Nano/Micromotors for Cancer Diagnosis and Therapy

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Nano/micromotors are artificial robots at the nano/microscale that are capable of transforming energy into mechanical movement. In cancer diagnosis or therapy, such “tiny robots” show great promise for targeted drug delivery, cell removal/killing, and even related biomarker sensing.

Keywords: nano/micromotor ; biocompatibility ; microswarm ; cancer diagnosis ; cancer therapy

1. Introduction

As is well-known, cancer is a serious disease that threatens human health. Today, it remains a medical challenge despite the progress achieved so far in pharmaceutical science and drug discovery [1]. Among the advanced techniques, nano/micromotors are man-made miniature devices that have arbitrary, directional, or even controllable motions to perform special tasks in microenvironments due to the energy conversion hiding behind their mechanical movement. Various types of energy, including chemical, light, magnetic, ultrasound, etc., have been employed for nano/micromotor propulsion and achieved the desired effect [2][3]. Such miniature devices can be integrated with fluorescence [4][5], electrochemistry [6][7], or the Raman signal-enhancement substrate [8][9] for biomarker detection or loaded with drugs for targeted site delivery and release [10][11][12], thus showing great potential for disease diagnosis and treatment. Indeed, a large number of nano/micromotors with different designs have already been introduced for cancer diagnosis and treatment. However, biotoxicity remains a challenge that restricts them from moving from the laboratory to clinical applications. These include (a) The toxicology of nano/micromotor components (for instance, by assessing cell viability, previous research reported that Mg/Pt Janus micromotors show a concentration-dependent toxic trend) [13]; (b) The generation of harmful byproducts [14]; (c) The use of toxic fuels at high concentrations [15]; and (d) The immune response caused by nondegradable components and the use of sperm derived from other species [16][17][18]. Recent advances show the great efforts made by researchers to improve the biocompatibility of nano/micromotors.

When using nano/micromotors for cancer treatment, the disadvantage of traditional chemical propulsion modes is the unavoidable use of toxic fuel, which hinders their future in vivo application [16][19]. The use of natural enzymes for catalytic bubble production [20][21] and magnetic fields [22][23], ultrasound waves [24][25], or light [26][27] shows great promise in providing a biocompatible method for nano/micromotor propulsion. Another major factor that leads to biotoxicity may be the materials used to produce nano/micromotors. For this reason, cell membranes have been used to coat nano/micromotors, which provide a biocompatible surface mimicking cells. In addition, living cells (such as sperm bacteria or red blood cells) have been used directly as a basic skeleton to construct nano/micromotors [28][29][30]. Apart from that, driving nano/micromotors in an in vivo microenvironment becomes much more complex since the blood flow or co-existing protein/cells/tissues can weaken their motion behavior. To address this issue, dual propulsion modes have been introduced due to their more controllable and powerful mechanical movement [31][32]. Finally, cooperation among groups of nano/micromotors (so-called microswarms) could help to achieve the delivery of larger doses of loading drugs and higher therapeutic efficacy compared to individual agents, thereby indirectly lowering the toxicity.

2. Bubble Propulsion by Inorganic Catalysts

Using inorganic catalysts to generate bubbles for nano/micromotor propulsion is the most traditional and well-known way to obtain in-depth information (such as motion mechanism or different applications) in studies in this research field. A typical example is the Pt-based nano/micromotor. For biocompatibility purposes, magnesium (Mg), with biodegradability and motion behavior in body fluids, has been employed.

Apart from cancer treatment, Mg-based micromotors also have been introduced for capturing and detecting CTCs using the electrochemical method, in which Mg particle surfaces are immobilized with Fe_3O_4/P/anti-EpCAM [7]. Another inorganic material with ideal biocompatibility is zinc (Zn), as reported by Zhou et al. The tubular micromotor consists of poly(aspartic acid) (PASP) with a thin intermediate Fe layer and internal Zn layer, and the outside surface of the

microtubes (negative charge) is further loaded with DOX (positive charge) via electrostatic interaction. Such Zn-based tubular micromotors can be propelled in the presence of gastric acid and permeate the gastric mucus layer, increasing their retention in the stomach [33]. It is well-known that acids like hydrochloric acid react with $CaCO_3$ to produce CO_2 bubbles. As expected, this chemical reaction can be utilized for nano/micromotor propulsion. Recent work reported by Zhang et al. is a typical example. They used yeast cells to synthesize a nano/micromotor by introducing inner- and outer-mineralized $CaCO_3$. Inner nano-$CaCO_3$ is generated by the reaction between Ca^{2+} ions (combined with proteins and polysaccharides while entering yeast cells) and CO_3^{2-} (changed from CO_2 produced by cell respiration in basic environment), while outer $CaCO_3$ is synthesized via the one-pot method using Na_2CO_3 and $CaCl_2$ to form crystals. This micromotor showed good self-propulsion behavior in gastric fluid). Even though the application is related to gastritis therapy instead of cancer treatment, this excellent work provides a new bubble-propelled micromotor with high biocompatibility that, importantly, could be adapted to work in vivo in the stomach [34].

3. Bio-Hybrid Nano/Micromotor

Natural entities such as sperm cells, red blood cells, etc., possess unique properties such as limited immunogenicity, high binding specificity, and the use of bio-safe fuel from the surrounding environment for propulsion. The introduction of biomaterials endows miniaturized actuators with high biocompatibility for working in biological systems [35][36][37]. Natural entity-based nano/micromotors can be categorized into the following four groups.

(A) Cell membrane. Inspired by nature, the cell membrane, which has unique properties such as immune escape, specific recognition, prolonged circulation time, and high biocompatibility, has attracted the interest of researchers, who coat them onto the surfaces of micromotors. The biological function of natural cell membrane endows the micromotor system with the ability to realize targeted drug delivery or specific binding of bacterial toxins [38][39][40]. One typical example is the use of the red blood cell (RBC) membrane. Hou et al. proposed a cell-mimetic micromotor fabricated using $Ca(OH)_2$ microparticles with biconcave discoidal morphology as the template, camouflaged with the RBC membrane. To explore further applications in tumor therapy, Fe_3O_4 nanoparticles and DOX(an anticancer drug) were loaded within the wall part of an RBC micromotor for magnetic navigation and tumor therapy [41]. Recently, Li et al. introduced a swimming micromotor with clawed geometry by synthesizing sunflower pollen covered with magnetic Fe_3O_4 layers as clawed microparticles, followed by the immobilization of an RBC membrane-camouflaged coating. This micromotor proved to have effective magnetic propulsion even against the flow in the rabbit jugular vein. Though no further anticancer experiments were conducted in this work, they provided a promising and safe method of drug delivery in vitro [42]. Another example is the introduction of the cancer cell membrane, which endows gold nano-shell functionalized $CaCO_3$ particles with the ability to modulate immune activity, as the coating membrane contains many membrane-bound tumor antigens. The coating layer also enables the micromotor to target corresponding cancer cells due to the homotypic binding of cancer cell membrane [43]. To summarize, these practical examples achieved mimicking of related living cells to endow the nano/micromotor with similar functions, such as biocompatibility or target recognition.

(B) Enzymes. These are mainly proteins that can transform biocompatible fuel into a driving force by catalytic reactions. For instance, by immobilizing urease, a silica-based tubular micromotor is able to catalyze the decomposition of urea, thus generating microfluidic flow via the production of NH_4^+ and OH^-. This micromotor will be endocytosed by the cells and can provide enhanced delivery of anticancer drugs into cells to achieve higher killing efficiency [44]. Another example is the modification of glucose oxidase and catalase in which glucose oxidase catalyzes endogenous glucose to produce H_2O_2, then the catalase catalyzes the decomposition of H_2O_2 (both as produced and natural) for micromotor propulsion. This design shows a synergetic effect for photodynamic-starvation therapy based on the consumption of glucose and the NIR-triggered generation of $^1O_2$ [45]. In addition, natural platelet cells have also been transformed into biocompatible micromotors, and such cell-based micromotors show propulsion behavior in the presence of urea fuel by the surface modification of urease [46]. An enzyme-based micromotor was also introduced for cancer-related detection, in which catalase was modified on the inner surface of the microtube for propulsion. Based on the decreased motion speed, the researchers realized the bio-sensing of DNA [47].

(C) Bacteria. Bacteria are born with self-swimming ability, which makes them ideal objects for fabricating biohybrid micro-swimmers for drug-delivery purposes [48]. In addition, some bacteria can selectively migrate to the hypoxic regions of solid tumors, which further promotes their development as chemotherapeutic drug carriers [49]. *Escherichia coli* has been incorporated with magnetic nanoparticles for spatial magnetic and hypoxia perception, which provides the collective perception and positive migration ability of microrobots in targeting the tumor microenvironment. Before magnetic modification, bacteria were encoded with bacteria-phage λ repressor cI857 for the triggered expression of the NDH-2 enzyme (respiratory chain enzyme II) and mCherry. Here, the expressed mCherry acts as an internal fluorescence reporter for imaging-guided tracking and actuation along with the NDH-2 enzyme, enhancing anticancer treatment by the

upregulation of H_2O_2 [50]. The gut-friendly bacteria *Lactobacillus rhamnosus* have also been employed for cancer therapy, in which the bacteria were modified with a photoluminescent (Au nanoclusters) and anticancer drug, which showed cytotoxicity to cancer cells [51]. In another work reported by Akolpoglu et al., *Escherichia coli* MG1655 was used as a biological unit to fabricate magnetically controlled microrobots for stimulus-responsive cargo delivery. The chosen bacteria expressed biotin attachment peptides, which allowed for the highly efficient modification of nanoliposomes (loaded with photothermal agents and chemotherapeutic molecules) and magnetic nanoparticles (controlled propulsion) via biotin-streptavidin-biotin connections. By applying an external magnetic field, the as-prepared microrobots showed a controlled swimming path (square shaped). In another application, bacterial microrobots were also used for the release of anti-cancer drugs by near-infrared light activation [52].

(D) Sperm. Similar to bacteria, sperm cells are natural self-moving microswimmers that can perform complex tasks at microscale [53]. In order to construct a sperm-based anticancer drug-delivery system, man-made tubular microstructures have been designed for the magnetic guidance and release of drug-loaded sperm to an in vitro cultured tumor spheroid [54]. In another work, to prevent the motility of sperm from being affected by surrounding threats such as the specific binding of anti-sperm antibodies, researchers wrapped sperm cells with a zeolitic imidazolate framework-8 (ZIF-8) to maintain their effective propulsion [55].

4. Ultrasound Waves for Propulsion

Ultrasound, which uses sound waves created by vibrating objects and is a type of mechanical wave, is not only widely used for medical imaging, but it also can serve as a biocompatible propulsion mode for nano/micromotors. Compared to other energy inputs, ultrasound has wide clinical use (for imaging), and its ability to penetrate deeply through tissue has been well-proved. By using ultrasound as the energy input, nano/micromotors can avoid using toxic fuel and, thus, be highly biocompatible. Ultrasound is also currently being widely used for nano/micromotor propulsion [56][57][58]. Both surface acoustic waves (SAWs) and ultrasonic standing waves (USWs) can be used for nano/micromotor manipulation. SAWs usually use piezoelectric ceramics (such as lithium niobate, $LiNbO_3$) as the substrate with interdigital transducers (IDTs) on the surface [59][60], and for USWs, researchers tend to carry out nano/micromotor propulsion in a tailor-made chamber (made from Kapton tape or PDMS) for ultrasound wave reflection and piezoelectric ceramics stuck to the chamber for ultrasound wave generation [61][62].

Wang et al. synthesized nanomotors using gold nanowires (AuNWs) modified with ovalbumin (OVA), which act as model protein antigen. The nanowires are also propelled by ultrasound and realized antigen delivery). Such acoustically active AuNWs@OVA nanomotors retain high speed (only a little decrease from average 90 to 61 μm/s), even coating the surfaces with protein) and show the ability to enter single cells without disrupting the integrity of the cell). This novel design provides a new strategy for solving the challenge of the degradation of internalized exogenous antigens in lysosomes while using the vaccine. These ultrasound-powered nanomotors help in the process of antigen cross presentation and cellular immunity (with upregulation of MHC I and MHC II-related molecule expression), which are critical components of the immunological effect of therapeutic vaccines for tumors or viral diseases [63].

5. Electromagnetic Wave (Light)-Based Propulsion

Electromagnetic waves such as light provide a clean, harmless, and noninvasive propulsion mode, making it a versatile and powerful candidate for driving nano/micromotors that are highly biocompatible. In addition, by using electromagnetic waves for this purpose, researchers can easily control the speed and direction of nano/micromotors, and no complex or special equipment is required compared to other propulsion modes such as magnetic or ultrasound waves [64][65][66]. Different wavelengths of electromagnetic waves, ranging from ultraviolet (UV) to visible (VIS) to near-infrared (NIR) light [67][68][69], as well as X-rays [70], have been used for nano/micromotor propulsion. In addition, materials such as Ag_3PO_4 [71], $BiVO_4$ [72], Ag [73], Cu@MoS_2 [74], carbon nitride (f-C_3N_4) [75], TiO_2 [76], BiOI/AgI/Fe_3O_4/Au [77], ZnO/Pt [78], and Cu_2O@CdSe [79] have been smartly designed and utilized for the construction of nano/micromotors driven by electromagnetic waves. The mechanism behind such propulsion is usually complex and can include electrophoretic and diffusiophoretic effects or the generation of an interfacial tension or temperature gradient [65][68][80].

Currently, using light-propelled nano/micromotors for cancer treatment is a research hot topic. For instance, Xing et al. fabricated jellyfish-like mesoporous carbon nanomotors integrated with single-atom copper (Cu-JMCNs) propelled by the thermophoretic effect after NIR light irradiation. By integrating single Cu atoms, H_2O_2 was catalyzed to produce toxic hydroxyl radicals for chemodynamic therapy, and an NIR-triggered motion of the nanomotor improved cellular uptake and tumor penetration [81]. With light propulsion, one major drawback is the inability to be propelled in solution with high ionic strength because existing ions would restrain the formation of concentration gradients, which would contribute to the self-

electrophoresis or self-diffusiophoresis effect around the nano/micromotors [82][83]. To overcome the above challenge, Sridhar et al. employed two-dimensional (2D) poly(heptazine imide) (PHI) carbon nitride to build light-propelled micromotors. Thanks to the proper interaction between the textural and structural nanoporosity and optoionic properties of particles, the proposed microswimmer achieved propulsion in a highly ionic solution. Compared to traditional one-dimensional (1D) CN_x, the PHI show both higher hydrogen evolution activity and the ability to store light-induced electrons. The researchers supposed that the mechanism of the light-simulated microswimmer motion mainly came from asymmetric illumination and photocatalysis, which caused ion flow around and through the materials. The cations move across the pores of the material to counteract Debye layer collapse, thus contributing to the ionic tolerance. In addition, a pseudocapacitive photo-charging effect occurs in the materials to further strengthen ionic tolerance with respect to 1D CN_x. The light-propelled PHI micromotors were further loaded with doxorubicin (DOX) and showed stimulus-responsive drug release when triggered by hypoxia, pH, and light [84].

6. Magnetic Propulsion

Nano/micromotors propelled by an external magnetic field have advantages including being fuel-free and having precise and controllable motion [85][86]. In this propulsion mode, nano/micromotors seem to be simply propelled by the external magnetic field, yet they also involve energy conversion during the self-propulsion process. Indeed, these nano/micromotors have potential magnetic energy that is relative to the outside magnetic source; during propulsion, the potential magnetic energy is transformed into kinetic energy. Various techniques have been proposed to enable the magnetic response of nano/micromotors for directional or propulsion purposes, such as the chemical synthesis of magnetic particles followed by the encapsulation or surface modification [87][88], physical vapor deposition [89][90], electrochemical deposition [91], 3D printing using direct laser writing [92][93], and the combination of microfluidic droplet printing and wettability-induced drawing photolithography [94]. For magnetic guidance or propulsion, various devices such as Helmholtz coil [95], Maxwell coil [96], and saddle coil [97] have been designed to provide a uniform or gradient magnetic field.

For cancer treatment, Mayorga-Martinez et al. used sunflower pollen deposited on thin-film metal layers (including Au, Co, and Au) on one side of the microsphere, thus endowing it with magnetic response ability for micromotor propulsion. This type of micromotor shows good performance in attracting cancer cells due to the electrostatic interactions between them and can be loaded with DOX to kill cancer cells [98]. In another work, researchers introduced magnetically actuated cystine micromotors by the zinc-mediated self-assembly of cystine and the encapsulation of Fe_3O_4 nanoparticles during the synthesis process. This cystine micromotor could be efficiently internalized in late endosome/phagolysosome compartments before Zn^{2+} ions are released for killing tumor cells after the bio-enzymatic degradation of micromotors due to broken disulfide bonds [99]. For cancer detection, magnetically propelled gold-nickel nanowires were fabricated by template electrochemical deposition and used for rapid/sensitive sensing of the cancer biomarker microRNA-21. The fluorescent dye-labeled ssDNA probe was first immobilized on a nanomotor, and its target-miRNA-21 present in the solution was hybridized with the ssDNA probe on the nanomotor surface, thus decreasing the fluorescence intensity and motion speed related to the target biomarker concentration. Au-Ni nanomotors were further physisorption loaded with DOX via the hydrophobic interaction after being modified with poly(sodium 4-styrenesulfonate) (PSS) to provide active chemical groups for DOX interaction. Related results show a pH-dependent drug release of DOX-loaded nanomotors, as well as the magnetic guidance of the nanomotors on MCF-7 cells, with efficient and controlled drug delivery [100].

7. Dual-Propelled Nano/Micromotors

Integrating two different propulsion modes into one nano/micromotor, also called a dual-propelled nano/micromotor, can realize more flexible and efficient movement. By employing two engines, nano/micromotors can carry out cargo transportation in more complex situations, and it is more convenient to control their speed and direction. The dual-propulsion mode can include bubble (chemical)/light [101], magnetic/bubble (biocatalytic enzyme) [102], light/magnetic [103], bubble (chemical)/ultrasound [104], ultrasound/magnetic [105], or ultrasound/light [106], which have also been used for cancer-related treatments or detection. For instance, a combination of enzyme-based chemical energy and magnetic field energy has been developed to drive micromotors for synergistic anticancer therapy. Here, the enzymatic-based decomposition of glucose leads to self-propulsion, and the magnetic energy provides controllable movement [31]. In a photodynamic-based cancer therapy strategy, ultrasound was employed as an efficient and bio-safe energy for the propulsion of blood cell-mimicking (RBCM) micromotors. By integrating Fe_3O_4 NPs, it is possible to orient the motion of RBCM micromotors under an external magnetic field [107]. The hydrothermal method was utilized to synthesize dendrite-shaped microrobots that exhibited dual light/magnetic propulsion. These micromotors showed negative phototaxis due to the self-diffusiophoresis effect under light irradiation, while the external magnetic field endowed them with rolling-motion

behavior. By exposure to light along with H_2O_2, such micromotors could realize on-site ROS generation to deplete GSH and enhance PDT efficiency for prostate cancer therapy [108].

Dual-propelled nano/micromotors not only have been well-designed for cancer treatment as described above, but they also have played an important role in related disease detection. For instance, Báezet et al. synthesized tubular micromotors propelled by chemical catalytic and magnetic energy for gastric cancer biomarker detection [109]. Ren designed nanomotors propelled by magnetism and bubbles, in which γ-Fe_2O_3 nanorods were used to coat the catalase. The modification of folic acid (FA) and hyaluronic acid (HA) carbon dots endowed the nanomotor with target recognition ability. This nanomotor showed the ability to capture specific circulating tumor cells, provide imaging (fluorescence from carbon dots), and achieve quantitative detection due to the modification of the recognition elements (FA and HA), thus providing a new possibility for cancer detection [110].

8. Microswarm

In order to use nano/micromotors for cancer treatment or imaging, they can be designed as individual units to perform the task or as microswarms. Microswarm, which refers to the concept that large numbers of micro/nanoparticles can work together cooperatively, seems to not be directly related to biocompatibility. Yet it represents an important and effective tool for further reducing nano/micromotor toxicity, thus promoting clinical applications. For cancer therapy, generally efficacy and safety are two critical aspects when evaluating a new drug. Thus, researchers need to design the correct dosage by balancing the benefits and harms to achieve the desired therapeutic efficacy. A higher curative effect means a lower dosage, which may reduce the side effects of cancer treatment. Similarly, improving the cancer treatment efficacy of drug-loaded nano/micromotors would indirectly affect their biosafety [111][112][113]. For drug delivery, the cooperative behavior of nano/micromotors would enable the use of larger doses of loading drugs for delivery compared to individual agents, therefore enhancing therapeutic efficacy [114][115]. A recent published review by Sun's group also pointed out that the swarm behavior of nano/micromotors provides possibilities for drug delivery with longer retention time, which in turn would allow significantly lower doses for higher biocompatibility [116]. For medical imaging, researchers have also proved that with ultrasound imaging, introducing microswarms acting as imaging contrast would reduce the minimal required dose of nanoparticles, indicating a lower dosage for potentially higher biocompatibility [117][118].

9. In Vivo Nano/Micromotor Visualization

When using nano/micromotors for targeted drug delivery in vivo, it is difficult to track them in real time because visible light cannot penetrate the tissues. In consideration of this, medical-imaging technologies based on different mechanisms such as ultrasound, positron emission tomography, or magnetic resonance have been introduced. Ultrasound not only can drive nano/micromotors, as mentioned above, but it also is a powerful imaging technique based on the reflection of mechanical waves. Wang et al. realized the manipulation of a magnetic microswarm near the boundary of vessels, with the ability to navigate upstream and downstream even in flowing conditions due to the reduced drag force from blood flow and strong interactions between nanoparticles. When ultrasound waves are produced and travel to the microswarm, the Doppler effect occurs, which can subsequently be detected by a Doppler ultrasound imaging device. Thus, the position of the microswarm can be tracked in real time [119]. It should be noted that the spatial resolution of ultrasound imaging technology is at the scale of millimeters, which is insufficient for imaging individual micro/nanorobots, which are typically at micrometer scale. Thus, it is necessary to use a microswarm with cooperation behavior. Another medical imaging technology, positron emission tomography (PET), has also been used to track microswarms, in which short-lived radioactive substances, including ^{124}I on gold nanoparticles or ^{18}F-labeled urease, were labeled on nanomotors with an enzyme-based engine that showed swarm behavior. During in vivo experiments, the radio-labeled nanomotors underwent positive beta decay and emitted positrons to further interact with ordinary electrons, followed by particle annihilation, γ-ray emission, and, finally, ray detection. Due to the highly efficient tissue penetration of γ-rays, researchers determined by analysis that the biodistribution of nanomotors could be realized after being injected intravenously in female mice [120].

Magnetic resonance imaging (MRI) has also been utilized for tracking or imaging purposes. In one study, Fe_5C_2@Fe_3O_4 nanoparticles were fabricated for both magnetic targeting and T2-weighted MRI and showed great potential for imaging-guided therapy [121]. The same group recently developed FeO@mSiO_2/Au-CAT Janus nanorobots for enhanced tumor penetration and therapy. With the high spatiotemporal resolution and deep penetration of MRI, the migration of nanorobots can be monitored in a non-invasive way in real time [122].

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