Forum Article

Pushing Bacteria Biohybrids to *in vivo* Applications

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Abstract

Bacteria biohybrids use the energy of bacteria to manipulate synthetic materials with the goal of solving biomedical problems at the micro- and nanoscale. We explore current *in vitro* studies of bacteria biohybrids, the first attempts at *in vivo* biohybrid research, and problems to be addressed for the future.

The Power of Biohybrids

The aim of biohybrids is to harness cell motility and energy for user-desired tasks, including transport of artificial cargo, drug delivery, or to power a tool for micromanipulation of other objects [1, 2]. Bacteria powered biohybrids (Box 1) present new micromachines to perform complex tasks at the micro- and nanoscale. *In vitro*, biohybrids have demonstrated the ability to selectively sort particles [3] and even build micro architectures [4], but real-world applications for bacteria biohybrids have yet to be achieved. However, the biomedical field offers many opportunities to utilize biohybrids micro-maneuverability and natural sensing capabilities for non-invasive medical applications that are not possible with current technologies and recent research

has been pushing biohybrids towards this goal. Current bacteria biohybrids have the potential to be used for cancer or disease detection, targeted drug release, and even disruption of infectious biofilm sites. Still many challenges with external guidance, cargo loading and unloading, and efficient swimming remain, hindering their use for clinical and therapeutic applications. Here, we present recent attempts and developments to improve bacteria biohybrid performance and move the field closer to *in vivo* medical applications.

Bacteria-Particle Swimmers

Bacteria attached to micro- or nanoparticles are some of the most well studied bacteria biohybrid systems. Bacteria adhere to the particle and carry it while swimming creating an effective cargo delivery system. Guided cell adhesion of the bacteria body to localized regions of the particle is essential for efficiently propelling the biohybrid. Random attachment of bacteria to the microparticle creates competing propulsion forces that cancel each other out, inhibiting the motility of the biohybrid [5]. Patterning particles with bacteria adhesive cites such as proteins or antibodies limits ubiquitous bacteria adhesion and ensures a more efficient swimmer.

Janus particles, where a single particle is divided into two sides, have shown to be an effective substrate for creating biohybrids. Janus polystyrene microparticles with metal caps were shown to attract and preferentially adhere *Escherichia coli* (*E. coli*) to the metal cap. This approach allowed the polystyrene portion to be coated with the anti-cancer drug, doxorubicin, demonstrating a dual-functional biohybrid with controlled cell adhesion and controlled drug attachment (Figure 1A). Magnetically active iron caps could also be deposited on the particle for guided swimming with an external magnetic source [6]. For other methods of magnetic control, magnetic beads were investigated as a cargo load for *Serratia marcescens* (*S. marcescens*) as a way to maneuver the biohybrid with four iron-core electromagnets and reduce random bacterial motion and improve the steering control of the biohybrid swimming [7]. 2D and 3D swimming

trajectories of the magnetic bead biohybrids (Figure 1B) were analyzed to gain insight into the mechanisms of magnetic controlled guidance for future *in vivo* biohybrid applications.

The first attempts at using bacteria biohybrids for *in vivo* medical applications have been performed using bacteria-particle swimmers. *Salmonella Typhimurium* (*S. Typhinurium*) were covalently bound to microparticles that targeted tumor cell lysates. The biohybrids exhibited chemotactic migration towards tumor cites in *in vitro* microfluidic chambers and *in vivo* mouse models [8]. More recently, the magnetotatic, *Magnetococcus marinus* (MC-1) bacteria, were used to carry drug-loaded nanoliposomes and were guided in a unified direction through a tumor in a mouse [9] (Figure 1C). These bacteria contain an internal chain of nano magnets (magnetosomes) allowing them to sense and be directed by external magnetic fields [10]. MC-1 bacteria also respond to oxygen gradients, so while biohybrids were initially magnetically guided to the tumor site, MC-1 bacteria preferentially located at oxygen-deprived regions of the tumor, which may be partially attributed to the bacteria's oxygen sensitivity.

Alternative Cargo Loads for Bacteria Biohybrids

To improve the swimming directionality and efficiency of bacteria biohybrids, cargo chassis shapes other than spherical particles have been investigated. *E. coli* swimming with non-spherical particles, with shapes such as 'barrel' and 'prolate spheroid', had increased directionally in their swimming compared to *E. coli* swimming with spherical particles [11] (Figure 1D). Adhesion of bacteria to spherical particles induced asymmetry and rotation of the particle during swimming, but with elongated particles, rotation around the short principal axis of the particle was restricted, improving the directionality of the swimming. However, in the presence of a chemical stimulus, *E. coli* carrying elliptical-shaped particles displayed no differences in their chemotactic sensing behavior compared to *E. coli* carrying spherical particles, suggesting that particle shape does not hinder the sensing mechanism of bacteria or their ability to collectively migrate towards

an attractant [12]. This opens up the idea of creating material shapes for biohybrids that can be multifunctional: improved biohybrid swimming and designed for maximum biological impact. In one study, the use of alternative micro- and nanoparticle shapes (e.g. cube, rod, and disk) improved the chances of particle uptake by cancer cells, making non-spherical shapes more advantageous for potential drug delivery applications [13].

Microtube chassis have also recently been investigated as a new type of biohybrid. In this strategy, *E. coli* were integrated with polymer microtube structures (Figure 1F), where a single bacterium became trapped within the tube and could push it through a biological solution (Figure 1G). Bacterial adhesion within the tube structure aligned the propulsion force of the *E. coli* creating a highly directional biohybrid without random swimming behavior. To increase the functionality of the biohybrid, the microtube was modified with a chemically activated 'kill switch' to terminate the bacteria swimming and prevent unwanted biofilm formation [14]. This type of biohybrid presents exciting opportunities for controlling the behavior of bacteria and for localized drug delivery. Other alternative shapes, such as microgears, have exploited the power and energy of large quantities of swimming bacteria. The collective motion of many *Bacillus subtilis* against the teeth of a polymer gear caused the gears in solution to rotate in a single direction (Figure 1E) [15] and represents a first step in designing a mechanical system that could be powered by bacteria energy.

Future Opportunities

There are opportunities for innovation and to improve biohybrid functions thus increasing their potential applications. In the future, it is conceivable to see biohybrids as potential micromachines working within the human body to improve the health of their host. While significant progress has been made for bacteria biohybrids in *in vitro* and *in vivo* systems and they have the potential to provide benefits for clinical and therapeutic applications, the disadvantages of such systems need to be addressed. For example, bacteria are ubiquitous in

the human microbiome, but they can cause infection and harm in specific locations of the body, such as the capillaries or muscle tissue. Future biohybrids will require designs tailored to localized regions of the body, to avoid triggering an immune response or impeding their functionality. One solution may entail employing the host's own bacteria to power the biohybrid or using foreign bacteria with a short life time and limited cytotoxic effects. Magnetotactic bacteria have shown promise for operating *in vivo* without causing an observed immune response [9], but further research should be done to confirm their effects on host health.

Another large disadvantage is the difficulty of tracking biohybrids *in vivo*. Bacteria or their chassis need to be labelled so they can be tracked in 3D environments with microscopic techniques or magnetic resonance imaging (MRI). This type of tracking is only possible with large quantities of labelled biohybrids and single or small groups of biohybrids cannot be monitored. High-resolution imaging techniques need to be explored to better understand bacteria swimming in confined environments and track single bacterium.

Finally, a better understanding of biohybrid swimming in native biological fluids must be investigated. *In vitro* biohybrid experiments are typically performed in buffer solutions with low viscosity, but this is not representative of all fluids in the human body. The swimming force of a bacterium is relatively low, so their swimming behavior in different fluid viscosities would vary largely. Exploring biohybrid swimming in fluids such as mucus, saliva, or hyaluronic acid would expand our knowledge of how bacteria biohybrids could operate *in vivo* and make them significantly more viable for targeted drug delivery and cell micromanipulation.

Box 1. Bacteria Biohybrids

Biohybrid microswimmers are the integration of a motile cell or cells with an artificial material. Mobile cells are powered by their surrounding biological fluid, turning chemical energy into work to operate as a bio-engine to carry their cargo load (Figure I). Biohybrid actuation has been demonstrated by contractile cells, such as skeletal or cardiac muscle, in microfluidic pumps or macroscale actuators [1]. However, flagellated bacteria have several advantages over mammalian cells for powering biohybrid micro-systems: bacteria occur in great abundance in various regions of the human microbiome as well as the environment, reproduce rapidly, and require little maintenance for viability. Bacteria biohybrid systems can utilize the sensing capabilities of the integrated bacteria to guide the biohybrid with temperature, pH, or chemical gradients, making them ideal to navigate complex biological microenvironments [2].

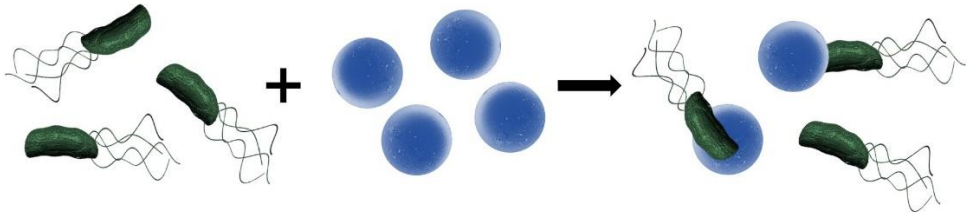

Figure I. Example of biohybrid formation. For bacteria biohybrids, free bacteria come into contact with a foreign object, adhere to the object, and then continue swimming with their new cargo load.

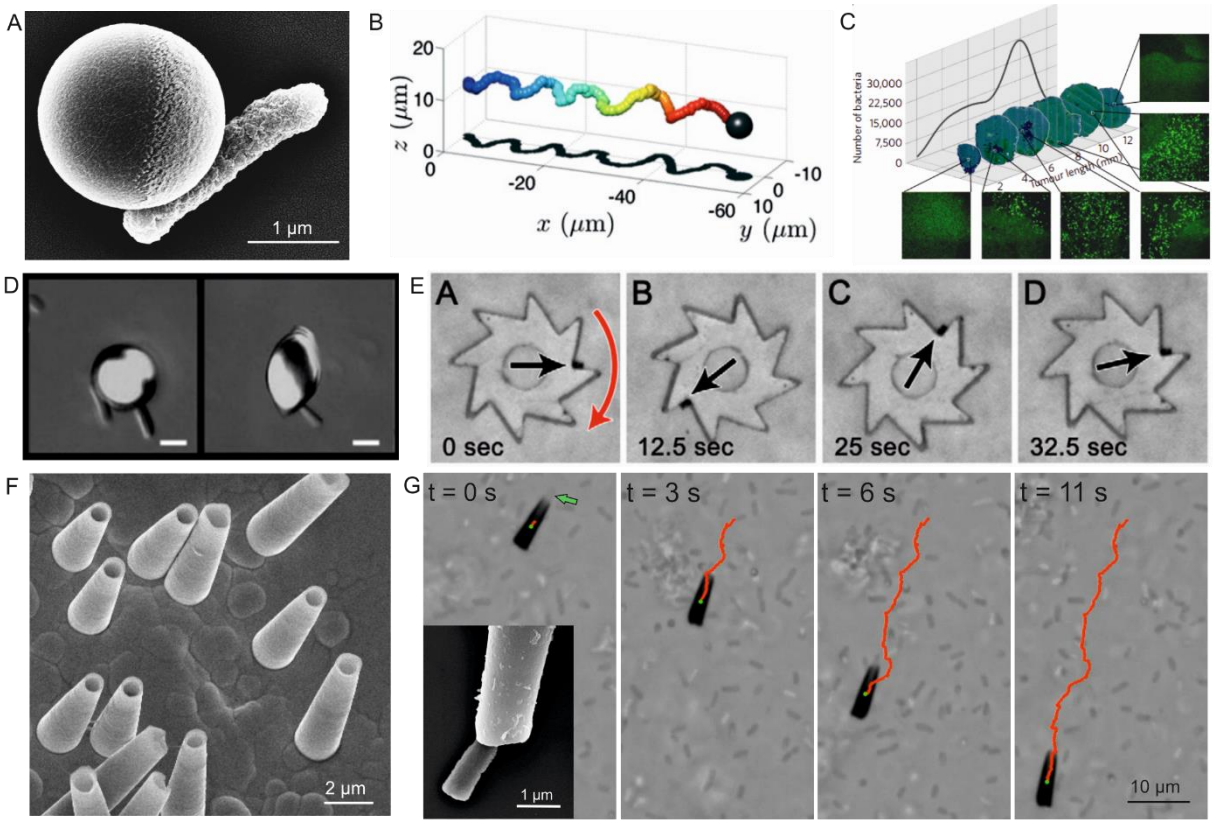

Figure 1. **Bacteria biohybrids with spherical and alternative cargo loads.** (A) An *E. coli* attached to the metal cap of a Janus particle [6]. (B) 3D trajectory of *S. marcescens* swimming with a particle [7]. (C) Distribution of MC-1 bacteria with nanoliposomes throughout tumor sections [9]. (D) *E. coli* attached to spherical (left) and prolate (right) particles. Scale bars = 2 µm [11]. (E) Snapshots of gear rotating with bacteria power. Black arrows indicate the gears' orientation obtained by computer processing and red arrows show the direction of rotation [15]. (F) Polymer microtubes for biohybrid chassis and (G) snapshots of a single *E. coli* bacterium pushing a microtube through solution. The green arrow in the first panel indicates position of *E. coli* partially trapped within the tube [14].

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