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Artificial bacterial flagella functionalized with temperature-sensitive liposomes for controlled release☆

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ABSTRACT

Inspired by flagellar propulsion of bacteria such as *E. coli*, artificial bacterial flagella (ABFs) are magnetic swimming microrobots with helical shapes. ABFs are capable of performing precise three-dimensional (3D) navigation in fluids under low-strength rotating magnetic fields making them attractive tools for targeted drug delivery. Further biomedical functionalization of these swimming microrobots is essential to enhance their biological and medical performances. We report the successful functionalization of titanium-coated ABFs with temperature-sensitive dipalmitoylphosphatidylcholine (DPPC)-based liposomes, known as "smart" drug carriers. Liposome coating on the surface of ABFs was confirmed using quartz crystal microbalance with dissipation monitoring (QCM-D) and fluorescent probes. The functionalized ABFs (f-ABFs) showed the ability to incorporate both hydrophilic and hydrophobic drugs. Finally, thermally triggered release of calcein (a common drug analog) from f-ABFs was demonstrated. These f-ABFs have the potential to be used in targeted and triggered drug delivery, microfluidic devices and biosensing.

1. Introduction

Magnetic micro/nanorobots, wirelessly powered by magnetic fields, have the potential to be used in biological and medical applications such as *in vitro* cell manipulation, targeted therapy and *in vivo* sensing [1–8]. Artificial bacterial flagella (ABFs) are magnetic helical microrobots that use a cork-screw strategy for self-propulsion and are of similar size as real bacteria such as *E. coli* [9–12]. ABFs can be actuated in liquid under weak rotating magnetic fields (1000 times lower than the fields used in MRI systems), which are not harmful to living cells and tissues. Their flagellar propulsion, i.e. cork-screw motion, is a promising approach for *in vivo* applications [13,14].

When the helical body of an ABF rotates by following a rotating magnetic field in liquid, the rotational motion translates to translational motion. By changing the rotational axis of the rotating magnetic field, the ABFs swim in 3-D allowing them to precisely target the desired sites. Previous work showed that ABFs can be used to manipulate cellular and sub-cellular objects by direct pushing [15,16] and non-contact methods (agitating the peripheral liquid when an ABF is rotating) [17,18]. However, for biomedical applications such as drug delivery and wireless sensing, further surface biofunctionalization with specific chemicals, such as drug molecules and chemicals, is required [19]. For example, biological modification of the surfaces of nano/micro motors has been used in DNA separation and drug delivery applications [2].

Liposomes have been extensively studied in various applications including drug delivery systems and cell membrane science [20–22]. A liposome is a lipid vesicle consisting of a self-assembled lipid bilayer in which DNA, drugs and/or chemicals can be encapsulated. Liposomes range in size from 20 nm to several hundred micrometers. Depending on the lipid composition of liposomes, their payload can be locally and remotely trigger-released by different stimuli, such as enzymes, pH, ultrasound, light and temperature [23]. Temperature-sensitive liposomes have been proposed for local hyperthermia treatments in cancer therapy [24,25]. Dipalmitoylphosphatidylcholine (DPPC) is commonly used as the key component for temperature-sensitive liposomes. DPPC has a phase

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transition temperature of 41 °C, at which liposomes switch from the solid phase to the liquid-gel phase and become leaky releasing their encapsulated cargo [26]. Adding small amounts of lysolipids (such as 10% 1-myristoyl-2-stearoyl-sn-glycero-3-phosphocholine (MSCP)) with DPPC liposomes increases the drug release rate of DPPC liposomes at 39–42 °C [27,28].

In this work, functionalized ABFs surface-coated with DPPC-based liposomes are reported. Quartz crystal microbalance with dissipation monitoring (QCM-D) was used to investigate the adsorption of liposomes onto a TiO_2 surface. Confocal laser scanning microscopy (CLSM) was used to detect fluorescently labeled (liposome membrane lipids are fluorescent) or calcein loaded (liposome encapsulated calcein is fluorescent) liposomes on the surface of ABFs. Finally, the calcein release from functionalized ABFs was studied.

2. Materials and methods

2.1. Materials

The photoresist IP-L was purchased from Nanoscribe GmbH, Germany. Dipalmitoylphosphatidylcholine (DPPC), 1-myristoyl-2-stearoyl-sn-glycero-3-phosphocholine (MSPC) and lissamine rhodamine B lipids were purchased from Avanti Polar Lipids, Inc. Sodium chloride (NaCl), calcein disodium salt and 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) were purchased from Sigma–Aldrich Chemie GmbH, Buchs, Switzerland. The HEPES buffer solution was prepared with 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid and 0.15 M sodium chloride in Milli-Q water (Milli-Q gradient A10, Millipore, resistivity 18.3 MΩ cm). The pH of the buffer was adjusted to pH 7.4 by a 6 M NaOH solution.

2.2. Fabrication process of ABFs

ABF arrays were fabricated using direct laser writing (DLW) and e-beam deposition methods. The process consisted of three steps (Fig. 1): Step 1, writing helical structures in a photoresist IP-L using DLW based on two-photon polymerization [29]; Step 2, developing the written sample in isopropyl alcohol (IPA) to remove un-polymerized resist; Step 3, coating the sample with Ni and then Ti layers (25 nm Ni and 15 nm Ti) using electron beam deposition.

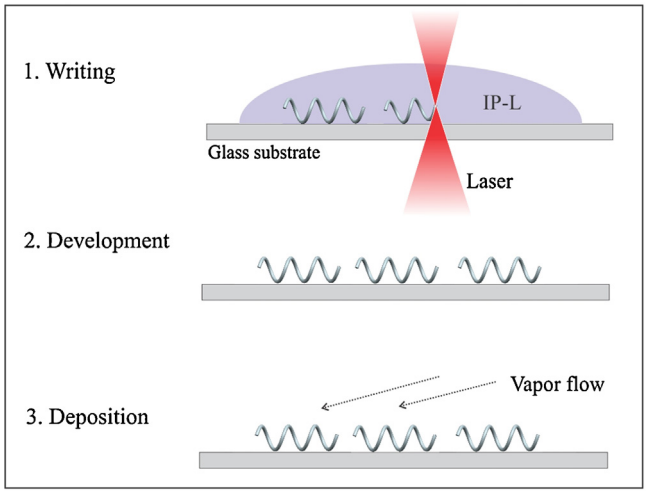

Fig. 1. Fabrication flow of ABFs. Step 1: Writing helical arrays in IP-L photoresist. Step 2: Development in IPA. Step 3: Coating the Ni/Ti bilayer using electron beam deposition.

The Ti layer is naturally oxidized to TiO_2 when exposed to oxygen. A more detailed fabrication process is described elsewhere [15].

2.3. Preparation of liposome-coated ABFs

Fig. 2 shows the three-part preparation flow of liposome-coated ABFs. First, unilamellar DPPC liposomes are prepared. Second, the ABF suspension is prepared, and third, the mixture of the two suspensions and washing generates functionalized ABFs (f-ABFs).

The unilamellar DPPC liposomes were prepared by extrusion [30,31]. DPPC lipids in chloroform were completely dried in a glass vial under a gentle N_2 flow for 30 min and rehydrated with HEPES buffer. In this step, fluorescent molecules can be dissolved in the HEPES buffer to be incorporated within the liposomes. The glass vial was subsequently vortexed to create multilamellar vesicles. The multilamellar vesicle suspension was transferred into a glass syringe and assembled to form the extruder (Fig. S1a). The lipid solution was extruded 31 times through two packed polystyrene membranes (Fig. S1b) to form uniform-sized (200 nm) unilamellar vesicles. Extra care was taken to keep the entire extruder system including the lipid solution above the transition temperature (41 °C) during the extrusion by pre-warming the system at 65 °C in an oven. The DPPC/MSPC (9:1 w/w) was prepared by adding 10% MSPC lipids in DPPC lipids before drying. All lipid mixtures were dissolved in buffer at 2.5 mg/ml concentration.

The second step was to prepare the ABF suspension. The ABF array was cleaned in an UV/ozone cleaner for 30 min followed by washing with Milli-Q water. The array was then detached from the

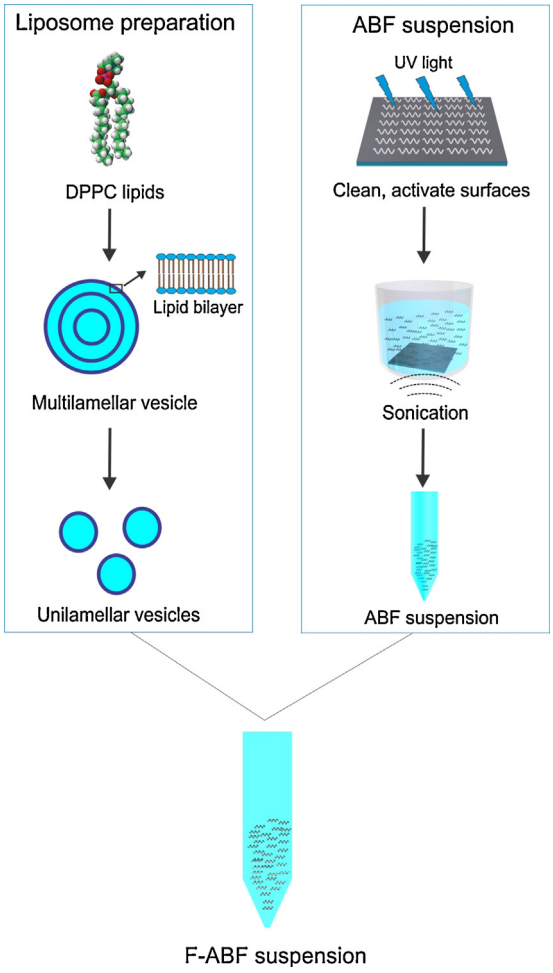

Fig. 2. Preparation flow for coating ABFs with unilamellar DPPC liposomes.