Analysis on Nanorobot Based on Bacterial Propulsion Method

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Abstract. Nanorobots have shown great potential in medical applications in vivo. Ingenious propulsion methods of nanorobots are significant for saving energy and accomplishing the difficult and complicated tasks. Considering variable ambient conditions inside human body, devices for propulsion methods are able to sustain in different environments. Bacterial propulsion method is one of the popular propulsion methods. Nanorobots are attached to bacteria which will carry them into the specific places in body. The in vitro experiments based on bacterial propulsion in recent ten years will be generalized and discussed here. It has proved that it is feasible to combine nanorobots with bacteria and this combination is steady. This paper aims to introduce bacterial propulsion method, which is one of propulsion methods with high efficiency, good biocompatibility and controllability.

1. Introduction
The concept of nanorobot was first proposed by Richard Feynman in 1959, which attracted researchers’ and surgeons’ interests. Nanorobots are nanometer-scale machines or robots that can work in minute places with a wide variety of functionalities. They are designed to satisfy various medical applications, such as the treatment of atherosclerosis, anticancer, removal of blood clots, cleaning wounds, coagulation, parasite removal, treatment of gout, crushing kidney stones, artificial insemination and activation of cellular energy, including bio-sensing, drug delivery, and actuating devices. There are two phases during its working processes: moving process and medicine-giving process.

The propulsion of nanorobots have been studied. Chemicals and bacteria have been combined together to produce biohybrid devices, which fulfills many expected tasks, such as directional swimming under artificial control. Various advantages of biohybrid devices have been revealed by a number of experiments. Nanorobots have to overcome the low Reynolds number environment which has always been a challenge for the motion of nanorobots. Moreover, artificial devices cannot be smaller, which results from the limitations of current technologies. Since bacterial propulsion method is biocompatible and efficient, more and more studies regarding nanorobots based on bacterial propulsion are emerging. This paper summarized the recent progress of bacterial propulsion method.

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2. Method and Analysis

2.1 Bacterial Propulsion Method

2.1.1 Foundations Some bacteria move by waving their flagella. Natural bacterial flagella are nanohelices that can self-assemble. By rotating their helical flagella, bacteria generate thrust force, which propels them to swim with a relatively high speed [1]. Bacterial flagella are composed of a single sub-unit protein called flagellin. Flagellin units are bound together through non-covalent bonds and they are arranged in repeated spiral patterns, thus forming the overall shape of flagella [2]. However, the shape of bacterial flagella is not fixed. Molecular switch that exists with flagellin, enables flagella to experience separate and reversible changes between different polymorphic forms in their helical structures [3]. Flagella can change shape and handedness, so they transfer stress generated during swimming process, which would otherwise lead to the deformation of flagella [4].

There are three fundamental beating patterns: the planar waviness of a form similar to Sine wave that was produced by regular successions of approximately equal active sliding at different two sides of the axis of axoneme of flagellum, the helical waviness produced presumptively by active sliding moving sequentially around 9 sets of doublets (as shown in Figure 1) around the flagellum, and the lateral pulsation. In this kind of beating, a sliding bends axoneme to one side, while the transmitting of wave on the other side will turn axoneme to its initial shape [5]. Due to the bundling effect of flagella that rotates independently, bacteria are propelled to move in one direction. However, if flagella point to various directions, the bundling effect disappears, and the direction of the motion of bacteria changes and randomizes [6].

By using bacteria to deliver medicine, bacteria can swim freely in vivo and they are able to maintain their moving directions even changes happened in surrounding environment. Therefore, they are ideal nanorobot carriers.

![AXONEME OF FLAGELLA & MOTILE CILIA](image-url)

Fig.1. Oaxoneme of flagella and motile cilia. 9 microtubule doublets are shown[7].

2.1.2 Experiments Fernandes et al. assembled the antibody-coated beads with E.coli on the Au patterns and then the assembly of beads and bacterium were released on-demand by using imidazole or ethylenediamine tetraacetic acid from Au patterns. They indicate that bacteria can be used to deliver cargo and the delivery can be triggered by certain chemicals. Figure 2(A) represents schematic of the attachment and release of cargo- carrying bacteria from patterned surfaces [8]. Recently, Traore et al. proved that the occurrence rate of attachment between nanoparticles and bacteria can be improved by increasing the initial particles to bacteria ratio. They also demonstrated the manufacturing of nanobeads of various assembly arrangements at nanometer scale. Figure 2(B) shows occurrence rate of 390 nm particles attachment to bacteria at different nanoparticles-bacteria ratio [9]. Singh et al. designed a surface patterning technique which is easy for replicating and achieving. By attaching E. coli bacteria to Janus biohybrid microswimmer via biotin–streptavidin bonding, they are able to achieve an assembly of strong robustness, irreversible integration, and stability. Those were proved by the observations of a trend of maximum bacteria adhesion density on biotinylated streptavidin surfaces compared with other samples and the least obvious effect of bacteria density trend decline. Experimental modeling results indicate that Janus patterned beads show increased average propulsion speed. Figure 2(C) displays data
of E. coli bond maturation of group with Janus microbeads using the streptavidin-biotin protocol and of other control groups. Figure 2(D) shows schematic of E. coli cell membrane with biotin linker when it binds to streptavidin through covalent bond [10]. Singh et al. synthesized quasi-monodisperse and doubled emulsions by water and soybean oil, which were loaded with various organelle-labeling dyes as the cargo. Then, streptavidin was added to the outer surface to ensure strong attachment of E. coli that bonded to biotin. They reported that the delivery efficiency of cargo of bacteria-driven emulsion is notably higher than that of passive emulsion, which is often applied in cargo delivery. The stability of bacteria attachment on the micro-emulsions is evaluated. It turns out that the biohybrid bonding is stable over time under the physicochemical conditions. The effective diffusion constant of the microswimmer will reach maximum, if bacteria with high mobility are used, and the emulsion size, the number attachment and locations of multiple bacteria are optimized. The microswimmers swim through the bottom membrane of Transwell actively when guided by glucose gradient. The capability of active and biocompatible cargo delivery of bacteria-driven emulsion microswimmer is testified. Figure 2(E) represents schematic of setup to analyze cargo delivery of microswimmer composed of E. coli MG1655 bacterial cells and double microemulsions with cargo [11]. Alapan et al. proposed a new cargo delivery method, combining erythrocytes (red blood cells, RBCs) which loaded with DOX (doxorubicin) and SPIONs (superparamagnetic iron oxide nanoparticles) with E.coli bacteria via biotin-avidin-biotin binding complex. The similar value of average speed of RBC microswimmers and the mean speed of natural E.coli not attached to RBC shows that the expression of biotin attachment peptides and subsequent functionalization on the membrane of bacteria have a negligible influence on the motion of the bacteria. Observation of the successful passing-through behavior between a narrow gap of RBCs suggests that the bacteria are able to generate enough thrust force to support the deformation and help loads pass through the opening. The bacteria propulsion can be remotely terminated by triggering ICG molecules. Bacteria’s capability to actuate cells is showed. Figure 2(F) and Figure 2(G) shows the microswimmer, composed of RBC with cargo and SPIONs, is attached to a bioengineered E. coli [12]. Stanton et al. grew electropolymerized polypyrrole (Ppy) microtubes electrochemically on Au layer. Polydopamine (PDA) deposited on the inner wall of tubes attract bacteria and an additional layer of Ni nanoparticles was designed for magnetic guidance. One single bacterium is trapped inside microtube. Biohybrid swimmers show their ability to move along directional trajectory paths. The directionality will be increased if bacteria inside tubes are properly arranged. Ppy-Ni-PDA tubes can trap bacteria and they can be guided under external magnetic field. The traces indicate that direction of motion is paralleled to that of external field when magnetic field of 8 mT is applied. It also shows that curved paths disappear due to the use of field and suggests the improved directionality. The motion of bacteria can be inhibited by triggering urease in the micro-tubes [13]. An intact microalga, C. reinhardtii, is attached to PE-functionalized magnetic polystyrene (PS) particles. Cargo is fabricated by using negatively charged magnetic PS particles that is functionalized with oppositely charged polyelectrolytes. Noncovalent electrostatic interactions between the positively functionalized magnetic PS microparticles and the negatively charged microorganisms help cargo to be attached the assembly [14].
2.1.3 Advantages of Bacterial Propulsion Compared with other mechanisms such as chemical propulsion and external-stimuli based propulsion, biological propulsion has good biocompatibility. Moreover, it can be controlled and it is capable of responding to a wide variety of stimuli applied by outside. In order to better control the motion of bacteria-based nanorobot working in vivo, researchers also combined the external magnetic field with magnetotactic bacteria (MTB). Magnetic Resonance Imaging (MRI) technology can be used to track bacteria nanorobot.
3. Discussion

3.1 Bacterial Propulsion Method Magnetotactic bacteria (MTB) are a kind of bacteria capable of directional motion under external magnetic field and forming magnetic nanoparticles called magnetosomes inside them. The magnetosome chain works to help bacteria self-orient along the lines in magnetic field. Therefore, MTB not only have the flagella which help them to move inside the small places such as blood vessels, but also can be controlled to swim along one certain direction by external magnetic field. These advantages of having motility and being controllable make MTB become the ideal bacterial carriers of nanorobot.

The possibilities of this propulsion method are supported by showing that in fresh water environment at room temperature. The chaotic displacements of MTB become regular when MTB are influenced by simulated experimental grids. The manipulation of MTB is controllably testified by the observation that the trace of a single MTB under the applied external magnetic field is conformed to that of the same MTB attached to microbeads. The video analysis shows that one MTB has an average speed of 7.5 µm/s with a maximum of 20 µm/s when a set of microbeads is attached to that MTB. Magnetospirillum gryphiswaldense bacteria are able to rotate 180° when a magnetic field of frequency of 2 Hz is applied. This experiment also demonstrates that this micro-actuation method can efficiently be applied in low Reynolds condition and controlled by programming algorithm [15]. Bacterial nanorobots are proved to have a superior performance of carrying loads, compared with the ferromagnetic microparticles in small diameter capillaries. Large Embolization and Transport (LET) entities are designed to contain layer of polymer and nanoparticles with bacteria. Nanoparticles can be Fe3O4 that is compatible, or FeCo that can enhance steering force. Experiments demonstrate that bacteria can efficiently reach capillary networks and improve the microsystem’s targeting [16]. MC-1 cells are proved to possess the ability to transfer drug-loaded nanoliposomes to hypoxic regions of the tumour due to their magnetotaxis and aerotaxis [17].

Fig. 3. Images show the chaotic movements of MTB without external magnetic field, path of a MTB when not attached to microbeads, and trace of displacement of a microbead pushed by a MTB when a change in direction occurs.

3.2 Limitations of Bacterial Nanorobots However, there are still many limitations of the actual applications of bacterial nanorobots. For example, living cells are often unstable under certain conditions. Additionally, new biocompatible and functional nanomaterials must be created to construct the structures of micro or nanorobots. Moreover, since nanorobots should be real-time tracked outside human body, appropriate technologies are required. The directional motion of bacterial nanorobots should be controlled more precisely. The material that builds up the structure of nanorobot needs to degrade in human body with the least toxic effects after the completion tasks of nanorobot.

4. Conclusions

A large amount of progress has been made in the field of nanorobot. Researchers developed many synthetic and biohybrid systems of bacterial nanopropellers and demonstrated the possibility of bacterial propelling method. Moreover, by introducing bacteria into artificial environment that mimics in vivo environment, nanorobots can be studied to develop multiple functionalities, such as motion with high speed, directional transport, and sufficient steering or thrust force. The superior biocompatibility allows
nanorobots to fit biomedical applications and surgeries. Researches concerning both in vitro and in vivo experiments not only describe the various capabilities of bacterial nanorobots used in medical applications, but also indicate the significant development of transiting nanorobots from chemical laboratories to important clinical applications. However, the bacterial propulsion is still in its infancy. Experiments mentioned above focus only on several aspects of the motion of nanorobots, such as the speed of the combination, but not the whole process. Moreover, biocompatible materials which comprise the nano-devices are necessary. The cargo should be delivered at the precise and required location. The process of loading and unloading should be controllable and nanorobots should be able to penetrate into cells or other microorganisms. The tracking of nanorobots in vivo should be available by using some current technologies. Finally, the biodegradability is significant, which protects human body from waste or toxicity produced by nanorobot.

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