4 cm below the skin. Drug localization phenomenon was further confirmed by Price et al., towards targeted delivery in lungs, for treating lung cancer. [77]. They demonstrated magnetic microparticles (50 μm), encapsulating doxorubicin for site-specific drug localization, *via* an external magnet (demonstrated 10-fold increase of particle deposition).

5. Chemical activation: utilizing body fluids as a fuel for propulsion *in vivo*

Chemically-propelled micromotors are another class of actively propelling microscale DDS that have been widely investigated. These effervescent autonomous microswimmers can be divided into two categories: (i) Catalytic micromotors and (ii) Chemical micromotors (Non-catalytic). As the name suggests, catalytic micromotors are specific geometries of heterogeneous catalysts, promoting high turnover numbers, under the aqueous reaction environment for bubble propulsion (*via* a gas evolution reaction) [78]. For example, hydrogen (H_2) evolution reaction in the presence of Pt/Ag as catalyst with hydrogen peroxide (which has been a key driver for the early reports of such catalytic micromotors) [79]. However, utilizing hydrogen peroxide as a fuel in living organisms, is not the most viable idea, owing to its potential toxic side-effects. Due to this reason, chemical or non-catalytic micromotors are of considerable interest. Mou et al. reported 'comparatively biocompatible' Mg-Pt micromotors, based upon passivation of $Mg(OH)_2$ in $NaHCO_3$, and reaction between Mg and H_2O, to generate H_2-bubbles for propulsion. Since, the active agent (propellant) got rapidly consumed in the chemical reaction (in this case, Mg), life span was reported to be 80 s only. Nonetheless, these chemically-activated micromotors, exhibiting gas evolution reaction, turned out to be the ones which were introduced *in vivo*. As it stands within the framework of chemistry, a catalyst, by definition, should not get consumed in a chemical reaction. However, catalytic and non-catalytic or chemical micromotors, have been used indistinguishably in the literature.

Conceptually, these chemically-propelled micromotors are very similar to effervescent floating-type drug delivery systems (FDDS) [80], which have found extensive application in P.O. delivery, for more than three decades. Based upon their mode of propulsion/activation, these FDDS can be regarded as effervescent FDDS (gas generation), or non-effervescent FDDS (buoyant preparations like hollow microspheres or 'microballoons', among others) [81]. In fact, chemically-activated micromotors have much in common with these effervescent FDDS, where active components like sodium bicarbonate, citric acid or tartaric acid (and acid-base mixtures) have been extensively employed in P.O./GI formulations — including antacids, anti-microbial drugs and overall improved bioavailability. [82–85] For instance, Özdemir et al. demonstrated enhanced bioavailability of furosemide drug *via* gas evolution reaction involving sodium bicarbonate and citric acid. [86] In terms of pharmacological significance, three main advantages can be cited for such a floating type or chemically propelled system in GI tract [87]: (i) avoid periodic emptying cycles in stomach (myoelectric cycles); (ii) increased gastroretention time (GRT); (iii) mucoadhesion properties for sustained drug release [88,89].

Fate of a drug along the GI tract (P.O.) depends on following parameters: (a) permeability of GI mucosa and transit rate in GI tract; (b) variable gastric emptying of pharmaceuticals, which is also dependent on the dosage form (including fast/fed state) and; (c) presence/absence of a protective matrix against highly acidic stomach microenvironment with aggressive enzyme cocktail. Likewise, for an effervescent FDDS, the main objective is to remain buoyant *via* a gas-evolution reaction (or entrapment), so as to avoid being cleared out from the stomach. At the same time, they protect the drug from harsh GI environment, achieve mucosal layer adhesion *via* polymer swelling and ultimately, sustained release of the chosen therapeutic. In this context, micromotors may be able to achieve the same, albeit faster/better, due to autonomous propulsion or motion control. With an aim of promoting open scientific discussions, part of us would like to present the first prototype concept (unpublished) of an 'oral – micromotor incorporated DDS' as shown in Fig. 6. Biocompatible polymeric microcontainers can be bulk-produced and loaded with both – therapeutics and a suitable propellant ($CaCO_3$ in our case). Gastroretention time (GRT) can be further enhanced, by covering these microcontainers with a polymeric cap, to avoid drug degradation. Finally, resulting drug-propellant microcontainers, can be easily filled into a capsule for oral administration; much like a normal capsule, with drug granules inside. Such a dynamic oral DDS safeguards the drug in harsh GI environment and facilitates characteristic micromotor propulsion (upon dissolution of outer capsule matrix *via* a polymer swelling process). Our initial studies (Fig. 6) have confirmed that these drug loaded microcontainers, provide a viable solution towards an age-old challenge of low bioavailability of orally administered drugs, which needs to be further investigated by the DDS community. In fact, we have dedicated an entire section towards micromotors in GI tract (per oral), owing to their growing significance under *in vivo* setting.

6. Micromotors in GI tract: exploring clinical relevance and future opportunities

One of the key differences between autonomously propelled micromotors and effervescent FDDS, is its size-scale. As the name suggests, micromotors are in the range of micrometers, while a normal FDDS formulation is in the scale of millimeters. However, if one truly talks about pharmacological significance or minimum effective concentration (MEC), it is imperative that significantly higher drug loading will be required for any P.O./GIT drug delivery [90]. This also requires pharmacological assessment, like gastroretentive dosage, which is not limited to single particle motion properties. For example, antibiotic administration against bacterial infections (like *H. pylori*) is difficult due to: [91] (a) stability of antibiotics in low pH gastric acids; (b) drug concentration in the deep gastric mucus where the bacterium actually resides and; (c) short antibiotic residence time in stomach.

Therefore, micromotors for GI tract drug delivery, needs to overcome above challanges, to move beyond a proof-of-concept phase.

Gao et al. demonstrated efficient micromotor propulsion under *in vivo* conditions (Fig. 7A) [47]. PEDOT/Zn tubular micromotors utilized gastric acid (HCl) in mouse stomach for self-propulsion (H_2 evolution *via* oxidation of zinc metal with acid). This autonomous propulsion allowed micromotors to penetrate into the soft mucosal layer, without inducing any destructive effect on the gastric epithelial cells, and increase their retention in the stomach tissue. This was confirmed by analyzing the stomach tissue post-administration (oral, 2 h), as compared to the non-motile control (Pt).

While, thickness of mucus layer is highly variable (40–200 μm), and any extra penetration may damage the underlying cells/tissues; swimming in such an environment is fairly 'difficult' — low Reynnold's number regime [93] [94]. While no actual drug was utilized in the above mentioned study, a pH-sensitive drug release mechanism was demonstrated using Au nanoparticles. It is advisable to demonstrate similar

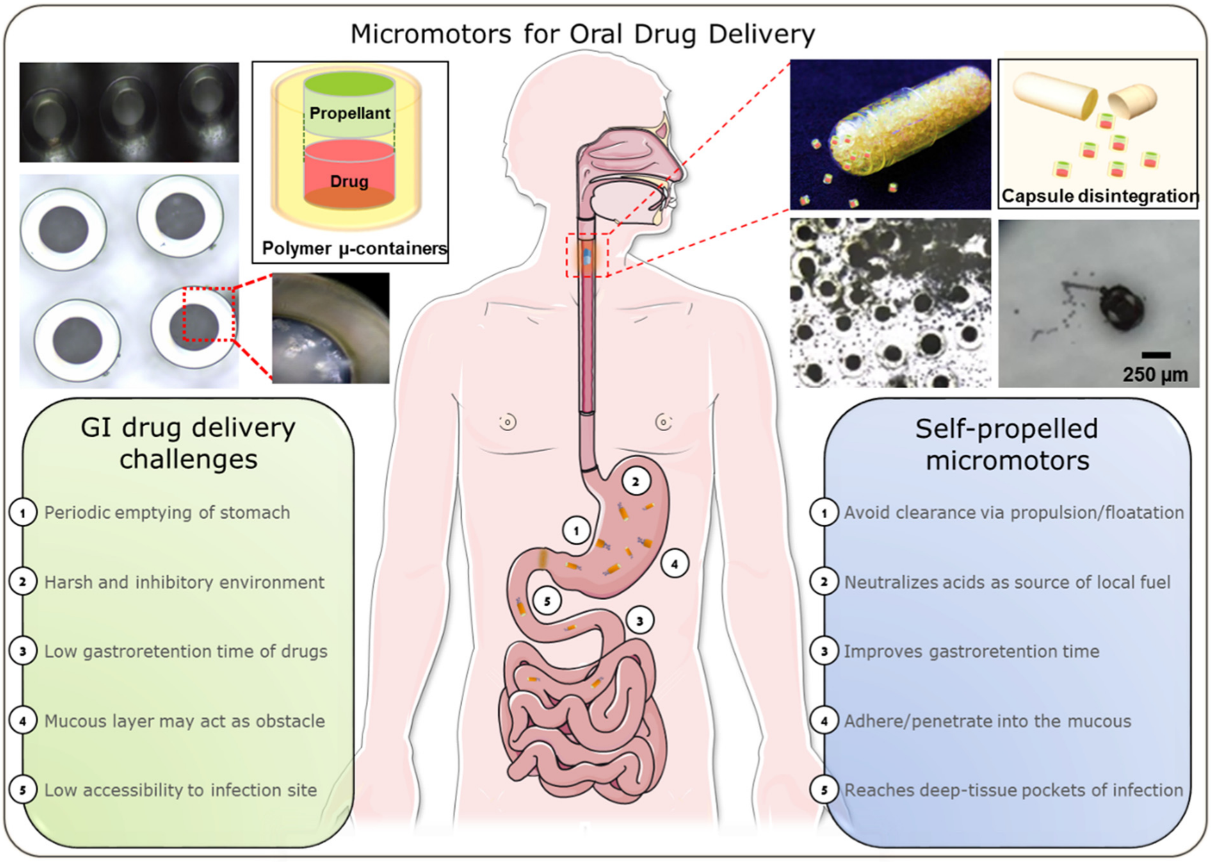

Fig. 6. Oral DDS depicting a capsule filed with drug loaded micromotors and comparative assessment of GI tract drug delivery between conventional *vs* dynamic DDS.

effects as a function of time, to confirm enhanced retention of such mobile DDSs across GIT [95].

Another key-area where micromotors find merit is towards an alternative for Proton Pump Inhibitor (PPI) drugs. PPIs are a class of drugs characterized by irreversible deactivation of the hydrogen-potassium adenosine triphosphatase enzyme system, commonly known as gastric proton pump, present on the lumen of parietal cells (responsible for HCl production); thereby, raising the gastroduodenal luminal pH from approximately 1.5 to 6.0. Similar to an antacid which neutralizes stomach acidity, these chemically propelled micromotors, also react with the stomach acids to reduce acidity. This is demonstrated by the reaction of stomach acid with alkali ions, or acid hydrolysis in presence of metals, each effectively reducing the protons (H^+) in the acidic microenvironment.

However, PPIs have been questioned over their long term side-effects, with reduced absorption of iron, calcium, magnesium zinc and vitamins (Vit B12) [96,97,98]. Interestingly, Mg and Zn have been used as an active layer (propellant), to react with the stomach acids, and simultaneously propelling the micromotors under *in vivo* conditions [12,48,50,92]. The choice of propellant becomes a relevant criteria (more than just propulsion), as severe hypomagnesaemia has been a challenge in long-term users of PPIs, to which this study sheds new light by forming Mg by-products, which can be absorbed by the body. [99,100] In fact, magnesium deficiency is not uncommon, 12% of the patients admitted in hospitals (with 60–65% in Intensive Care Unit), have been diagnosed with hypomagnesemia [101]. Further, inherent H_2 gas production, upon reaction of HCl with Zn, is a common feature of gut microflora activity, and should not be of greater concern within safe limits [102]. While one may argue, that the mode of action of a PPI is completely different than that of an antacid, to which such a class of micromotors actually resembles; the inherent design novelty, may actually be a clinically relevant idea, which needs to be further investigated.

The innate ability of chemically-propelled micromotors, to neutralize stomach acid *in vivo* (utilizing it as a local fuel), constitutes as a major improvement. This can be extrapolated in the field of clinical drug delivery in GI tract (P.O.) as many drugs suffer from very low bioavailability [103]. Li et al. presented Mg-based Janus-like micromotors (Fig. 7B), that reacted spontaneously with the protons in the gastric acid, to rapidly neutralize the stomach pH, without affecting the normal stomach function. [48] The pH-dependent release was demonstrated by fluorescent imaging (DiD dye) of the stomach, which confirmed homogeneous distribution along the entire tissue (Fig. 7B). To ensure normal stomach function was preserved after the treatment, stomach pH was recorded with a microelectrode sensor coupled with a pH meter. Their results demonstrated that neutral pH 7.81 obtained after the micromotors administration, returned back to pH 2.16, within 24 h post-treatment (Fig. 7B). However, care should be taken as single pH electrode measurements have demonstrated regional variations across four quartiles of the stomach [104,105].

Another key-aspect of P.O./GIT drug delivery is acknowledging the fact that, different bacterial population (*good* or *bad*), colonize different segments of the GI tract [106]. Several drugs need to pass through the stomach, into duodenum and further, for effective absorption. Therefore, there is a need to elucidate, if these micromotors remain in the stomach, or can actually pass through pylorus to duodenum and beyond? In this regard, Li et al. (Fig. 7C) demonstrated shielding of Mg-micromotors in the stomach (pH 1–2), by incorporating an enteric coating around it. [49] These tubular micromotors were able to passively diffuse through the stomach, via a pH-sensitive enteric coat, which disintegrated in intestines; thereby, exposing the underlying Mg microparticles for self-propulsion (*via* oxidation of Mg in water). This was confirmed by the ICP-MS analysis and fluorescent images of the mice GI tract (6 h after administration). Also, tuning the thickness of enteric coating controlled micromotor propulsion in

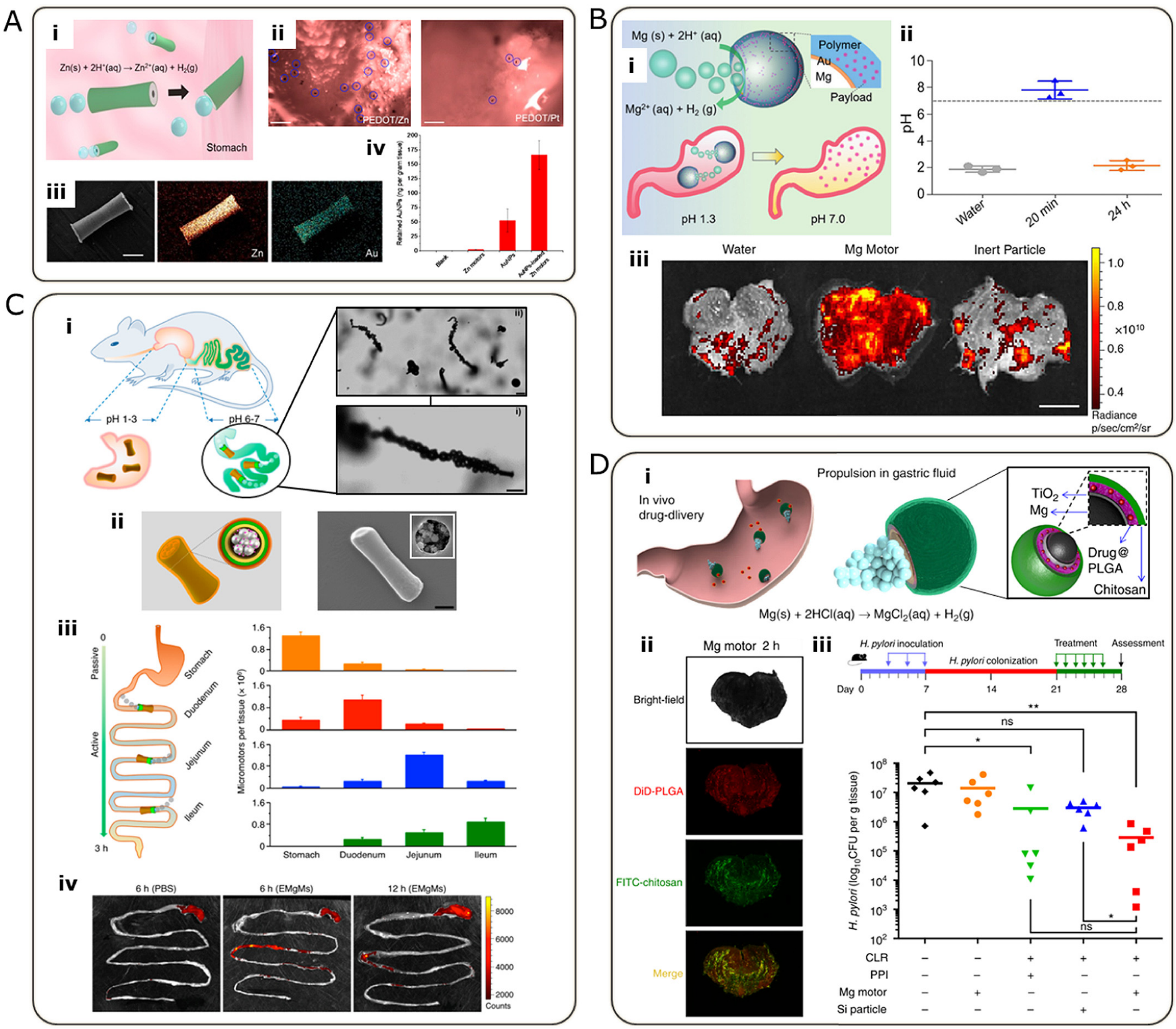

Fig. 7. Examples showing the potential of using micromotors *in vivo*. (7A) Zn-micromotors propel in the acidic environment of mice stomach (i) enhancing their retention to the stomach wall *via* mucoadhesion. (ii) Micrographs, (iii) SEM and EDX analysis showing enhanced retention of the micromotors on the mice stomach. (iv) Inductively coupled plasma-mass spectrometry (ICP-MS) analysis highlighting AuNP retention 2 h after administration. (7B) pH-sensitive Mg-micromotors (i) effectively neutralize gastric acid for cargo release upon pH activation. (ii) Recovery of the gastric pH after treatments in mice ($n = 3$) measured 20 min and 24 h post administration of 5 mg of the Mg-micromotors. (iii) Superimposed fluorescent images of the whole stomach of mice collected 20 min post-administration of DI water, Mg-micromotors and inert PS microparticles (both DiD-loaded within the pH-sensitive polymer coating as model drug). (7C) Micromotors with enteric-coating towards site-specific drug delivery in GI tract *via* autonomous propulsion. (i) Schematic illustration of *in vivo* operation of the enteric Mg-micromotors (EMgMs) and snapshots showing their propulsion in intestinal fluid. (ii) Schematic of an EMgMs showing the loaded Mg microspheres and payload into PEDOT (green)/Au (yellow) microtubes coated with an enteric polymer (orange), and SEM imaging of the EMgMs. (iii) *In vivo* biodistribution and retention of EMgMs in the GI tract using ICP-MS to determine the number of micromotors with different enteric coating thickness (Mg micromotors without enteric coating, orange; EMgMs with thin, medium and thick polymer coating, red, blue and green respectively) retained in the stomach, duodenum, jejunum, and ileum 6 h post-oral administration. (iv) Superimposed fluorescent images of mouse GI tracts at 6 and 12 h post-administration of EMgMs loaded with the dye Rhodamine 6G and covered with medium polymer coating. Reprinted (adapted) with permission from Wang et al. Enteric Micromotor Can Selectively Position and Spontaneously Propel in the Gastrointestinal Tract, ACS Nano. 10 (2016) 9536–9542. doi:https://doi.org/10.1021/acsnano.6b04795). Copyright (2016) American Chemical Society. (7D) Micromotor-enabled active drug delivery for *in vivo* treatment of stomach infection. (i) Schematic representation Mg-micromotors composition and their *in vivo* drug delivery. (ii) Bright-field and fluorescence images of the luminal lining of freshly excised mouse stomachs 2 h after oral gavage of the Mg-based micromotors. (iii) Study protocol and therapeutic outcome of CLR-delivery using micromotors stomach for the treatment of *H. pylori* infection. Image reproduced with permission from [92] [48,50].

different parts of the GI tract, including stomach, duodenum, jejunum and ileum (Fig. 7C).

The ability of these micromotors to effectively propel itself, by reacting with its immediate biological environment (like stomach acid in GIT), is relevent for antimicrobial drug delivery. Stomach infection with *H. pylori* has been reported as one of the main inducers of digestive tract disorders [107]. Causative bacteria adheres to gastric mucosa and induce gastritis, a chronic inflammation of the mucosa, that makes this condition particularly tricky to treat with conventional antibiotics. Drug efficacy is further reduced, owing to harsh acidic environment in the stomach, and potential side-effects with prolong usage of PPIs [108]. To this end, a study done by Esteban-Fernández de Ávila et al. demonstrated synthetic micromotors for drug delivery *in vivo*, with an aim to evaluate their therapeutic potential. [50] Enhanced antibacterial efficacy was demonstrated, with clarithromycin (CLR) loaded Mg-micromotors, against *Helicobacter pylori* stomach infection in a mouse model. Mg-micromotors were prepared by coating Mg-microparticle core with a thin TiO_2 film, followed by a layer of PLGA-CLR matrix, and finally, a positively-charged surface coating of chitosan (Fig. 7D). Such a micromotor design configuration offer several advantages as follows: (i) exposed Mg-core generates a thrust of bubbles for propulsion (*via* oxidation of Mg in H_2O); (ii) simultaneous reduction of stomach pH, creating favorable microenvironment for the drug; (iii) uni-directional propulsion promoting mucoadhesion, followed by polymer swelling (in this case, chitosan), towards sustained-release of drug.

Further, CLR-Mg-micromotors also demonstrated greater penetration into the stomach mucosa, where *H. pylori* resides locally. The antibacterial activity of CLR-loaded Mg-micromotors was investigated in C57BL/6 mice, infected with *H. pylori* every day for 5 consecutive days, with 30 mg/kg of CLR drug co-administered with PPI omeprazole. This is a conventional treatment regime to neutralize gastric acids and preserve the effectivity of co-administered antibiotics [109,110]. Results demonstrated moderate reduction of *H. pylori* by CLR-loaded Mg-micromotors as compared to their non-motile control (Fig. 7D) Authors claimed that active-propulsion was a key-factor for enhanced therapeutic outcome. In addition, while the antibacterial effect of Mg-micromotors and free CLR + PPI were similar, oral treatment with CLR alone, required prior administration of PPIs to increase pH of the stomach. In this regard, the built-in proton depletion capacity of the Mg-micromotors, offers a unique ability to temporarily and reversibly neutralize the gastric pH, and avoid dependency on PPIs with their potential side-effects [111].

These unique properties actually place chemically-propelled micromotors (like Mg-micromotors), in a prominent position for alternative therapeutic strategies, or combinatorial approach for oral/GI tract drug delivery. This becomes important as *H. pylori* infection is the major cause of stomach ulcers, often requiring PPI administration. Ironically, PPI administration, in turn, has also been cited as a causative agent for other enteric infections (*Clostridium difficile*) [112,113] [114] While one may point to inherent similarities between FDDS and micromotors, we strongly believe that these two distinct technologies, have excellent potential to work in synergy together.

7. Can size and shape of micromotors affect their drug efficacy *in vivo*?

Finally, it is imperative to discuss size as well as shape related effects, which will be of considerable importance for the micromotors community. Shape and size of microparticulate DDS affects drug metabolism across all types of DDS. In fact, size influences almost every aspect of DDS, ranging from uptake mechanism, flow properties, degradation, and clearance. As their scale decreases, drug delivery devices may be delivered by ingestion (~1 mm), injected into tissue (<200 μm), inhaled (<100 μm) or even released into circulation (<10 μm) [115]. This can be understood as particle size affects its diffusion and uptake in blood vessels, airways or GI tract. Microparticles of smaller size (1–5 μm), are typically cleared out in the liver by Kupffer cells, while larger particles are trapped in capillary beds [116]. In case of pulmonary administration, particle range of ~3 μm gets deposited in the alveolar region, while large particles are trapped in upper airway (smaller particles exhaled) [117]. Regardless of the method of administration, particles larger than 500 nm can be phagocytosed by macrophages, while smaller particles can be cleared off *via* endocytosis [118,119].

Even for imaging purposes, particle size is important. For example, one of the essential features of ultrasound contrast agent (UCA) is the size of the hollow microparticle. The DDS must be smaller than 8 μm in diameter for intravenous administration [120]. Interestingly, contrary to the belief that larger particles will experience more stearic hindrance, studies have indicated that despite the order-of-magnitude difference in bead diameters (0.02 to 2 μm), consistent delivery was achieved using US, showing no significant dependence on permeant size [42].

Further, P.O./GI drug delivery systems have been reported for several formulations, including floating systems, mucoadhesive/bioadhesive systems, expandable systems and magnetic systems — to prolong gastrointestinal (GI) residence time and to improve drug effectiveness [121]. In this regard, reservoir-type microcapsules, acting as drug microcontainers, will be of considerable interest [122]. Owing to their sufficiently large internal volume (100–250 μm) for drug loading, they have demonstrated excellent pharmacological effects under *in vivo* setting [103,123,124]. Similar design concept may be tested with autonomous propulsion or external guidance.

Interestingly, there has been a renewed interest in shape related effects of DDS. A basic premise for drug release, as governed by the Fick's law of diffusion, can be controlled *via* uni- or omni-directional continuum of the carrier [125]. Langer et el. demonstrated zero-order release, which is the main goal of many sustained release formulations, with a hemispherical particle facilitating release from the face-end only [126]. This single-phase release feature, has been highlighted as a significant advantage for top-down fabricated DDS, without compromising the continuum-like behavior. Therefore, polymer engineers and microfabrication experts have a common ground, in not only deciding the material, but also the design aspects of these micromotors. Schoellhammer et al. demonstrated US mediated delivery of microparticles deep into colonic tissue *ex vivo*. [42] Delivery was relatively independent of size and charge, but did depend on conformation; spherical particles were delivered to a greater extent than long-chain polymers. Finally,shape of particle also modulate immune cell response. Champion et el. demonstrated phagocytosis of PS shape (non-spherical, elongated ellipsoid) with rat alveolar macrophages, and concluded that, the local shape of the particle where the cell attached (not the overall shape), determined whether or not, a macrophage would initiate internalization [127].

Much like a conventional DDS, externally actuated DDS are also disintegrated, and cleared out by the body (mainly by kidneys and liver). Clearly, fabrication and administration of these micromotors should adhere to compounds/materials, accepted as generally recognized as safe (GRAS), to minimize any potential risk of toxicity [128]. This is where the choice of material and underlying chemical interfaces becomes important. Metallic architecture employed, is generally non-toxic (like Fe & Au), and is present in trace quantities (a few nm thick layer) [129]; much like trace metals required by the body. For instance, Wu et al. demonstrated bio-degradable protein-based drug micromotors,which upon enzymatic treatment under physiological conditions, exhibited limited *in vivo* toxicity [130]. Having said that, an in-depth toxicity and ADME profile assessment for micromotors *in vivo*, has been long overdue.

8. Conclusion

Research presented here clearly demonstrate the suitability of micromotors towards drug delivery *in vivo*. After a decade long research, vanguard advances made in the area of micromotors research, is clearly observable. However, in order to successfully transition towards clinically relevant DDSs, it is imperative to identify and establish their pharmacokinetic and pharmacodynamic profile. (**1**) A key-issue involves animal testing, which requires significant infrastructure, as well as stringent ethical norms. Protocols required for such experiments, are co-developed with clinicians and veterinarians, on a case by case basis. (**2**) Since, micromotors in GIT (P.O.) promises to be a key-research area in the near future, one may explore artificial stomach-duodenum model, which is well established towards *in vitro* studies with *in vivo* significance [131,132]. (**3**) Biocompatibility of a material is critical, be it a pill or drug eluting devices; Given a broad range of such materials, relevant information can be looked up elsewhere [133–135]. Further, protein adsorption layer (protein corona), that forms on to the surface of nano/microparticles, plays an important role in their interaction with living matter, and needs to be investigated [136].

(**4**) Another key area of research will be to integrate multiple exogenous elements over a single microcarrier. For instance, US, NIR or magnetism, each technology has its distinct set of advantages, and a combinatorial approach will enable better theranostic systems in the future. Reservoir-type drug delivery systems will be of considerable importance, as they not only load higher amounts of drug, but can also be used as an ultrasound contrast agent (UCA).

(**5**) A fundamental strength of micromotors *in vivo* has been its potential towards site-directed drug delivery (not entirely dependent on the circulation system). However, similar to a conventional DDS, it should incorporate toxicity and immune response studies. *ing*. (**6**) Therefore, fundamental research in the area of better control and imaging modalities, will continue to develop at a rapid rate, to better support these demanding pharmacological benchmarks..

(**7**) While being a ‘motor' certainly enhances some properties, in terms of drug delivery *in vivo*, microscale (<100 μm) DDS may find greater relevance towards delivery of APIs required in low quantities (like hormones, enzymes, nucleic acids *etc.*). The tight interconnection between what can be termed as a nano/micromotor (including exogenous control schemes), and associated pharmacological benefits, will expand the future of drug delivery. This will not be limited in terms of size-scale alone.

Another, newly emerging class of biogenic and bionic microsystems have been tested for drug/cargo delivery applications, both *in vitro* and *in vivo* [137,138]. While biogenic microsystems involve materials isolated from nature, bionic microsystems tag living cells (like sperm cells, mammalian and microbial cells) with advanced functionalities [137,139]. A recent study by Martel et al. [140] demonstrated magneto-aerotactic migration behavior of magnetotactic bacteria (Magnetococcus marinus MC-1), towards drug delivery in a tumor mouse model. These bacteria were magnetically guided, resulting in enhanced penetration into hypoxic regions of HCT116 colorectal xenografts. An updated assessment of bioinspired microrobotics, with multiple biological locomotion strategies, can be looked up elsewhere [141,142].

The discussion presented here, not only highlight the advances made by propelling μ-DDS, but also provide a toolkit for exploring design-application relationship for advanced applications.. With interdisciplinary boundaries blurring fast, greater cooperation should be sought between disciplines, as diverse as space or clean energy, towards bio-medical applications [143,144] [145,146]. Micromotors are here to stay — although, they move fast, really fast.

Acknowledgements

SKS would like to thank H.C. Ørsted COFUND for funding. SKS & AB would like to acknowledge the Danish National Research Foundation (DNRF122) and Villum Fonden (Grant No. 9301) for Intelligent Drug Delivery and Sensing Using Microcontainers and Nanomechanics (IDUN).

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