MICROFLUIDICS FOR MICROSWIMMERS: engineering novel swimmers and constructing swimming lanes on the microscale, a tutorial review

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This paper provides an updated review of recent advances in microfluidics applied to artificial and biohybrid microswimmers. Sharing the common regime of low Reynolds number, the two fields have been brought together to take advantage of the fluid characteristics at the microscale, benefitting microswimmer research multifold. Firstly, microfluidics offer simple and relatively low-cost devices for high-fidelity production of microswimmers made of organic and inorganic materials in a variety of shapes and sizes. Microscale confinement and the corresponding fluid properties have demonstrated differential microswimmer behaviors in microchannels or in the presence of various types of physical or chemical stimuli. Custom environments to study these behaviors have been designed in large part with the help of microfluidics. Evaluating microswimmers in increasingly complex lab environments such as microfluidic systems can ensure more effective implementation for in-field applications. The benefits of microfluidics for the fabrication and evaluation of microswimmers is balanced by the potential use of microswimmers for sample manipulation and processing in microfluidic systems, a large obstacle in diagnostic and other testing platforms. In this review we introduce various ways in which these two complementary technology fields will enhance microswimmer development and implementation in various fields.

1 Introduction

The microscale was an unexplored terrain, present but invisible, for researchers until the first engineered magnifying lenses and simple microscopes. From there on, science began a more collective exploration of the microspace, leading to new fields of science, technology and research: across more established fields such as microbiology, colloids or microsensorics, and emergent areas like active matter and microfluidics, which have surged in the last couple decades. Evolving independently at first, active matter and microfluidics, recently, have become more intertwined. This tutorial review starts by defining active matter, i.e. microswimmers, and microfluidics separately. At this point, any curious reader might then ask: what do these two fields have in common? What are the benefits resulting from merging them together? What are the difficulties associated with the integration of microswimmers in microfluidic devices? What are the perspectives of combining the two fields? This manuscript will answer all of these questions in a non-exhaustive way. The focus will be on recent advances in microfluidic systems implemented to fabricate artificial or biohybrid microswimmers and to set up specific experimental conditions and environments as illustrated in **Figure 1**. Recent related reviews focus either on microswimmers' fabrication with microfluidics[129] or on potential microswimmer applications.[53,68] Our goal is to update the reader on the fast evolving field, while highlighting the three main areas where combining microswimmers and microfluidics has been highly beneficial in the recent past.

2 Basics of microswimmers

Motors are engineered devices that translate energy into motion and are commonly found throughout our everyday lives. From the power engines in cars to the blenders in our kitchens, motors are well known machines in the macroscopic world. Recently, with technological advances, synthetic motors have been integrated into the microscopic world under the name micromotors. Even at this scale micromotors are still defined by their ability to convert energy into motion. To conquer this new microscale world, novel propulsion mechanisms have been developed to enable micromotors to navigate the conditions posed by

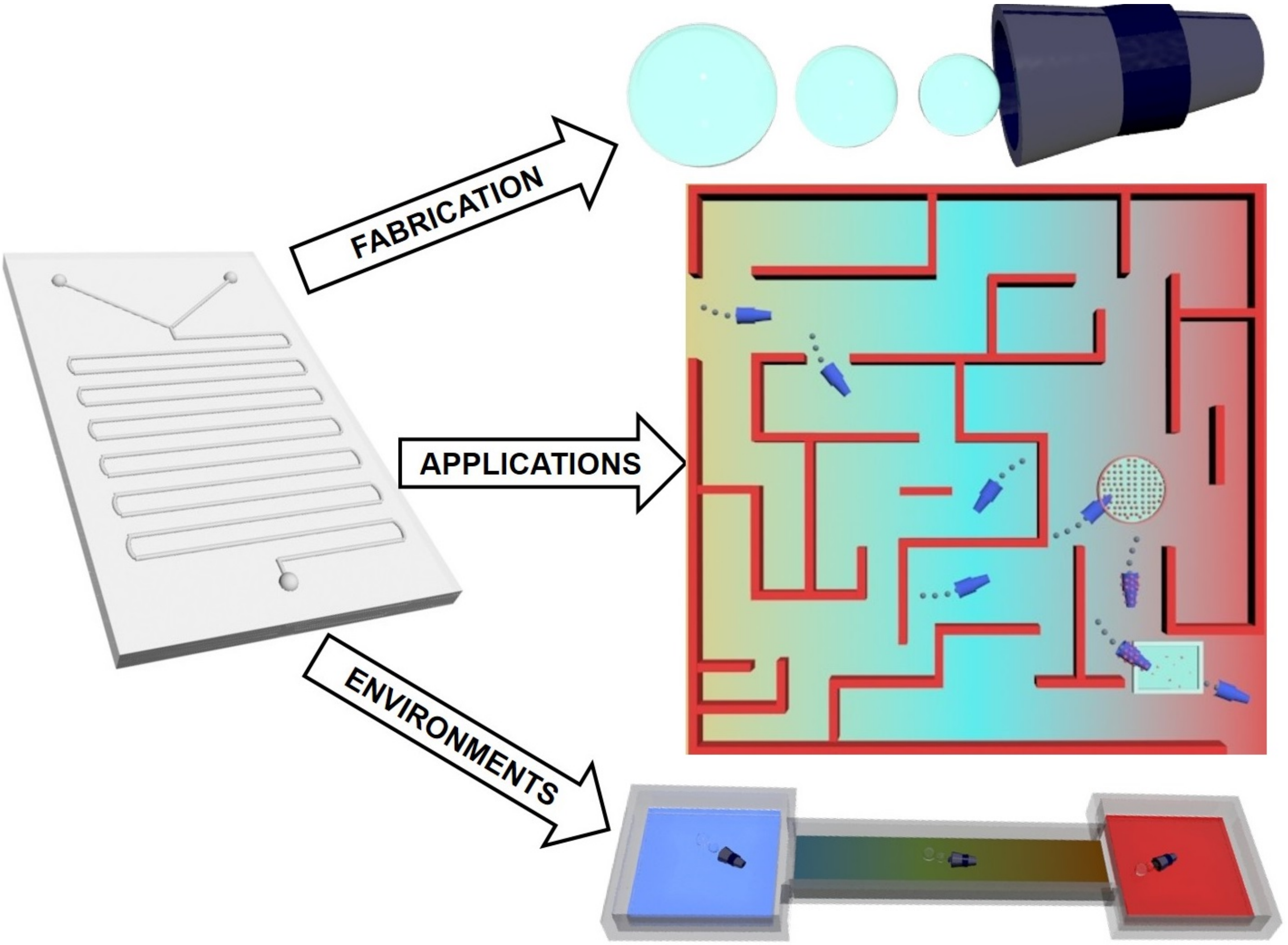

Figure 1: Schematic highlighting the synergistic combination between microfluidics and microswimmers in three areas (Fabrication, Environments, Applications) covered in this review.

the different physical phenomena predominant at this length scale. More generally micromotors have been called active particles, to incorporate the concept of energy conversion and motion into the terminology. Although, when referring to the ability to move through a solution, they are referred to as microswimmers; since neither the biological components nor the swimming ability of the micromotors are excluded from this review, we find the term microswimmers more adequate.

Different forms of energy, i.e. magnetic, chemical and mechanical, drive the propulsion mechanisms of a diverse array of microswimmers, which come in distinctive shapes, size and composition.[18] The diversity of these microswimmers results in three major categories: artificial, biological and biohybrid. Artificial microswimmers are made of inorganic or a mixture of organic/inorganic materials and are generally asymmetric to ensure a directional motion instead of an enhanced Brownian motion. Motile biological microorganisms, such as sperm cells or bacteria, are natural microswimmers often propelled by flagellar beating. Biohybrid microswimmers take advantage of the already optimized geometries and/or motion mechanisms of living microorganisms and combine or replicate these properties in a synthetic component or swimmer.

Many chemical and biological processes in the macroscale are governed or limited by physical principles in the nano/microscale such as mixing and diffusion transport, consequently self-propelled microswimmers can induce a significant improvement on the control we have over these macroscopic processes. Although it is still early for microswimmers to be applied to real, large-scale applications, various efforts are being put into exploiting the potential of artificial and biohybrid microswimmers. Applications in biology such as drug delivery[14,124], in vitro fertilization[71] and cancer therapy[101] are being investigated in addition to environmental remediation applications.[92,42]

Another aspect in this research area is that microswimmers have often been observed in milliliter or greater-sized volumes, with little or no control over the flow profile of the surrounding fluid. While these testing conditions facilitate experiments, looking prospectively towards applications, e.g. in the biomedical field, these simple and clean setups do not resemble real environments. To better account for and describe the scope of microswimmers' abilities and limitations, they need to encounter complex surroundings such as flows, passive objects and limited spaces. Microswimmer behavior, similar to microorganisms, is dependent on the environmental and boundary conditions; microfluidics offers new possibilities for initiating and observing these under-characterized behaviors.

3 Basics of microfluidics

Microfluidics came out as an emerging field from the development of microelectromechanical systems (MEMS) in the early 90's.[73,112] Influenced by molecular biology and microelectronics,[120] the goal was to integrate sample testing processes and the corresponding analytical components on a single chip to create so called lab-on-a-chip (LOC) devices. Emulating the miniaturization of microelectronics, a big advantage of microfluidics results from miniaturization to provide small and low-cost devices, greatly reducing the sample volume and reagents consumed. The reduction in size does not involve a loss in quality, on the contrary, microfluidic devices can perform experiments with high sensitivity and reproducibility in shorter times as compared to their corresponding conventional methods. To better understand the physical principles behind this technology, a few fundamental concepts need to be introduced.

A rather astonishing property of fluids at the microscale is the fact that fundamental physics that we are accustomed to in the macroscale, change quite drastically when size is decreased. This leads to a series of curious phenomena that affect microfluidics strongly and are frequently described in a set of dimensionless numbers. Here, we want to offer a brief overview to the reader, without claiming to be comprehensive; excellent specialized reviews are available on the topic.[76,102] To understand microfluidics we consider the fluid physics of mostly Newtonian fluids that occurs within channels. A typical example are small channel dimensions, often less than a few 100 μm transverse dimension (channel height and width), leading to nano- to microliter volumes. The physical phenomena at this scale are dictated and characterized by various dimensionless numbers that describe the physical corner stones. Some of these numbers we discuss below. One of the best-known examples is that mass transport in microfluidic devices is generally dominated by viscous dissipation, and inertial effects are generally negligible. This fact is best characterized by the Reynolds number Re

$$Re = (\frac{\rho u L}{\mu}) \tag{1}$$

which relates inertial forces to viscous forces, with ρ the density of the fluid, u the flow speed, L length and μ the dynamic viscosity. Generally speaking, the velocity field for a Newtonian fluid obeys the Navier–Stokes equation:

$$\rho(\frac{\partial}{\partial t}\mathbf{v} + (\mathbf{v} \cdot \nabla)\mathbf{v}) = \eta \nabla^2 \mathbf{v} - \nabla p \tag{2}$$

where the inertial acceleration terms appear on the left and forces on the right. On the microscale it is simplified to the Stokes equation:

$$(\frac{\partial \rho}{\partial t} + (\mathbf{v}\rho \cdot \nabla)) = 0 \tag{3}$$

For both microfluidics and microswimmers the Reynolds numbers are small enough that inertial effects are negligible resulting in linear and predictable, laminar Stokes flow. Laminar flow results in many counter-intuitive behaviors, with mixing being one of the most drastic examples. These behaviors are best characterized using the Péclet number, which describes the relation of convective to diffusive flows. Purely diffusive mixing is generally slow. Typical diffusion rates for small ions are in the range of $2 * 10^3$ $\mu m^2 s^{-1}$ while cells of a size of 10 μm diffuse about 0.02 $\mu m^2 s^{-1}$, illustrating that mixing, reaction kinetics and other operations in microfluidics depend strongly on the species of interest. In most microfluidic devices fluid interfaces are largely parallel to the fluid velocity, which simplifies handling because diffusion occurs rather slowly. If mixing is desired, the Péclet number becomes more important and can influence different mixer designs implemented. Generalizing, to minimize the relative distances and enhance diffusion in the devices, the more effective microfluidic mixers use stirring motions to reduce distance; some example device design approaches are flow-focusing, minimizing volume by packing with microbeads and rotary mixers.

Besides the inherent physical principles of flow in microfluidic devices, other properties in these systems need to be accounted for when working with microswimmers. For example, density mismatched microswimmers are strongly dependent on the surface properties of the substrate, hence the chip material and its wetting properties (hydrophilicity or hydrophobicity) will be crucial for their swimming behaviors. For other types of swimmers, namely the oxygen producing catalytic ones, the gas permeation properties of the chip material can dictate the success of working in a confined environment. While comprehensive reviews on the advantages of microfluidics and the fabrication techniques for microsystems already exist[85,87,72], our aim here is to briefly present the advantages specific to and aligned with active matter applications.

In the early days of microfluidics, chips were commonly made of glass or silicon. The fabrication processes often involved bulky and expensive equipment along with harsh treatments. As the field grew, these materials were replaced by plastics. One polymer in particular - polydimethylsiloxane (PDMS) - attracted a lot of interest and became the leading material for microfluidic chip fabrication. PDMS and other polymers are highly advantageous compared to glass and silicon in terms of flexibility, biocompatibility, transparency and gas permeability, which are convenient properties when working with microswimmers; although it is the ease of fabrication and low cost that boosted the competitive advantage of polymer devices.[120,72] PDMS chips are fabricated with standard photolithography, followed by soft lithography techniques to produce a variety of designs.[85] Additionally, micromachining is another way to categorize the top-down microfabrication techniques developed for MEMS and microfluidic systems.[138] The "hard-micromachining" techniques include laser photoablation, etching and micromilling and is mainly employed for hard materials such as glass, silicon or hard plastic, whereas "soft-micromachining" mostly refers to soft lithography as well as hot embossing that are used for soft materials like elastomers and thermoplastics.[61]

The microfluidic chip geometrically defines the fluid channels and device properties, however when flow conditions are applied in experiments, these are often actuated by external pumps. Syringe pumps are the most commonly used to ensure a constant flow rate within the microchannels. For more precise control, especially at low flow rates, other instruments are also used such as pressure controllers and electro-osmotic pumps.[131,119] Since their introduction 30 years ago, microfluidic systems have grown into a diverse set of enabling technologies for applications in various emerging fields including, but not limited to, droplet microfluidics[96,69], mixing[60], separation[32,83,11], gradient generation[109,118,34] and lab-on-a-chip.[27,1] Microfluidics was initially adopted into the microswimmers' field with Clemmens *et al.* work on protein motors.[12] This first combination paved the way for numerous studies that have since benefited from the synergy of microfluidics and active matter.

4 Microfluidics for active particle production

Considerable efforts have been made to design artificial microswimmers with complex geometries capable of specialized functions and applications, such as biomimicry of biological swimmers and targeted biochemical delivery, respectively. Various fabrication techniques such as electrodeposition, solvothermal reactions or template-based synthesis have been extensively adopted for active particle generation. However, most of the particles fabricated using these strategies are inorganic materials with rigid shapes. In addition to their sharp and cutting edges, these active particles exhibit a non-tunable Young's modulus and a lack of biodegradability, making them hardly relatable to biological swimmers. Recently, microfluidics has emerged as a powerful tool to design microswimmers with high tunability in their shape and structure. Changing simple parameters such as the fluid flow ratio in microfluidic channels enables the generation of particles, in various shapes, with high throughput production. These particles can propel themselves exploiting various propulsion mechanisms which are discussed in this section.

4.1 Chemical swimmers

Chemically-responsive microswimmers, one of the earliest researched microswimmer types, are widely used for their robust swimming properties. Their defining characteristic is chemically responsive propulsion, traditionally due to self-generated, local chemical gradients. The generated gradients can occur via the decomposition of the surrounding fluid, or even the release of solutes from the microswimmers body (detailed in 4.1.1). Chemical swimmers can be broadly sub- divided into Marangoni-driven, bubble-propelled, self-diffusiophoretic, or electrophoretic. Although microfluidics has been extensively adopted to fabricate microswimmers, to our knowledge, there are no reports of diffusiophoretic or electrophoretic swimmers being microfluidically produced. Diffiusiophoretic and electrophoretic swimmers are mostly below 8 μm size, which may explain their lack of fabrication with microfluidics. While micron or submicron particles have been produced microfluidically, microfluidic systems generally produce microparticles 10 μm in diameter, and larger, much more robustly and reproducibly than smaller particles. The combination of the microswimmers size (>10 μm) and the catalytic processes that propel these chemical swimmers dictates the swimming behavior, ensuring the need to tailor swimmer size based on its propulsion mechanism.[117]

4.1.1 Marangoni

Marangoni microswimmers, are often active droplets, i.e. self-propelling droplets driven by Marangoni stresses and internal flows. These stresses stem from the different interfacial tensions along the surface of the droplets.[133] Most of the time, the difference in interfacial tension involves a change in the coverage of surfactant around the droplet. The droplets can be driven by different propulsion mechanisms including chemical reactions, solubilization and phase separation.[64] Microfluidics has been widely used as a tool for droplet generation[96] and it has recently been applied to active droplet production.[41,40,108,64] As the size and shape of droplets will impact their movement[8], microfluidics provides an unprecedented advantage over conventional techniques for droplet production because of its precision to control droplet features.

Jin *et al.* described a microfluidic hydrodynamic flow focusing device to produce highly monodispersed ($< 5\%$) self-propelling droplets (**Figure 2A**).[41,40] The droplets are made by emulsifying an oil phase of a nematic liquid crystal into an aqueous phase mixed with surfactant. The surfactant, here tetradecyltrimethylammonium bromide (TTAB) stabilizes droplets, to avoid droplet coalescence. Additionally, TTAB initiates the autonomous motion of the droplets. By adding the droplets into a solution of higher TTAB concentration, Marangoni flows are created by the micellar solubilization of the oil phase. Resulting in droplets to be attracted along micellar surfactant gradients by chemotaxis, i.e. the directed motion towards or away from a chemical gradient.

In another study, a microfluidic PDMS chip was used to produce droplets containing the common liquid crystal 4-Cyano-4'-pentylbiphenyl (5CB) in an aqueous solution of SDS surfactant.[108] These droplets were then set into motion by adding a solution of high surfactant concentration, above the critical micelle concentration (CMC). Their autonomous motion and collective behavior was studied under several boundary counditions.

4.1.2 Bubble propelled

In this propulsion strategy, gas bubbles emanate from a mostly concave space in the microswimmer body. The origin of the gas is typically the catalytic decomposition of hydrogen peroxide (H_2O_2) by either enzymes or inorganic catalytic materials. It is still up for debate whether the bubble growth or ejection is the origin of these microswimmers' propulsive forces. Regardless of origin, the consequent momentum transfer of these events leads to a jet force acting on the microswimmer body and the propulsion of the swimmer.[22] The propulsion speed of these swimmers relies highly upon the bubble generation parameters, and increases if the bubbles can be generated easily.[51] Bubble generation is dependent on size of the concave cavity and the catalytic properties of the microparticle. Another important parameter, dictating the microswimmers' trajectory type and velocity, is the pinning of bubbles, which is dependent on the surface roughness of the cavity. The highest speeds, correspond to run-and-tumble trajectories, which are obtained in microswimmers with a rough surface resulting in the pinning of multiple bubbles at the same time(**Figure 2B**).[50]

Droplet-based microfluidics employing immiscible phases have been heavily applied to fabricate bubble propelled spherically shaped microswimmers. Through the droplet-shaping control in microfluidics, the diversity of microswimmer structures has expanded.[51,50,88,9,139,135,2]. For instance, Zhou *et al.* used a droplet microfluidic based fiber-confined approach to engineer microswimmers with spindle, drum, zigzag and bi-layer shapes (**Figure 2C**),[135] while others have utilized a microfluidic lithography technique with spinning and spiraling systems to engineer bubble driven and magnetically actuated helical shaped swimmers.[130] Recently, our group employed a stop flow lithography technique to fabricate enzyme driven 'S' shaped rotors, 'U' shaped propellers and 'l' shaped pumps.[99] This fabrication strategy is known to engineer particles with tunable structures and thus provides an edge over other methods, particularly the freedom in selecting shapes and controlling the active regions in the fabricated structures.

Most of the reported work has used inorganic materials such as Pt[50,88,139,135,9,130,2] to drive bubble-propelled swimmers, with some exceptions striving to develop a biocompatible microswimmer using an enzymatic biocatalyst.[51] Keller *et al.* fabricated catalase loaded poly(ethylene glycol) diacrylate (PEGDA) microswimmers using droplet microfluidics where the enzyme induced decomposition of H_2O_2 fuel led to the hydrogel swimmers' propulsion.[51] Besides the advantage of generating diverse morphologies using microfluidics, another very useful aspect is the ease of catalyst loading into the microswimmers, which gives

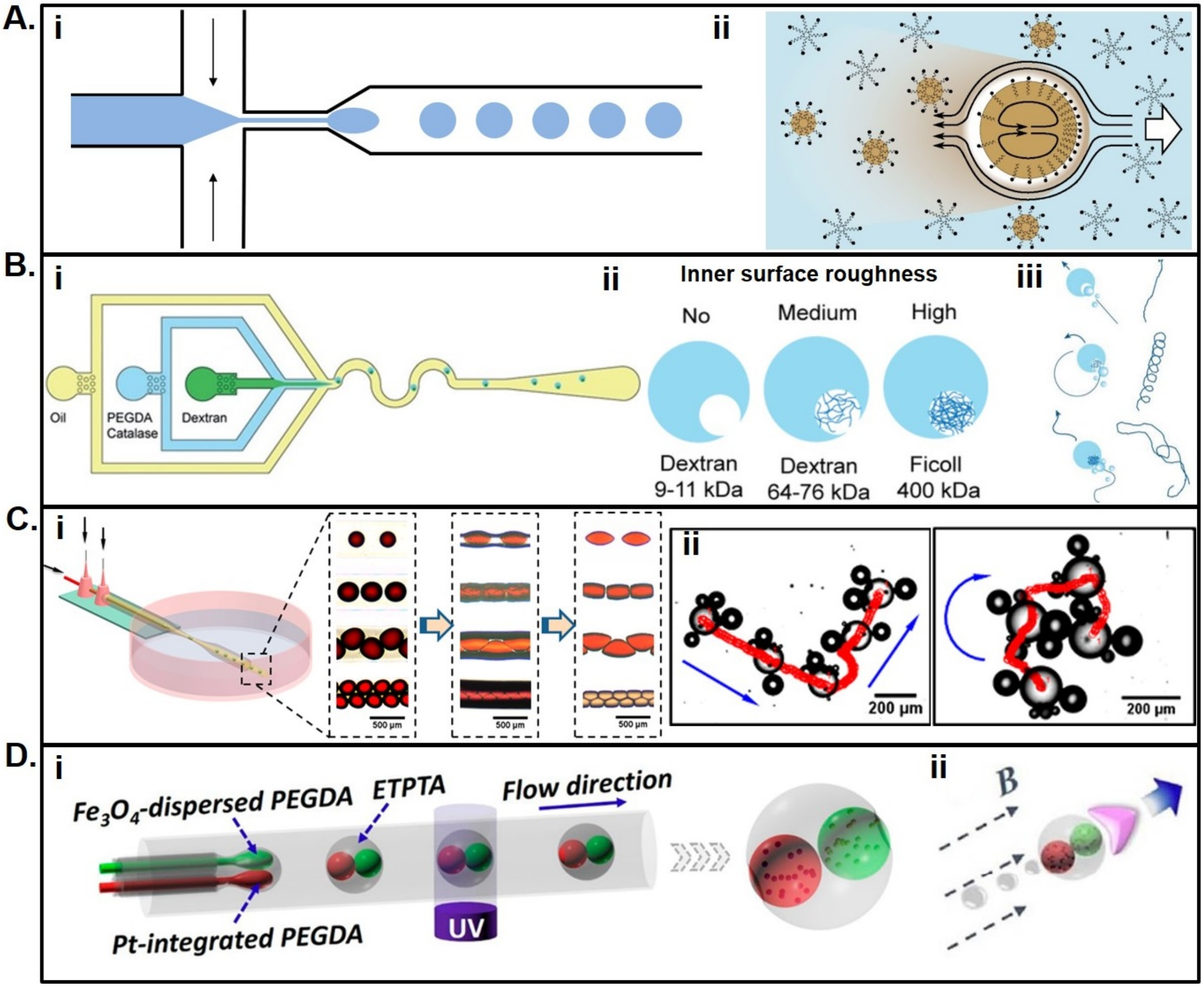

Figure 2: A. Illustration of (i) the microfluidic flow focusing device for active droplets production and (ii) schematics of Marangoni propulsion mechanism. Reproduced with permissions from [41]. B.(i) Schematics of the microfluidic setup used for fabrication of bubble propelled hydrogel microswimmers, (ii) inner surface roughness of the swimmers can be tuned from low to high depending upon molecular weight and branching of polysaccharides, (iii) linear, circular and tumble-and-run trajectories are obtained depending upon the extent of surface roughness (and hence bubble pinning). Reproduced with permissions from [50]. C.(i) Schematics of the capillary microfluidic channels and the sequential steps for engineering droplet templated microswimmers with spindle, drum, zigzag and bilayer shapes, (ii) magnetic field assisted guidance of bubble propelled microswimmers. Reproduced with permissions from [135],Copyright © 2019, American Chemical Society. D.(i) Illustration of the capillary microfluidics with dual injection cores for microswimmer generation, (ii) magnetic guidance of bubble propelled microswimmers. Reproduced with permissions from [139], Copyright © 2018, American Chemical Society.

this technique an advantage over other complex methods such as thermal deposition and sputtering. Using microfluidics, the so called "engines", or catalyst, of the microswimmers can be directly encapsulated inside the swimmers by suspending it in either the dispersed or continuous phase or both.[9] Combining the advantages of phase separation and microfluidics, multifunctional microswimmers with complex compositions have been engineered (**Figure 2D**).[88,9,139]

Bubble-propelled microswimmers are particularly interesting for water remediation applications, stemming from the enhanced mass transfer, due to the generated gas bubbles. This makes hydrogel microswimmers loaded with various catalysts especially attractive for water cleaning and other functions.[88,9] For instance, Chen *et al.* developed a multifunctional microswimmer simultaneously loaded with various nanoparticles (NPs) both on the surface and within the bulk of the swimmers for motion-facilitated dye degradation.[9] We expect bubble-propelled swimmers to continue fluorishing through microfluidic fabrication techniques, benefitting from the geometric tuning of the microparticle shapes and size as well as the controlled localization of catalysts. Interestingly, bubble-propelled swimmers while readily fabricated in microfluidics, will most likely remain ill-suited for use in these devices. Implementing microswimmers in microfluidic devices is complicated by swimmer-generated bubbles as the bubbles become trapped in the channels, increase the fluid resistance and eventually block the fluid flow. Thinking ahead, any kind of air (bubbles) is highly undesirable for biological applications, especially if the bubbles cannot be vented or pulled off without disrupting biological samples.

4.2 Bioinspired and biohybrid microswimmers

Microswimmers are often compared to biological microorganisms because of their energy dissipating properties and their life-like motion. While few artificial microswimmers can compete with the evolution-refined properties of living microorganisms, the latter provide a design template for future synthetic microswimmers. Critically, microswimmers for biomedical applications need to fulfill certain conditions such as biocompatibility and biodegradability. This has resulted in various efforts to create self-propelling microswimmers that do not require the use of toxic fuels.[101]

Ideally, one could mimic and tune artifical microswimmer properties to mirror biological swimmer behavior, such as velocity, tunable direction or collective behavior. Microswimmers made to emulate these behaviors are called bioinspired microswimmers. Whereas other approaches fuse biological swimmers and artificial components, to take advantage of their respective properties, creating biohybrid microswimmers.

Microfluidics has effectively been implemented to produce both bioinspired and biohybrid microswimmers, in a variety of unique approaches. Huh *et al.* are one of the rare groups to have performed effective coupling of bacteria with artificial microparticles in a microfluidic device to produce biohybrid microswimmers.[36] Their innovative approach to fabricate bacteriabots uses microfluidic devices consisting of a microchannel with V-shaped microstructure "trapper" arrays to trap cubic polymeric, biodegradable microparticles (**Figure 3A**). Once the microparticles are trapped, a solution of attenuated bacteria (*Salmonella Typhimurium*) is pumped inside the microfluidic channel. The bacterial flow process is done under laminar flow, which increases the efficiency of bacterial attachment to the microparticles. Laminar flow also ensures an anisotropic attachment of the bacteria on the hydrophobic microcubes; whereby the bacteria attach to the side of the cube experiencing the flow. The microcubes are then released by reversing the flow direction. As for bioinspired microswimmers, researchers have attempted to mimic bacterial flagella with artificial helical microswimmers. Although they are inspired by the geometry of natural flagella, the microswimmers are fabricated at a much larger scale, mainly due to technological limitations of the fabrication techniques.

Yu *et al.* used microfluidic spinning and coiling technology to fabricate self-propelling helical microfibers (**Figure 3B**).[130,127,128] These microfibers are made of a polymeric material and are sometimes coated with biocompatible methacrylate gelatin (GelMA). The advantage of this co-flow polymerization method is that functional nanoparticles (e.g. magnetic or catalytic) can be easily encapsulated into the microfibers

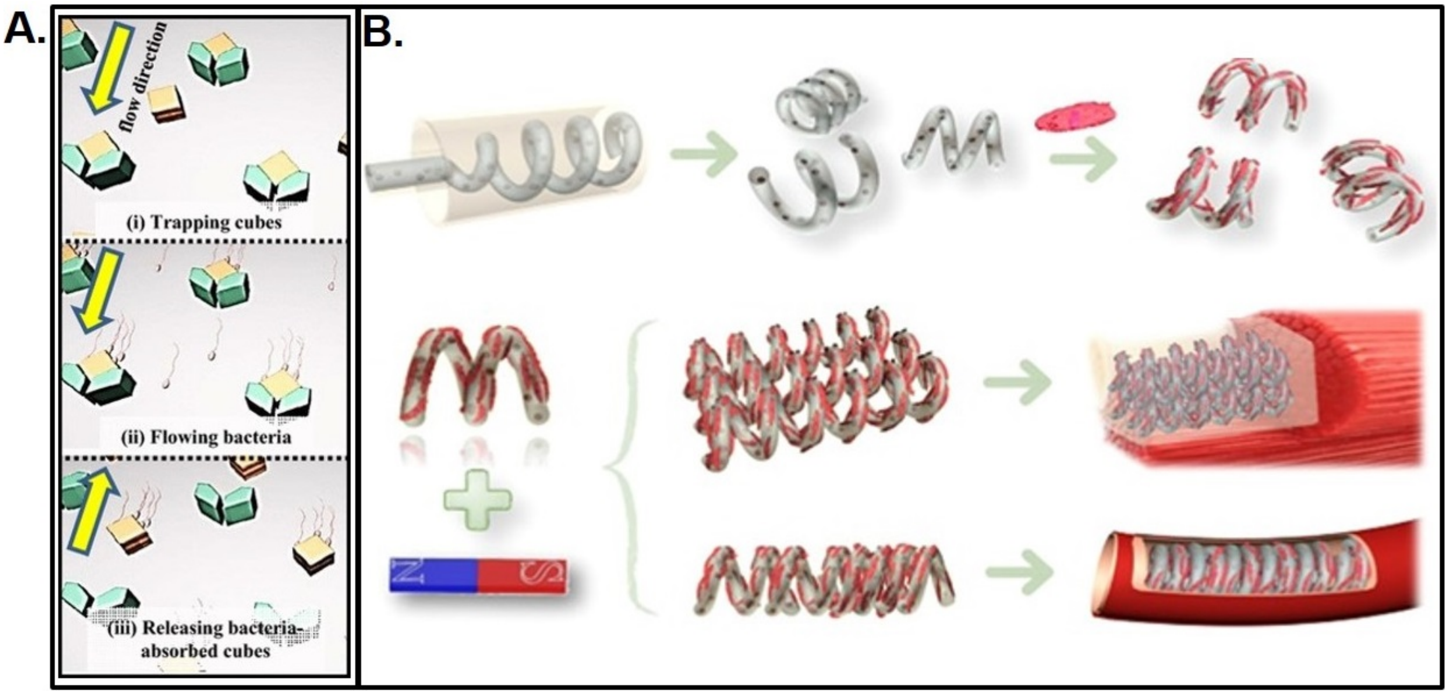

Figure 3: A. Trap and release method for the bacteria-bots fabrication in a microfluidic channel. Reproduced with permissions from[36]. B. Biohybrid soft microswimmers fabrication in a microfluidic capillary device and potential application of the magnetic nanoparticle embedded cell carriers. Reproduced with permission from[128].

to ensure their self-propulsion. The GelMA coating also serves as scaffold for seeding and culturing cells on the microfibers, consequently enabling the microfibers use in tissue repair applications.[128] Despite the clear advantages of biohybrid or biological micromotors, researchers who wish to benefit from these features have to take into account that biological entities mostly have a defined life-time and require rather specific conditions such as high ionic strengths, which might complicate scale up and re-usability.

4.3 Swimming based on physical interactions

4.3.1 Mechanical

Most biological motile entities move through mechanical propulsion mechanisms such as helical flagella motion, coordinated cilia waves[31] or twitching on surfaces *via* pili.[67] Artificial structures attempt to simulate these propulsion mechanisms by using soft or flexible structures that resemble the bending motion of their biological analog.[110] The actuation mechanisms in artificial appendices are varied and include magnetically influenced colloidal beads, strain-inducing multilayers, and optically responsive materials. Though there are different actuation mechanism, the resulting bending motion is well understood theoretically[55,91] because of the well-documented descriptions of surface influences[29] that can be extended to walls in microfluidic channels.[91,38] Artificial mechanical swimmers that move by bending waves have been constructed using different principles[17] and influenced by different biological systems. One useful example is the extrusions on the flagellum, which have been analyzed in biology[39] and reproduced artificially.[111] Complex shapes have been fabricated using optofluidic[82] and microfluidic techniques such as stop flow lithography,[121] however, to the best of our knowledge none of these artificial, flexible flagella have been produced in a microfluidic setup or studied in a microfluidic device. A promising candidate to evaluate in microfluidics is the magnetically actuated ciliary microswimmer produced by Kim *et al.*. This swimmer was generated using 3D lithography, and with controlled sputtering the cilia were covered by a magnetic layer (**Figure 4A**).[54] The net propulsion force to create motility was produced by a stepping magnetic field to actuate the cilia in a non-reciprocal fashion and the final microswimmer was able to perform cargo transport followed by drop off.[54] The tunability and control of its directed motion could answer questions on how it interacts with different channel geometries. More thorough understanding of how the mechanical

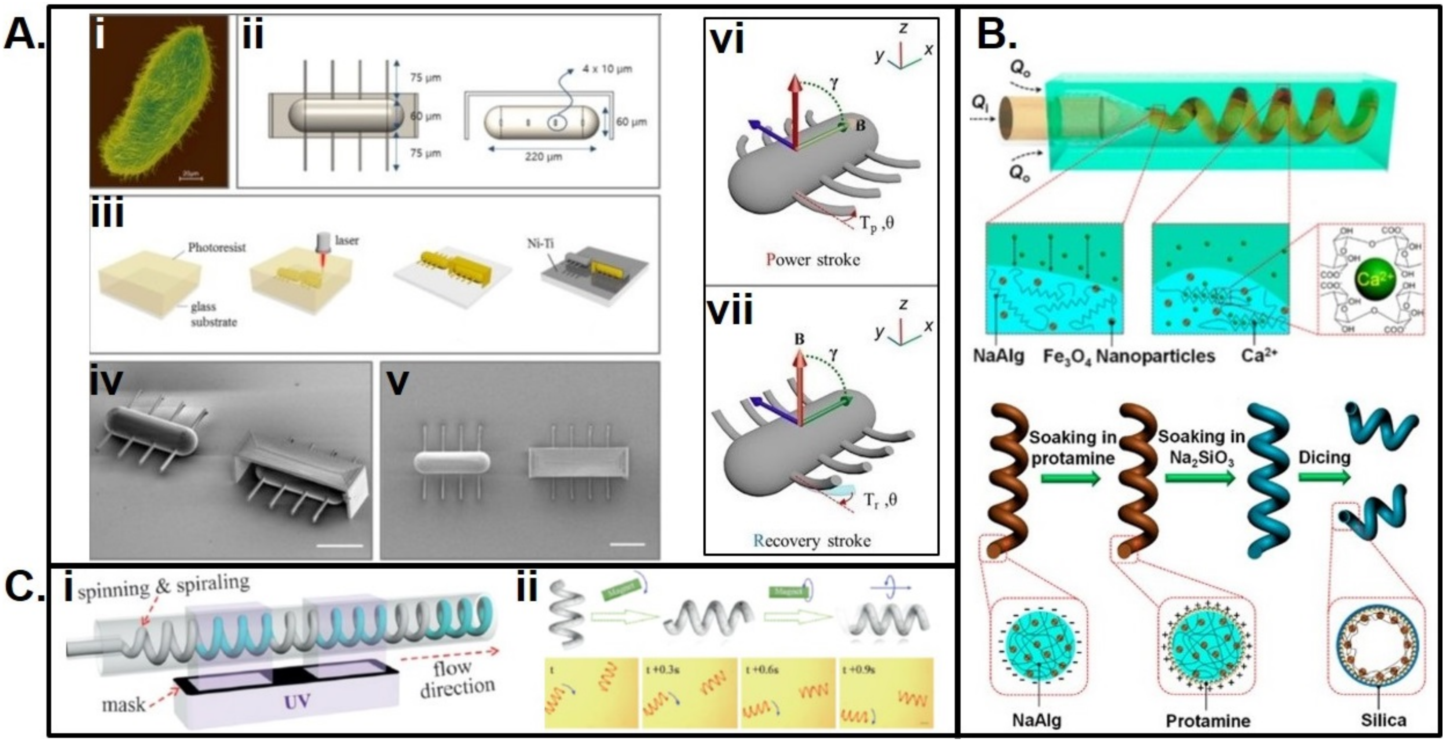

Figure 4: A. Fabrication of microrobots. (i) SEM image of Paramecium, (ii) Scheme of artificial ciliary microrobots, (iii) Schematics illustrating the fabrication process of ciliary microrobot, (iv) Scanning electron microscopy images of fabricated ciliary microrobots with and without mask structure in trimetric and (v) top view. (Scale bar: 100 μm), (vi) Power stroke and (vii) recovery stroke motion generate different magnetic actuation forces.[54] Copyright © 2016, Springer Nature. B. Schematic illustration of microfluidic fabrication of magnetic hybrid microswimmers with hollow helical structures, their microfluidic generation of coiled flow templates for synthesizing magnetic helical Ca-Alg microfibers. For Biosilicification of magnetic helical Ca-Alg microfibers two sequential soaking steps for protamine coating are followed by Silica coating and Ca-Alg decomposition. Controlled dicing creates magnetic hybrid microswimmers made from hollow helical structures,[106] Copyright © 2018, American Chemical Society. C. (i) Schematics illustrating fabrication of helical microswimmers using spinning and spiraling integrated shuttered UV lithography technique, (ii) Schematics and microscopic images illustrating response of helical micromotors to clockwise rotary and 3D rotating magnetic field.[130] reproduced with permission of Wiley 2017.

propulsion is influenced by confined surfaces and channels would have great impact on the engineering of different biomimetic of biohybrid microswimmers.[52]

4.3.2 Magnetic

Despite the fact that many of the magnetic microswimmers are often driven matter, i.e. propelled by an external field instead of self-propelled, they have received a lot of attention due to their resemblance of flagellar microswimmers. The creation of artificial flexible flagella is still challenging, so helical structures that are able to perform the spiraling motion are currently the primary option to mimic biological swimmers' structure and motion. These complex geometries are often produced by lithography based techniques, such as the aforementioned artificial ciliated swimmer[54] and other microswimmers[35], as well as complex deposition strategies[30] that mostly fabricate rigid swimmers. Tang *et al.* used a microfluidic spiraling approach to produce helical alginate microfibers containing Fe_3O_4, and subsequently biosilicificated them to obtain magnetic rigid helical microswimmers and demonstrated simple cargo transport (**Figure 4B**) .[106] In order to take advantage of microfluidics and achieve flexible structures, Yu *et al.* used the previously explained integrated microfluidic spinning and spiraling strategy to produce helical structures.[130] PEGDA photopolymerization enabled flexible encapsulation of magnetic nanoparticles, conferring a magnetic moment to the helices. (**Figure 4C**).

5 Microfluidics for specific environment design

5.1 Microfluidics to create confinement

In a series of early publications[77] the use of microfluidic channels offered confined geometries with predetermined paths to highlight the capabilities of different microswimmer types, such as electrophoretic,[6] bubble driven,[28] diffusiophoretic, biohybrid and others. In these studies, the microswimmers were used to demonstrate pick up and/or delivery applications in channels; while the channel geometries provided confined paths, the channel dimensions were significantly larger than the microswimmers.

Some of these studies also evaluated the effects found on swimming behavior. Despite the theoretical assumption that confinement would enhance the chemical gradient formation and consequently increase propulsion, a reduction in speed was often observed.[6] Burdick *et al.* observed a drastic decrease by almost 75 % for carbon nanotubes and hypothesized that it was due to the partial absorption of H_2O_2 by PDMS. Kreuter *et al.* observed that confining driven colloids in a narrow channel strongly modifies the interactions among them.[57] The influence of walls and surfaces on active biological microswimmers has been known for decades[24] and with the growth of microswimmer manufacturing techniques, the interest in the corresponding influence on artificial swimmers has also grown. More specifically, characterizing the interactions between microswimmers and the physical boundaries, such as walls and patterned substrates, was done in micropatterned topologies and microfluidic chips.[114] The modification of the flow fields *via* surfaces was indeed observed, characterized and modelled using diffusiophoretic Pt-coated Janus particles.[100] This observation could be transferred to side walls, where microswimmer guidance *via* topological features or walls was achieved.[13,100] These findings have since been extended to induce dynamic motion of passive microgears, which instead of immobilized guidance walls and features, positioned precisely engineered mobile objects in the microchannels.[66] It was predicted, that using these approaches of controlled guidance of microswimmers can open up ways for developing active and smart actuation systems.[21]

5.2 Microfluidics to create interfaces

Solid surfaces can be created independently of microfluidics, whereas liquid–liquid interfaces, predominantly found in laminar coflow, commonly occur in specific microfluidic systems. It was shown that bacteria in the vicinity of fluid interfaces are not only confined, but actually interact with the interface. In droplets at inclined surfaces, a highly concentrated suspension of microorganisms was able to achieve depinning and subsequent sliding of the droplets,[33] as soon as the motile forces created by the bacteria exceed the capillary forces. Ramos *et al.* created droplets containing bacterial suspensions in hexadecane and observed persistent random walks of the droplets, driven by the collective motion of the bacteria that create vortices, which are subsequently enhanced by the drag effect stemming from the bottom surface.[86] Interface observations have also been extended to artificial swimmers. One of the pioneers in this approach, Ding *et al.*, used a microfluidic setup to explore the effect of a liquid-liquid interface on magnetic microswimmers by encapsulating helices into a droplet.[15] Even though the size and geometry of the microswimmers inhibited close contact with the interface, the authors demonstrated the motion of the helix within a confined volume and additionally introduced cells to prove the suitability of helical magnetic microswimmers to manipulate soft materials.[15] A similar static oil-water interface was created by Palacios *et al.* using a microfluidic channel and was subsequently used to study Pt-driven diffusiophoretic Janus particles in the vicinity of an oil-water interface while still swimming on a solid surface.[79] Recently, our group used a special microfluidic chip geometry to create a flat liquid-liquid interface. The high surface energy of the flat surface resulted in partial wetting of the substrate, easing direct comparison of motion of Pt@SiO_2 particles at a liquid and a solid substrate.[98]

5.3 Flow

In laboratory settings active matter is mostly observed in static conditions, but theorists noted early on that the occurrence of flows is pervasive in most real, variable-rich situations and applications. Microfluidics can nicely fill this gap by applying controlled and variable flows, which provide the needed complexity to test active matter (or microswimmers). Tao and Kapral looked at partially catalytic nanodimers moving in a Poiseuille flow within a square channel, which resulted in upstream swimming as a function of the fluid flow velocity.[107] Frankel and Khais looked at active Janus particles in shear flow, finding that the flow disturbs the self-generated solute gradient by advection, altering the particle dynamics.[23] The authors predicted that this distortion would lead to cross-streamline migration. Related work by Lauga's group took a more general approach that is applicable to biological swimmers.[107] While upstream swimming has been observed for both, sperm and bacteria,[132] Sun *et al.* used quantitative analysis of human sperm flagellar beating to come to the conclusion that passive hydrodynamic interactions cause the directionality. The conical shape and the chirality of the sperm flagellum result in a net lift force against the flow direction. Similarly, bubble-driven microtube artificial swimmers can be guided to move upstream.[93] While the initial assumption was that an elongated shape is necessary to have a substantial rheotactic response (i.e. directed motion against an imposed flow), this was disproven by tests performed with spheroidal hematite surfers that demonstrated rheotaxis independently of shape.[78] For Pt@SiO_2 Janus particles, a different response to flows was found despite their spherical geometry; their flow response was characterized as cross-stream migration.[48] This points towards the fact that the impacts from flow maybe more multifaceted than expected and should be rigorously considered for new types of swimmers, in addition to evaluating the effects of chemical fields or interactions with adjacent walls. A research effort driven by Gompper and Winkler, in addition to studying the shape influence, systematically evaluated the different impacts of flow on microswimmers. It is obvious, that these effects cannot be independent of wall hydrodynamic interactions, active stresses or thermal fluctuations, so the authors gave a detailed overview of individual implications and respective interplays.[84]

5.4 Microfluidics to generate gradients

5.4.1 Gradients to guide artificial microswimmers

Microswimmer motion is generally described by their speed and direction. Controlling the speed of the microswimmers is the most frequently reported outcome, in some applications it is crucial to be able to control their orientation and direction of motion as well. In order to achieve directional control, researchers can either use external fields (e.g. magnetic field) or design the microswimmers' environment to drive them towards the desired destination. Tailoring the microenvironment by integrating gradients is a useful microswimmer control strategy. Depending on the microswimmer type, they can be attracted or repelled by chemicals (chemotaxis), a change in flow resistance (rheotaxis) or light (phototaxis).[40,75] In some cases, the microswimmers themselves create a local chemical gradient and respond to it (autochemotaxis).[41] Externally established gradients are difficult to create and maintain but have been proposed to guide particles' paths. Contrarily, microfluidics are well-known for efficiently generating gradients in a highly controlled and tunable fashion.[109,47] In 2013, Baraban *et al.* described a three-inlet microfluidic device to create a H_2O_2 gradient around the catalytic artificial microswimmers flowing in the central channel (**Figure 5A**).[3] They found that the microswimmers had a chemotactic behavior towards their fuel, H_2O_2, causing them to deviate from their initial trajectory, along the gradient, towards the higher H_2O_2 concentration region. It is critical to note, that the gradient responsive deviation was found to be impacted by the size and shape of the swimmers. This study specifically evaluated microtubes and Janus spheres, and while these findings cannot be transferred to other swimmer types without more investigation, it is our expectation, that size, shape and propulsion mechanism will play a critical role, in defining chemotactic swimming responses.

Recently, an innovative chemotactic microswimmer has been studied in a microfluidic-generated gradient by Mazur *et al.*.[70] In this study, spherical silica microparticles were coated with liposomes; the swimmers' movement is initiated by solubilization of those liposomes. Solubilizing agents, Triton X-100 (TX) or bile, were used to generate gradients in commercial microfluidic chips with different surface treatments (uncoated, Poly-l-lysine (PLL) coated and ibiTreat coated) and the resulting microswimmer motion was tracked. The researchers initially evaluated the movement of PLL-coated silica microparticles in the different microchannel coatings, and found that Brownian motion was signicantly enhanced in the coated microchannels. The different behaviors disappeared when the same experiment was conducted with liposome coated microparticles. Critically, the control experiment highlights the importance of taking into account the surface properties and the material of microfluidic chips when dealing with microswimmers. As Brownian motion will affect the ballistic motion depending on the sampling velocity and might even change the directionality of microswimmers, it needs to be evaluated in the absence of flow or any self-propelling effect before drawing any conclusions and especially taken into account during video evaluation and data analysis. Modifications of the microswimmers' surface chemical properties as well as the microchannels walls should be assessed or at least theoretically predicted in order to prepare a more comprehensive hypothesis about the behavior of certain microswimmers. In uncoated microfluidic chips, the aforementioned liposome-coated microswimmers are propelled by self-diffusiophoresis as the liposomes are degraded asymmetrically by the solubilizing agent gradient. Surprisingly, when using bile in the experiments the microswimmer movement was dependent on the gradient steepness. The microswimmers moved towards the higher concentration in a steeper gradient, and conversely reversed direction, towards lower concentration, when exposed to a shallow bile gradient. A similar propulsion mechanism was demonstrated by Fernandez-Medina *et al.* with silica microparticles coated with multilayers of pH sensitive polymers.[19] When placed in a steep pH gradient, these microswimmers self-propel with directionality due to the asymmetrical degradation of the polymers layers around the particles.

Microfluidic channels have also been used as a platform to perform maze solving experiments with microswimmers. Jin *et al.* used active liquid droplets that were chemically attracted by a higher concentration of empty micelles (**Figure 5B**).[41,40] Two reservoirs were connected through a microfluidic maze and a higher dose of TTAB surfactant was added to the exit reservoir. After some time, with no external flow, a diffusion-driven gradient was established in the maze; where the steepest gradient indicated the shortest path between the entry and exit. When entering the maze, the swimming droplets were directed up the surfactant gradient *via* the alignment of their Marangoni flows and the majority of droplets reached the exit reservoir by taking the shortest path. While many microfluidic systems have been designed for gradient generation, they fall into two categories, diffusion-based and convection-based, depending on the governing principle that creates the gradient.[109] Though we primarily present examples of microfluidic-generated chemical gradients, several groups have used pH gradients as a way to initiate self-propulsion of microswimmers.[19,136] Because gradients can be rapidly established at the microscale, we expect other microfluidic-generated gradient types, such as temperature and viscosity gradients, to emerge for evaluation and control of microswimmers.

5.4.2 Gradients to guide biohybrid microswimmers

Many of the biohybrid microswimmers that have been designed are sensitive to external stimuli such as pH change or certain chemicals. The ability to react to these stimuli and orient their swimming direction is greatly valuable in active matter applications such as cargo transport and drug delivery. Like it does for artificial microswimmers, microfluidics provide excellent tools to study chemotactic[81,137,10,97] and pH-tactic[136] behavior of biohybrid microswimmers through gradient generating platforms. Many of these microfluidic systems create diffusion-based gradients. The simplest design comes from Shao *et al.* where they connected two microfluidic chambers through one single central microchannel for a flow free assay (**Figure 5C**).[97] A hydrogel containing *E. Coli* bacteria was added in one chamber and biomolecules produced by the bacteria formed a gradient in the microchannel. This initiated the propulsion of biohybrid neutrophils loaded with bacterial membrane-coated mesoporous silica nanoparticles. A similar approach, using cellular sources to generate the biochemical gradient, was employed to evaluate a bacteria-based robot in a design composed of three microfluidic chambers.[81] In a similar fashion, other groups used an agarose hydrogel to separate three channels of a microfluidic chip and create a gradient in the central channel *via* diffusion of chemicals flowing in the outer channels.[10,137] The main difference in this configuration is that the gradient was formed along the width of the microchannel, orthogonal to the flow direction. This design was also used to create a pH gradient in another study (**Figure 5D**).[136]

The previously mentioned articles focused on motion studies of chemotactic or pH-tactic biohybrid microswimmers, where the gradients driving the microswimmers were localized to a single microchannel. However, to study the effect of different chemical concentrations on the speed of the microswimmers, it might be useful to perform the experiments in parallel, differential gradient channels, as Sun *et al.* did with their bioinspired soft robot.[104] Their microrobot, made up of a complex assembly of hydrogel layers containing carbon nanotubes, magnetic nanoparticles and cardiomyocytes was able to mimic the motion of snakes or caterpillars. After fabrication the soft microrobot was integrated into a 2-inlet, 5-channel multimixing microfluidic system with different drug concentrations in each channel. This work provides a glimpse into the potential of combining biohybrid or bioinspired microswimmers into a microfluidic platform. We envision that this could be a screening tool to evaluate drug candidates and optimal concentrations, by evaluating the "heart" microswimmer moving in their respective channels, faster or slower based on optimal drug presentation. Similar to heart-on-a-chip systems, which aim to emulate specific heart functions to test new drugs for heart, we imagine this "racing heart-on-a-chip" could evaluate optimal drug regimens by assessing microswimmer speeds. This approach may increase the ability to do drug screening, in novel parallelized ways. At the very least, by removing the "heart" system from being built into the chip and making it into a heart microswimmer, researchers can increase parallelization and manipulation of the "heart" component on the same chip and then use microfluidics in a more common format, i.e., multiplexing on the same chip.

6 Envisioned applications

6.1 Pick up - different chambers/environments

A series of research articles have demonstrated the ability of microswimmers to recognize, isolate and extract different biological[28,27,43,16,71,134] and environmental[125,113,58]targets from a complex raw medium. The surface of the microswimmers can be functionalized with various groups which helps the microswimmers to hybridize specific targets *via* electrostatic, magnetic or antigen-antibody interactions. The binding efficiency between the target and probe is enhanced, if the probe is an active particle, due to the rapid propulsion of the microswimmer and the corresponding fluid convection.[43] This synergistic effect of the microswimmer propulsion at the microfluidic scale has the potential to create powerful LOC microsystems with the advantage to selectively capture and perform downstream analysis of targets.

For instance, Garcia *et al.* demonstrated a microchip capable of 'on the fly' immunoassays that selectively capture target proteins with bubble-propelled, antigen-functionalized PEDOT/Ni/Pt microtubes.[27] Whereas, Vilela *et al.* reported the capture of heavy metals by self-propelled graphene oxide tubes because of strong complexation interactions, useful for environmental remediation.[113] For targeted delivery, an ideal cargo carrier should release the payload once the target destination is reached. Some promising release mechanisms reported in the literature include ultrasonic[125], chemical[113,134] and magnetic[71] stimulation or treatment. An interesting strategy was demonstrated by Sanchez *et al.* to capture, transport and release immotile sperm to oocytes for fertilization using magnetic microhelices. Rotating magnetic fields actuated the microhelices to transport and then release the sperm by simply inverting the rotation direction of the magnetic field (**Figure 6A**).[71]

In a bulk medium, the target capture by microswimmers mostly relies on the random collisions and sufficient proximity between the microswimmer and target, making the process inefficient and time consuming. This issue can be addressed using microchannels with limited width with respect to microswimmer's dimension. Another challenge that occurs even within microchannels, is the inability of a microswimmer to capture more than one target despite the presence of several receptors on its surface. Zhou *et al.* tackled this problem when working with Au/Ni/Au microswimmers as bacterial transporters in microchannels.[134] The spatial confinement of the channels and oscillations of the microswimmers helped them increase the

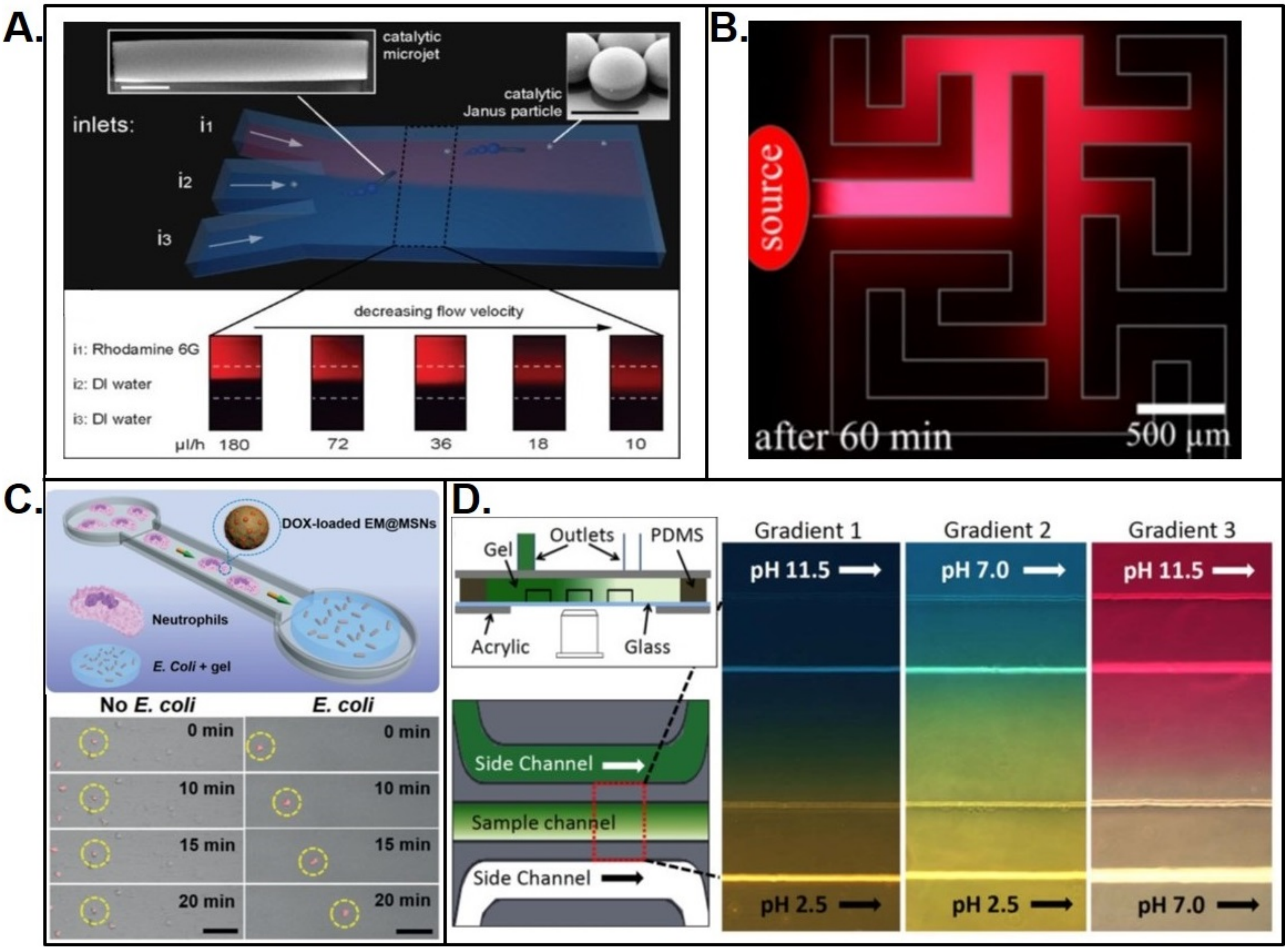

Figure 5: A. Illustration of 3 inlets microfluidic gradient generator used to evaluate H_2O_2 fueled microswimmers. Reproduced with permission from[3]. B. Microfluidic maze with TTAB surfactant gradient. Reproduced with permissions from[41]. C. Simple microfluidic device to study chemotactic behavior of biohybrid neutrophils. Reproduced with permissions from[97]. D. Diffusion-based gradient generator to evaluate pH-taxis of bacteria-bots, reproduced with permission from[136].

average bacteria loaded per microswimmer by about 3-4 times. Furthermore, by implementing this compartmentalization strategy and increased bacterial loading, the capture efficiency of the system increased by 96%.

6.2 Cargo transportation

One of the most exciting prospects of microswimmers is the directed capture, transport and release of cargo to advance both, biological research and medical applications. In practice, realising these applications with microswimmers still remains a challenge due to complex nature of biofluids. For example, one of the many problems associated with blood is that it is composed of red blood cells (in addition to other cell types) which can interact and obstruct the motion of the swimmers.

Similarities between streamlined flow in blood vessels and microfluidic channels, makes microfluidic devices promising to study swimmers' propulsion and cargo-towing ability in blood-like flow regimes. While other literature has covered advances focusing on cargo transport in microfluidic channels,[115] we touch on some key studies in this area. For instance, in earlier work, Burdick *et al.* guided the transport and on-demand release of polystyrene beads by doped carbon nanotubes within complex microchannel networks (**Figure 6B**).[6] An important aspect in payload transportation, touched on in section 6.1, is the specialized interaction acting between cargo and swimmer which guides their loading and unloading. Several chemical interactions such as electrostatic[105], biotin-streptavidin[105] and non-chemical interactions such as magnetic[6,4,44,26] helps the payloads adhere to microswimmers. On-demand cargo release is then triggered by either changing the direction of motion very rapidly in case of magnetic interaction (magnetic force between the cargo and swimmer becomes less than the viscous fluid drag on the swimmer)[6] or by terminating the interactions between the payload and swimmer by using different stimuli such as pH.[7] Most cargo towing studies rely on modifying the swimmer surface with receptors or by adding a magnetic component to it, which ensures their target specificity.

However, there are some other reports which demonstrate cargo loading without any functionalization[124,63,94,123,95,80]. For instance, Sanchez *et al.* reported that multiple cargoes can easily be loaded in Ti/Fe/Pt rolled-up microtubes without any functionalization because of their pumping propulsion mechanism which helps to suck in the payloads towards the front opening of the tubes.[94]

Most of the early studies of targeted delivery using microswimmers have been done in artificial environments making them unrelatable to the real, complex nature of blood flows in the human body. The main reason behind this is the low thrust force of most artificial microswimmers resulting in their inability to deliver cargo against blood flows. The first study of active propulsion of microswimmers and transport of therapeutics in blood was done in microfluidic channels and was reported by Baylis *et al.*.[5] They reported that $CaCO_3$ microswimmers could propel through blood with velocities up to 1.5 cm/s to deliver thrombin in wound regions. Recently, Xu *et al.* reported successful propulsion of a hybrid sperm microswimmer against blood flow for targeted cargo delivery.[124] Especially in the earlier stage of micromotor research, cargo transportation was considered one of the very promising prospects of micromotor research, specifically because precision transportation of matter on the microscale is intricate. Nowadays it is well known that the interactions between microswimmers and their passive counterparts are complex and strongly dependent on the medium,[116] so expectations on cargo transportation have become more realistic and the prospects on controlled drug delivery have been tempered. However, in biomedical applications such as IVF or gene transport the potential benefit is high, so despite many hurdles new transport modes are being researched to build theoretical models and actively contribute towards a more comprehensive understanding of microswimmers added value in these fields.[103,65] One application we foresee of high potential and more realistic milestones in the near future, is the use of the microswimmers as a vector to transfect cells, since a low quantity of cells and exact targeting can more easily be tackled. While we are excited at the potential of these different biomedical applications, optimization is still required for control mechanisms and enhancing precision, as well as defining increasing biocompatible microswimmer systems.

6.3 Concentration device

Concentration devices are particularly interesting in LOC systems for concentrating samples to combat the limitations such as low sensitivity and ultra trace detection. Generally, pre-concentration relies on using external power sources, limiting the development of fully automated portable systems.[62] In the field of active matter, pre-concentration was first observed for *E. coli* bacteria in a microstructured wall of funnels. When constrained geometrically, interesting hydrodynamic and mechanical interactions take place between the boundary and the bacteria resulting in an increased concentration along the funnel wall.[25,59] Later, Kaiser *et al.* published the first theoretical model on artificial microswimmers in concentration devices and showed that a chevron shaped wall at a defined apex angle, can act as a perfect trap for self-propelled rods (**Figure 6C**).[45]

Restrepo-Perez *et al.* achieved the first concentrating device based on catalytic microswimmers using a very specific design of microfluidic channels,[90] a concept that was later applied for rectifying the inherent random motion of Janus particles.[49] They demonstrated that the physical boundaries of the chevron and heart-shaped geometries sterically restrict the motion of the swimmers forcing them into closer packed confinement. In a later report by the same group, streptavidin-functionalized, microtubes towing biotin decorated cargoes were trapped in a heart shaped reservoir using the same concept, for on-chip bioassays.[89] Connecting these chevron shapes into a continuous circular unit ensures that not only trapping, but guidance can also be achieved.[49]

6.4 Computation

Since the first mechanical computers, electronic computing has drastically increased its capabilities to size ratio over the last decades by scaling down feature sizes. Alternative forms of computing, based on different approaches are frequently discussed as more apt way to solve issues such as combinatory logic, among them are several microfluidic based strategies.[20] In combinatorial tasks, that require calculation and sorting of several different solutions in classical computation, novel approaches such as microfluidic computation promise a reduction in memory requirements and therefore an increase in efficiency. Active matter has also been suggested to perform computing tasks, with early approaches using single microbe strains. More complex operations have been made possible by dividing tasks between "sending" and "receiving" microbe strains and genetic engineering tools. Some early approaches have been reviewed elucidating the contributions of microfluidic structures on these active matter computing approaches.[37]

Artificial objects such as regular droplets have been shown to perform different operations such as AND, OR, XOR, NOT and NAND when driven by an external field,[46] these microfluidic-generated droplets have been used in multiple types of logic operation.[8] While genuine active matter has only been examined theoretically, it could overcome complications of microfluidic computation using regular droplets (non-active matter) that stem from the need to apply pressure or external fields.[122] The use of active matter offers the potential to work around this constraint. Woodhouse and Dunkel predicted that active flow networks, operating far from thermal equilibrium, are able to perform logic functions through a combination of global incompressibility and local energy conversion constraints.[122] Microswimmers were indeed implemented in computation, using a combination of microtubuli moved by kinesin motors in a network on chip (**Figure 6D**).[74,56] The authors show, in proof of concept papers, that a combinatorial subset sum problem, which frequently presents a problem to normal computers could be solved more efficiently using motile microtubuli in a microfluidic channel setup.

We are excited to see and contribute to the further development of computational applications beyond theoretical designs, as the potential of this alternative computing is just starting to be exploited. While microswimmer computing requires further refinement, critically needed is an example breakthrough, such as solving a complex combinatorics problem to demonstrate the feasiblilty of microswimmer logic and establish its computing.

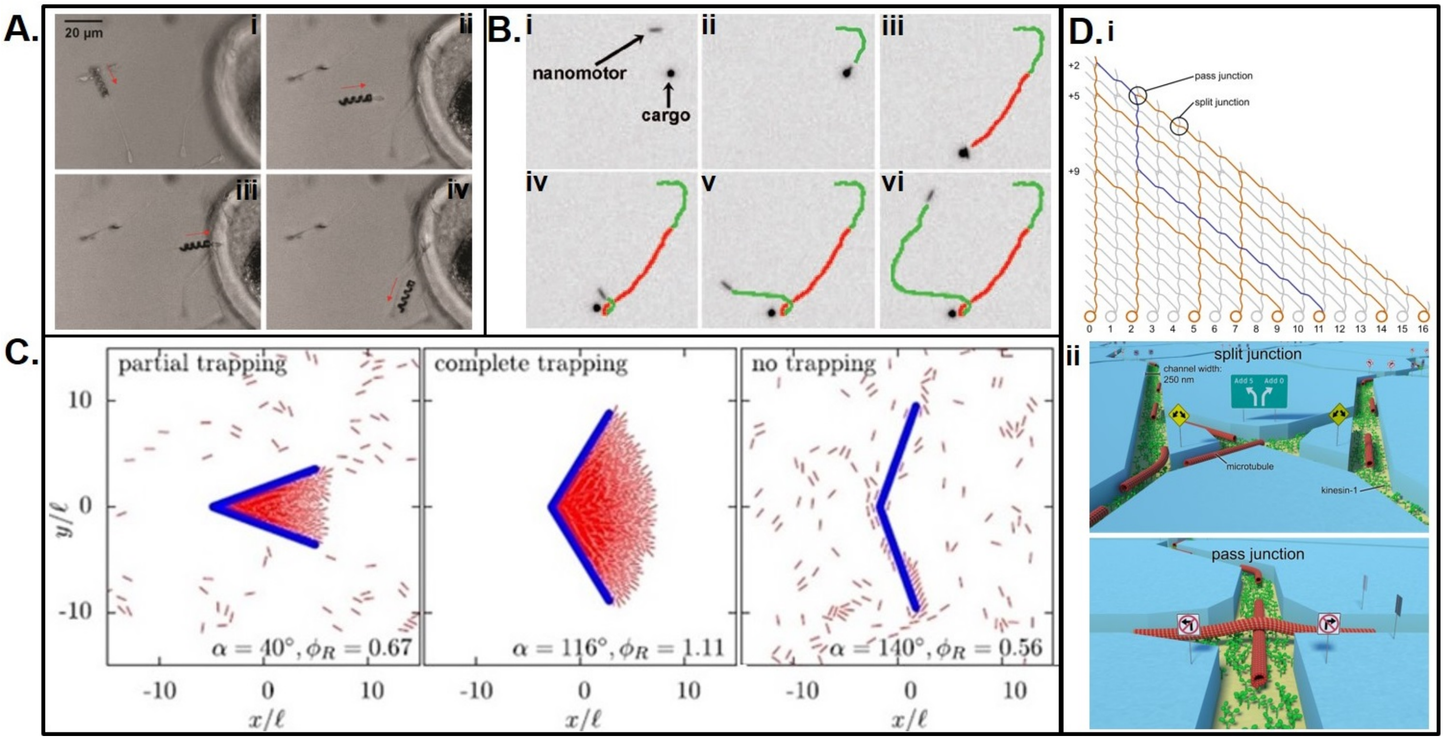

Figure 6: A. Demonstration of an immotile sperm cell's pickup using a helical microswimmer and its delivery to the oocyte for fertilization in four steps: (i) coupling of sperm cell and magnetic spiral, (ii) its transport, (iii) oocyte membrane approach and contact, (iv) release. Reproduced with permissions from [71],Copyright © 2016, American Chemical Society Copyright © 2008, American Chemical Society. B. Series of optical images showing (i,ii) cargo pickup by an Au/Ni/Au/Pt-CNT nanomotor, (iii) transport, (iv) release and (v,vi) motion of the nanomotor after the payload delivery. Reproduced with permission from [6]Copyright © 2008, American Chemical Society. C. Snapshots illustrating three different stationary states: at a small trap angle partial trapping occurs, medium trap angle results into complete trapping and further increasing the trap angle results into no trapping at all. Reproduced with permission from [45]. D. (i) Design of a network for solving a subtotal addition problem: green represent pathways that lead to a solution, grey ones do not, (ii) The upper crossing is distributing, i.e. the probability is half that the microtubuli moves left/right. The lower crossing is just for passage and microtubuli move straight, courtesy and copyright of Till Korten. [56]

6.5 Final remarks on applications

If microswimmers are to be fully integrated in biomedical applications in the future, many challenges remain. The main one is about the biocompatibility and biofunctionality of those microswimmers. Microswimmers size, primarily tens of microns in size, does not present major concerns for toxicity. However, the materials used to make microswimmers need to be selected to ensure the reduced toxicity for the cells and organisms they come in contact with. For example using biocompatible hydrogels, ceramic, and biosilicifated microswimmers would ensure low levels of toxicity. Furthermore, considering photocatalytic microswimmers, an issue arise with the use of toxic fuels such as hydrogen peroxide. Finding non-toxic fuels that keep swimmers active in physiological fluids is also a key necessity to realistically consider these microswimmers for future biomedical applications.

Besides biomedical approaches, many of the envisioned applications of microswimmers have been focused on environmental remediation. Especially in this area, we have to consider the material component. Even though the use of PDMS and other polymers has made chip fabrication much more flexible and economical, it has also led to a major single-use waste stream. The generated waste is a counter intuitive, and major, problem, if the environmental remediation goal results in fossil-fuel produced plastic devices and a direct use-to-waste life cycle. The polymers used for microfluidics are generally not compatible with all types of fluids namely organic solvents, alcohols and minerals oils, reducing the ability to effectively clean and reuse the devices. Contrarily, glass chips remain expensive, with multi-step process-intensive fabrication techniques, that require specialized equipment, which we believe will continue resulting in the limited adoption of these devices. Furthermore, due to the high resistance channels in microfluidic devices, cleaning devices is not always possible. Looking forward towards environmental remediation (and other) research and applications the development of solutions should not create a larger burden, and consequently a moral constraint, regarding sustainability. As such, we believe there is still room for improvement regarding the identification of materials for chips that can be cleaned, reused and/or recycled without losing their integrity. If this alternative material option is not identified, there are other materials such as ceramic, paper and hydrogels, which have also been used for microchip fabrication, though to a lesser extent, that could be implemented and reduce the environmental impact of the microfluidic devices.

7 Conclusion

We have reviewed the tremendous potential of the combination of microfluidics and microswimmers grouped into three main areas: The first one are synthetic possibilities opened up by microfluidics from which especially soft robots will benefit further, in particular hydrogel based microswimmers and related materials will benefit from advanced fabrication techniques.[121] Currently, scale up of microswimmers is difficult due to the more conventional fabrication process including deposition techniques. Using microfluidics, the production of larger batches simply requires longer times, given that all (active) compounds are stable and adequate conditions selected. In the past, micromotors have been largely optimized for efficient movement, or adapted to the use of non-toxic fuel. Due to the interest and their potential in future biomedical applications, the next step needs to be the optimization of biocompatibility of microswimmers, which is already off to a good start with the shaping of biopolymers in microfluidic droplets.

Additionally, microfluidics can also provide very controlled environments for motile objects. This control is accomplished through defined volumes and geometric constraints, flows or interfaces, as described in the second section of this manuscript. Down stream of fabrication and evaluation, 'traditional' applications for LOC devices can also benefit from adding microswimmers, for example to cause advanced mixing effects[126] or increasing sensitivity by concentrating analytes.

Generally, operations at the microscale are very efficient, but this limits the ability to scale - up and process larger volumes. This volume scale discrepancy results in a practicality issue. For most envisioned applications, the amount of fluid that comes into contact with a micromotor needs to be increased. Unfortunately, just increasing the sample volume eliminates the high-surface area-to-volume ratios common to microfluidics, and highly synergistic for effective micromotor function. If this volume scale discrepancy can be overcome, we expect micromotors in microfluidics processing large volumes to become a game changer in a variety of applications.

The synergy found at the intersection between microfluidics and microswimmers, becomes obvious when evaluating the overlapping benefits these two fields, and we expect that overlapping research will continue to produce exciting scientific advances from the combination of these technologies.

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